SPEAKER ABSTRACTS

Lecture.1

The Pharmacology of Hypertension- New Challenges

Michael J. Mulvany, Department of Pharmacology, University of Aarhus, University Park 1240, 8000 Aarhus C., Denmark

The introduction in 1957 of thiazides for the treatment of essential hypertension (EH) changed a life-threatening disease into one which could be controlled. Modern therapies using e.g. angiotensin receptor antagonists are effective and almost without side-effects. Significant questions, however, remain. First, although treatment reduces risk, the risk is not normalized. Second, the cause of EH remains unknown. Third, EH is still a disease requiring life-long treatment. The hallmark of EH is, apart from increased blood pressure, the structural change of the resistance vasculature, with reduced lumen and increased wall:lumen ratio. This structural change is the earliest type of target-organ damage, and recent evidence shows structural change in the resistance vasculature is a strong risk factor for later cardiovascular events. Treatment should thus seek not only to reduce blood pressure, but also to normalize vascular structure. We and others have shown that this requires vasodilator treatment, and recent trials show reduced risk with such treatment compared to traditional EH therapy (beta-blockers and diuretics). Normalization of vascular structure is one of the new challenges for treatment of EH.

Key words: antihypertensive therapy, vasodilators, vascular structure, cardiovascular risk

Acknowledgements: The author is supported by the Danish Medical Research Council and the Danish Heart Foundation.

L1

Muscle-derived Cytokines: Pharmacological implications

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For most of the last century researchers have searched for a muscle-contraction-induced factor, which mediates some of the exercise effects in other tissues such as the liver and the adipose tissue. IL-6 and IL-8 are produced by contracting muscles and released into the blood. We have suggested that at least muscle-derived IL-6 fulfills the criteria of an exercise factor and that such classes of cytokines should be named "myokines". The biological roles of IL-6 are many: 1) Activation/inhibition of the transcript of metabolic genes, 2) Induction of lipolysis, 3) Inhibition of TNF, and 4) Enhancement of glucose uptake. Carbohydrate supplementation during exercise has been shown to inhibit the release of IL-6 from contracting muscle, but not hepatic clearance in humans. Supplementation with vitamin C and E inhibits exercise-induced release of IL-6, but not muscle-IL-6 mRNA. IL-8 is produced and released by working muscle fibers and may play a role in angiogenesis. The clinical consequences of modification of the cytokine response to exercise may include both risk of obtaining infectious diseases, training adaptation including angiogenesis and insulin resistance.

Key words: cytokines, muscle

L2

Serotonin 5-HT2 Receptors: Molecular and Genomic Diversity

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An overview of the pharmacology of serotonin receptors in the 5-HT2 subfamily will focus on genetic and molecular events that create functional variants of 5-HT2A and 5-HT2C receptors. Evaluation of single nucleotide polymorphisms (SNPs) that alter the structure of the 5-HT2A receptor reveals prominent alterations in intracellular signal transduction and in receptor desensitization. SNPs in the noncoding, regulatory region of the 5-HT2A receptor also have functional consequences, as revealed by in vitro promoter assays and by endogenous expression of 5-HT2A receptor mRNA. Unlike the 5-HT2A receptor, functionally significant genetic variation in the 5-HT2C receptor seems to be replaced by another mechanism -- RNA editing. RNA editing of the 5-HT2C receptor changes the genetic code at the level of RNA, generating as many as 24 receptor isoforms, some of which have prominent functional deficits. RNA editing and genetic modifications have also been examined at the level of human psychiatric diseases.

Key words: Serotonin receptors, genetics, polymorphisms, RNA editing

This research was supported by NIH research grants MH34007 and DA05181.

L3

Annexin 1: a mediator of glucocorticoid action at the neuroendocrine-immune interface.

J Buckingham, C John, E Solito, 1R Flower, 2H Christian &2J Morris, Neuroscience & Mental Health, Imperial College London; 1Biochemical Pharmacology, William Harvey Research Institute, London; 2Human Anatomy & Genetics, University of Oxford.

Glucocorticoids (GCs) play an essential role in the maintenance of homeostasis and aberrations in the mechanisms which control their secretion and/or activity are strongly implicated in the pathogenesis of a number of common diseases including depression, hypertension, diabetes/obesity and immune/inflammatory disease. Annexin 1 (ANXA1), a protein mediator of GC action, is a key regulator of GC secretion, acting within the brain and pituitary gland to depress the release of the hormones which normally drive GC production. Its mode of action is unusual as it acts by a juxtacrine/paracrine mechanism and, following secondary processing, appears to interact with formyl peptide receptors (FPRs). Ligands for FPRs include bacterial peptides, mediators of the resolution of inflammation and peptides concerned with the pathogenesis of Alzheimer's disease, suggesting a complex interaction between GCs and inflammatory mediators in the brain and pituitary gland. Early life events (e.g. stress) exert long-term effects on ANXA1 expression and function in adulthood. ANXA1 may thus contribute to the altered disease susceptibility linked to adverse events in perinatal life.

Supported by the Wellcome Trust.

L4

TOWARDS HIGH RESOLUTION STRUCTURES OF G-PROTEIN COUPLED RECEPTORS

Hartmut Michel, Nicolas Andre, Jan Griesbach, Christoph Krettler, Cecile Prual, Chandramouli Reddy, Christoph Reinhart, Arun Shukla, Ankita Srivastava
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G-protein coupled receptors constitute the most important class of drug targets. Despite this fact the information about the precise structure of such receptors is very limited. The only high resolution structure of a G-protein coupled receptor is that of bovine rhodopsin which can be isolated from bovine eyes in sufficient quantity and homogeneity. Lack of suited starting material is the reason for the lack of structural information on other G-protein coupled receptors. In order to solve this bottleneck we have expressed in a structural genomics type of approach the cDNAs of more than 100 G-protein coupled receptors in Escherichia coli, the yeasts Saccharomyces cerevisiae, Schizosaccharomyces pombe and Pichia pastoris, in insect cells using the baculovirus system, and in various mammalian cell lines using the Semliki Forest virus system. At the moment we express and routinely purify about a dozen of receptors, mainly in Pichia pastoris and in insect cells, and have started promising crystallization attempts. In parallel we study the conformation of peptide ligands using solid state nuclear magnetic resonance spectroscopy and compare the receptor bound conformation with that in solution.

S1.1

Drug Resistance in Cancer Chemotherapy

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Recent progress in molecular cancer therapeutics has revealed various approaches for the development of novel anticancer drugs. We have been focusing on molecular cancer therapeutics mainly in the research area of drug resistance. P-glycoprotein (P-gp) has a major role in multidrug resistance. We have developed a P-gp inhibitor MS-209, which is currently under clinical study. Although P-gp is a typical and well-known mediator of drug resistance, cancer cells have other mechanisms of drug resistance Apoptosis is a pathway that modulates drug sensitivity, while apoptosis resistance is directly related to drug resistance. We have identified glyoxylase 1 as an apoptosis resistance protein, which plays a role for drug resistance in solid tumors. Solid tumors have another mechanism of drug resistance, which is called UPR (unfolded protein response). We have identified several compounds showing rather selective cytotoxicity under UPR conditions. P53 mutation and apoptosome defect are critically involved in drug resistance. We found agents that bypass these defects and induce apoptosis rather selectively in cancer cells.

Key words: Drug resistance, Apoptosis resistance, P-glycoprotein, Glyoxyla

S1.2

INTERFACIAL INHIBITION: TOPOISOMERASE I INHIBITORS, ONE OF NATURE'S PARADIGMS FOR DRUG DISCOVERY

Yves Pommier, Laboratory of Molecular Pharmacology, NCI, NIH.

Interfacial inhibitors are uncompetitive inhibitors that bind with high selectivity to a specific site involving two or more macromolecules within macromolecular complexes undergoing conformational changes (TiPS, 2005, 28:136). Interfacial binding traps (generally reversibly) a conformational state of the complex, resulting in kinetic inactivation. The paradigm interfacial inhibitors are camptothecins. We also recently demonstrated that interfacial inhibition applies to the non-camptothecin topoisomerase I inhibitors, the indenoisoquindines and indolocarbazole (Mol Cancer Ther 2006, 5:287). We will also provide examples generalizing the interfacial inhibitor concept to inhibitors of topoisomerase II (anthracylines, ellipticines, epipodophyllotoxins), gyrase (quinolones, ciprofloxacin, norfloxacin), RNA polymerases (amanitin and actinomycin D), and ribosomes (antibiotics such as streptomycin, hygromycin B, tetracycline, kirromycin, fusidic acid, thiostrepton, and possibly cycloheximide). We discuss the implications of the interfacial inhibitor concept for drug discovery, and especially testing for drugs that trap (stabilize) macromolecular complexes.

S1.3

mTOR as a Target for Cancer Therapy

Peter J. Houghton, Department of Molecular Pharmacology, St. Jude Children's Research Hospital, Memphis, TN 38105. U.S.A.

The serine/threonine kinase, mTOR (Target Of Rapamycin), regulates initiation of translation through phosphorylation of p70 S6 kinase (S6K1) and the 4E-BP proteins that suppress eIF4E, the RNA cap-binding protein. mTOR lies downstream of Akt in the PI3K pathway. Inhibition of mTOR leads to decreased translation of mRNA species that have structured 5'-UTR's (untranslated regions), and causes selective inhibition of cell cycle regulators (cyclin D1), and transcription factors (cMyc, HIF1-alpha). Inhibition of mTOR leads to accumulation of cells in G1 phase, but induces apoptosis in some cancer cell lines deprived of exogenous growth factors.

Considerable data support dysregulation of the PI3K-mTOR pathway in human cancer, and the suggestion has been made that such cancer cells become hypersensitive to inhibitors of mTOR. Currently, all mTOR inhibitors in the clinic are derivatives of rapamycin, a natural prooduct macrocydic lactone derived from S. hygroscopicus. Here we will consider the current status of rapamycin analogs, and the potential for mTOR as a target for cancer therapeutics.

S1.4

Anticancer Agents Discovery from Nature Products

Jian Ding; Division of Anti-Tumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China.

Natural Product is a very important resource for anticancer lead finding. One of the most promising research areas of Shanghai Institute of Materia Medica (SIMM) is novel anticancer agent discovery from Traditional Chinese Medicine. Camptothecin is a natural alkaloid isolated from Camptotheca acuminata. SIMM developed its derivative 10-hydroxycamptothecin in 1970s and it is still widely used in China. Recently, we synthesized a novel amphoteric 9-substituted camptothecin, Chimmitecan, which confers improved anti-cancer pharmacological profiles both in vitro and in vivo. Salvicine, a natural product derivative from Salvia prionitis, is a new topoisomerase II inhibitor. It also showed prominent anti-multidrug resistance effects and transcription factor c-Jun played a principal role in exerting this effect. Pseudolarix acid B got from Chinese plant Pseudolarix kaempferi. It caused depolymerization of tubulin by binding to a novel site, and abrogated secretion of VEGF due to reducing hypoxia-inducible factor 1a protein by promoting proteasome pathway, which may responsible for its powerful anti-angiogenic effect.

Key words: natural product, Camptothecin, Salvicine, Chimmitecan, Pseudolarix acid B

S1.5

TARGETING CELL SURVIVAL IN CANCER CHEMOTHERAPY

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Viable cancer cells, capable of proliferation, are different from normal, healthy cells in two key respects: they express abnormal levels of oncogene activity and are genetically unstable, with aberrant chromosomal integrity reflecting high levels of genomic instability. In normal cells both of these events initiate either cell senescence or cell death, often by apoptosis. Thus, in order for a tumour cell to survive and replicate, mechanisms to avoid senescence and apoptosis need to be initiated. It is important to stress that these changes are independent of proliferative status: both slow and fast growing tumours resist cell death and senescence. Since many carcinomas are slow growing (tumour doubling time for breast cancers has a median of 100 days) drugs targeted to proliferation biochemistry are clearly going to have limited efficacy. Targeting cell survival, with the goal of inducing cell death is attractive for both slow and fast growing tumours which are currently chemoresistant, since the inherent tumour-specific load of oncogene activity/ DNA damage shoud permit these cells to die preferentially compared to normal cells (without oncogene expression and inherent DNA damage).

S2.1

Pharmacologic or gene therapeutic inhibition of PKC increases contractility and attenuates heart failure

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Deletion of PKC in the mouse results in augmented sarcoplasmic reticulum Ca^{2+} loading, enhanced Ca^{2+} transients, and augmented contractility, while overexpression of PKC in the heart blunts contractility. Here we show that acute inhibition of PKC with the pharmacologic agents Ro-32-0432 or Ro-31-8220 significantly augmented cardiac contractility in wildtype mice, but not in PKC deficient mice. Ro-32-0432 also acutely increased cardiac contractility in two different models of heart failure in vivo. Moreover, acute or chronic treatment with Ro-32-8220 in a mouse model of heart failure significantly augmented cardiac contractility and restored normal pump function. Finally, adenoviral-mediated gene therapy with a dominant negative PKC cDNA rescued heart failure in a rat model of post-infarction cardiomyopathy. PKC is also the dominart cPKC isoform expressed in the adult human heart, suggesting relevance of these findings to human pathophysiology. Pharmacological inhibition of PKC may serve as a novel therapeutic strategy for acutely enhancing cardiac contractility in the setting of severe functional deterioration, or even as a longer-term treatment for certain stages of heart failure.

S2.2

Protein Kinase G-delta signaling in the heart

Susan F. Steinberg, Columbia University

Protein kinase G-delta (PKG-delta) is an important target for G-protein-coupled receptor signaling pathways in the heart. Conventional models view PKG-delta as a lipid cofactor-activated enzyme that plays a key role in the regulation of cardiac contractile function, ischemic preconditioning, and structural remodeling of the heart. Our recent studies identify novel PKG-delta activation mechanisms through tyrosine phosphorylation by Src family kinases in cardiomyocytes subjected to oxidative stress. Tyrosine phosphorylation alters the co-factor requirements and substrate specificity of PKG-delta. Tyrosine phosphorylation also generates docking sites on PKG-delta for SH2-domain containing binding partners, such as the adapter protein Shc. Studies that implicate phospho-tyrosine residues on PKG-delta as a mechanism to alter PKG-delta's enzymology and confer kinase-independent actions as a scaffold will be discussed.

S2.3

Spatial and temporal controls of PKC isoform activation

Yasuhito Shirai and Naoaki Saito; Biosignal Research Center, Kobe University.

Protein kinase C (PKC) plays an important role in various cellular events including differentiation, proliferation and gene expression etc. How can PKC give distinct roles in such large number of cellular responses One of the reasons is that PKC family contains many isoforms. However, they show similar substrate-specificity in vitro and multiple subtypes are expressed in the same cell. We have so far investigated PKC movement in living cells using green fluorescent protein, and found that lipid messengers induce isoform-specific translocation of PKC, and spatial and temporal localization of each isoform is distinctly regulated. For example, PKC is translocated from the cytoplasm to the Golgi complex by arachidonic acid, but to the plasma membrane by saturated fatty acids. In contrast, PKC doesn't respond to any fatty acids. In addition, different PKC translocation results in distinct cellular responses. These results indicate that spatial and temporal activation, which is termed as "the targeting", contributes to multiple functions of PKC. In the symposium, diversity and mechanisms of the PKC targeting is discussed.

Key words; translocation, lipid messenger

S2.4

IL-6 Mediates β2-AR–induced STAT3 Activation and its Signaling Pathway in Mouse Heart

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This study was aimed to determine whether β-adrenoceptors (βAR) activate STAT3 and to examine the underlying mechanism in mouse heart. We recently reported that β2-AR stimulation leads to a delayed STAT3 activation via an IL-6 family of cytokines-mediated pathway, and that cardiac fibroblasts is likely the predominant source of IL-6 in response to ISO stimulation in mouse myocardium. Surprisingly, the effect of cAMP was independent of protein kinase A and the Epac (exchange protein directly activated by cAMP)-Rap1 pathway. p38 MAPK inhibitor SB203580 abrogated isoproterenol-induced IL-6 release in cardiac fibroblasts. p38 MAPK could be positively regulated by Gs-AC-cAMP but negatively regulated by Gi-PI3K pathway. Multiple transcription factors (AP-1, C/EBP, NF-κB and CREB) regulating the IL-6 gene are activated in response to isoproterenol stimulation, which may provide essential linkage between upstream cAMP-p38 MAPK signaling cascade and downstream IL-6 gene transcription. The results suggest that β2-AR mediates IL-6 production through a noncanonical cAMP responsible pathway and p38 MAPK.

Key words: adrenoceptor, STAT3, heart.

Acknowledgement: this work was supported by NSFC No30470691.

S2.5

Potent Inhibitors of Vascular Oxidative Stress: Specific Block of Nox4-type NADPH Oxidase for Cardiovascular and Neurological Disease

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NADPH oxidases (Nox) are major sources of oxidative stress in artery walls and underlie cardiovascular disease. The Nox4 isoform is the main source of superoxide generated in vascular cells of mice and humans. We have identified potent inhibitors of the Nox4-dependent enzyme, and here we report their cardiovascular protective actions in animal disease models. Substituted benzamides suppress superoxide production in mouse vascular smooth muscle cells (typical IC_{50} = 1.6 μM), and are at least 50-fold more potent than in a phagocytic cell line (J774). Over 2 wk, one compound reduced the hypertensive response to angiotensin II, attributable to vascular oxidative stress, but had no effect in control rats. In apolipoprotein E-KO mice this compound (15 mg/kg per wk for 16 wk), but not apocynin, reduced atherosclerotic lesion area in aorta from 40 ±3 to 33 ±3 % ($P < 0.05$). Finally, development of a neointima induced by periaterial collars in rabbits is accompanied by Nox-dependent oxidative stress and compromises endothelial NO function, and all effects were prevented by a Nox4 inhibitor or apocynin given locally via the collar. These compounds are selective and potential therapies for artery disease and stroke

S3.1

Involvement of transporters in nephrotoxicity

Hitoshi Endou[1,2], and Naohiko Anzai[1]; 1 Department of Pharmacology and Toxicology, Kyorin University School of Medicine; 2 Fuji Biomedix Co., Ltd.

Numerous drugs and endogenous compounds are efficiently excreted from the real proximal tubule via two carrier-mediated pathways: organic anion transport systems and organic cation transport systems. These transport systems seem to be an early event for nephrotoxicity because most nephrotoxicants are taken up into renal target cells for further actions. Recent advances in the transporter research have made it possible to investigate the mechanisms of transport of those toxic compounds and their transporter-mediated organ toxicity at the molecular level. An organic cation transporter 1 (OCT1) was cloned in 1994. On the other hand, we have identified 6 isoforms of organic anion transporters (OAT1-4, URAT1, and Oat5) since 1997. Through these transporters with broad substrate selectivity, namely "multispecific" transporters, exogenous compounds including drugs and environmental toxicants enter the cells and exert their toxic effects. Such transporter-mediated nephrotoxicity are observed in β-lactam antibiotics cephaloridine, mycotoxin ochratoxin A, and Sevoflurane degradation products compound A, mediated by organic anion transporters.

Key words: Organic ion transporter, multispecific transporter.

S3.2

INVOLVEMENT OF TRANSPORTERS IN NEUROTOXICITY

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The blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB), are the first lines for protecting the brain. A complex network of transporters expressed at BBB and BCSFB participates to solute exchanges between blood and brain. Influx transporters belonging to the Solute Carrier superfamily may facilitate the occurence of neurotoxic effects. Thus, the monocarboxylate transporter MCT1 transports across BBB the recreational drug of abuse g-hydroxybutyrate leading to seizures, respiratory depression and impaired consciousness. In contrast, efflux transporters like P-glycoprotein (Pgp), acts by pumping out endothelial cells towards the blood a wide variety of substrates including potential neurotoxic compounds. More recently, a second member, the Breast Cancer Resistance Protein (BCRP) was found co-localized with Pgp at BBB. The neuroprotective effect of these ABC transporters was demonstrated against xenobiotics like ivermectin, an anti-parasite agent substrate of Pgp and dietary phototoxins which are substrates of BCRP. All these transporters play a critical role for protecting the brain from neurotoxic events.

Key words: neuroxicity, ABC, SLC, transporter

S3.3

ABCB1 related Adverse Effects of Drugs including Drug-Drug Interactions

Oliver von Richter, Ph.D. Division of Drug Metabolism and Pharmacokinetics, ALTANA Pharma AG, Konstanz, Germany

ABCB1 (P-glycoprotein/MDR1) translocates a broad variety of xenobiotics out of cells. In conjunction with drug-metabolizing enzymes such as CYP3A4, ABCB1 provides a protective physiological barrier against xenobiotics. ABCB1 limits drug entry into the body after oral drug administration, promotes drug elimination into bile and in addition, once a xenobiotic has reached the systemic blood circulation, limits drug penetration into sensitive tissues, e.g. into the brain, heart, testes, lymphocytes, and fetal circulation. Therefore, the identification of ABCB1 substrates, inhibitors, inducers, and combinations is relevant for drug development and drug safety. However, due to its functional complexity, the identification of ABCB1 substrates and inhibitors is difficult. Furthermore ABCB1 function is influenced by a compound's intrinsic passive permeability and its fraction unbound in plasma. These factors will be discussed in the context of ABCB1 related drug-drug and drug-food interactions affecting drug absorption and blood-brain distribution.

Key words: ABCB1, drug-drug interaction

S3.4

Transporters and adverse effects of drugs

Philippe Lechat; Pharmacology department, Pitié Salpêtrière Hospital, UPMC, Paris, France.

Among drug transporters, the ABC family is one of the most important with MDR and MRP transporters playing the role of efflux pumps. They basically extrude xenobiotics and drugs out of the cells. Gene polymorphisms, inhibition and induction of such ABC transporters may modulate such efflux pump activity and induce cellular accumulation and toxicity in case of loss of function. Such toxicity has been observed with renal tubular cell toxicity with methotrexate and tenofovir in case of MRP2 polymorphisms and according to different haplotypes. Preservation of cidofovir renal toxicity has been obtained with co-administration of probenecide which inhibits tubular organic anion transport, then preventing its extensive accumulation in tubular cells. Accumulation of hydroxychloroquine or chloroquine in retinal cells may involve ABC function as suggested by experimental works and could participate to retinal toxicity during chronic treatment. A similar mecanism of toxicity with intracellular accumulation could be involved for neuro-toxicity and hepatic toxicity of some drugs. Further investigations of genetic-kinetic-dynamic interactions will have to be undertaken to provide complementary informations.

S4.1

The current situation of medicinal products in children: Therapeutic orphans for 50 years

Dr Madlen Gazarian, Paediatric Clinical Pharmacologist, Sydney Children's Hospital, Randwick and Senior Lecturer, School of Women's and Children's Health, University of NSW, Sydney, Australia

The term "therapeutic orphan" was first used over 40 years ago as a colourful description of children's limited access to medicines with demonstrated efficacy, safety and quality. Even now, the majority of marketed medicines worldwide have not been studied in children and so are not approved by regulatory authorities for use in children. Children remain therapeutic orphans because they are either denied the use of many new medicines, or are given medicines that have bypassed rigorous evaluation, exposing them to potentially ineffective or harmful therapies. Unapproved medicines use is very common, with rates up to 40-90% in hospitalised paediatric patients. This situation has a multifactorial aetiology and a number of important consequences, including increased risk of harm; unapproved medicines use leads to increased incidence and seriousness of ADRs in children. Recent major initiatives in the US and Europe have stimulated increased medicines research in children, producing much needed information, so it looks like the therapeutic orphan may finally be adopted. However, many challenges remain to be overcome before we can consider that this orphan has found a happy home.

S4.2

Why do we need pediatric studies? Why not extrapolate from adult data?

Hidefumi Nakamura, Division of Clinical Research; National Children's Medical Center, National Center for Child Health and Development; Tokyo, JAPAN

Although many equations are proposed to extrapolate pediatric dosage from adult data, none has been proven to be exact. The dynamic process of growth, differentiation and maturation sets children apart from adults. In addition to growth in physical size, dramatic changes in body proportions, body composition, and physiology take place during infancy and childhood. Age-dependent changes in body composition influence drug distribution. Maturation of liver and renal function influence drug clearance. Maturation can also influence pharmacodynamic response of child to certain drugs. These changes do not occur simultaneously and the influence of these changes on drug response differ for each drugs. It is impossible to exactly determine pediatric dosage unless pediatric studies are performed. Children can also differ from adults in the types of diseases and/or manifestations of diseases (e.g., newborn respiratory distress syndrome and Wilms' tumor). A drug that has been tolerated well by adults may cause adverse events in children (e.g., tooth staining after tetracycline treatment). Therefore we do need to perform pediatric studies on drugs to ensure the safety and efficacy of drugs in children.

S4.3

The specificities of paediatric drug trials / paediatric devlopment plan

Pons G., Head Department of Clinical Pharmacology, Groupe Hospitalier Cochin St Vincent de Paul, Université Paris V René Descartes, Paris, France

CTs in children are more difficult than in adults due to ethical reasons, recruitment difficulties and therefore CTs take longer and cost more During CTs children should be protected and informed consent should be obtained from their parents and themselves whenever possible. Procedures should be as less harmful as possible regarding pain, anxiety, blood loss. Often the number of available patients is limited. Due to insufficient information and prejudices regarding CTs and use of placebo, randomization, parents are reluctant to give consent. The number of children exposed to investigational new drugs (INDs) should be limited to the minimum required. These constraints impact the methodological choice in favour of population approaches, modeling (PK, PK/PD, maturation, simulation of CTs), sequential approches (phase II: continuous reassessement method; phase III: triangular test) Children cannot express their symptoms like adults and specific tools have to be developed and validated. Delayed side effects consecutive to exposure during growth require long term follow-up. Time to initiate drug development in children as compared to adults varies from phase I to phase IV depending on disease and IND.

S4.4

The new initiatives for better medicines for children

Kalle Hoppu; Poison Information Centre, Helsinki University Central Hospital, and Hospital for Children and Adolescents & Department of Clinical Pharmacology, University of Helsinki, Helsinki, Finland

After over 30 years of therapeutic orphans - status the outlook for children's medicines is more positive than ever. The breakthrough was the Better Pharmaceuticals for Children Act passed by the US Congress in 1997 and providing a six-month extension of market exclusivity for on patent drugs in return for the drug company testing the drug in children. The Pediatric Rule adopted by the FDA in 1998 made it possible in US to require testing of new medicines expected to have significant use in children. The result has been more than 100 paediatric labellings. In the European Union a Paediatric Regulation, in preparation since 2000, is expected to be adopted in its final form in June 2006 and to come in force before the end of 2006. It requires that all medicinal products, therapeutically relevant for children and still under patent protection, applying for market authorisation undergo paediatric development. The fulfilled paediatric development, including development of age-adapted formulations, will be rewarded with a six-months extension of in effect patent protection. Similar measures have been implemented or are under discussion in many other countries.

Key words: child, development, medicines

S5.1

"Overview of potential targets for disease-modifying drugs in Alzheimer's disease"

A. Claudio Cuello; McGill University, Dept. of Pharmacology and Therapeutics, Montreal, Quebec, CANADA

Alzheimer's Disease (AD) is the most common cause of progressive cognitive decline in the elderly. Genetic, molecular and cellular studies have implicated the dysmetabolism of Amyloid Precursor Protein as a causal event of familial and sporadic forms of AD. This has led to the "Amyloid Hypothesis" which signals that the excess of extracellular A-beta peptide is responsible for synaptic and neuronal degeneration. Most current therapeutic approaches are of symptomatic nature (e.g. anticholinesterases or glutamate receptor antagonists), however, the present detailed knowledge of the AD molecular neuropathology has opened opportunities to investigate novel therapeutic targets that might generate disease-modifying therapies. These new potential targets will be discussed in this introductory overview. This Symposium will revise the status of therapeutic strategies geared at diminishing A-beta peptide generation interfering with APP cleavage sites (inhibition of gamma or beta APP secretases) as well as the immunological removal of amyloid material and the search for newer, safer, CNS specific anti-inflammatories.

Key words: Alzheimer's therapy

Acknowledgements: Supported by CIHR

S5.2

Beta-Secretase as a Therapeutic Target

Martin Citron, Department of Neuroscience, Amgen Inc, Thousand Oaks

Finding inhibitors of Aβ42 generation is a major goal of Alzheimer's disease drug development. Two target protease activities, β and γ secretase, were operationally defined in the early 90s, but progress in this area was slow, because the actual enzymes were not understood at the molecular level. Some years ago we have identified a novel membrane bound aspartic protease, BACE1, as β secretase. This finding has been confirmed and BACE1 and its homolog BACE2 have been characterized in detail by many groups. Major progress has been made in two areas: First, the x-ray crystal structure, which is critical for rational inhibitor design, has been solved and shown to be similar to that of other pepsin family members. Second, knockout studies show that BACE1 is critical for Aβ generation, but the knockout mice show an otherwise normal phenotype, raising the possibility that therapeutic BACE1 inhibition could be accomplished without major mechanism based toxicity. However, target-mediated toxicity of β secretase inhibition cannot be ruled out based on the currently available data alone. While various peptidic β secretase inhibitors have been published, the key challenge now is the generation of more drug-like compounds that could be developed for therapeutic purposes. Other current areas of investigation, including identification of additional BACE1 substrates, the potential role of BACE1 overexpression in AD and the phenotype of BACE2 knockout mice will be discussed.

S5.3

Experimental studies of traditional Chinese medicine to treat Alzheimer disease

LI Lin, ZHANG Lan, ZHAO Ling, WANG Wen; Department of Pharmacology, Xuan-wu Hospital of Capital University of Medical Sciences, Beijing 100053, China

AIM: To investigate the effects of Chinese herb compound Shen-wu Capsule (SW) on Alzheimer disease (AD)-like animal models. METHODS: SW was intragastrically administered to the animals for 1 or 2 mon. Morris water maze was used to detect learning and memory, microarray and RT-PCR to measure gene expression, Western blotting and immuno-histochemistry to determine content of related proteins. RESULTS: In 8 kinds of AD-like animal models (including APP transgenic mouse model), SW improved learning and memory ability, decreased brain b-amyloid (Ab) content, inhibited b- and g-secretase; decreased microglial activation, IL-1b and TNFa content; inhibited oxidative stress; increased ratio of cholinacetyl-transfetase (ChAT)/cholinesterase (AchE); decreased hyperphosphorylation of tau protein, increased protein phosphortase 2A (PP2A); enhanced expression of neurothophic factors and their receptors, and decreased cholinergic cell death. CONCLUSION: Shen-wu Capsule (SW) acts on multiple targets in the complicated pathogeneses of AD, and may become promising drug to treat AD.

Key words: Alzheimer disease; traditional Chinese medicine; animal model

S5.4

IMMUNOTHERAPY OF ALZHEIMER'S DISEASE

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Immunization against beta-amyloid can reduce amyloid plaque load and improve impaired behavior in transgenic mice. We established that antibodies against amyloid beta-peptides (Abeta) generated in response to active immunization are associated with significantly slower rates of decline of cognitive functions and activities of daily living in patients with Alzheimer's disease (AD), and that the beneficial clinical effects were also present in patients who had experienced prior transient episodes of immunization-related aseptic meningoencephalitis as an unwanted side effect of immunization. Because hippocampus-dependent neuropsychological tests were among the most sensitive measures of the clinical efficacy, we measured changes of hippocampal volumes over a course of three years following the initial active immunization by MRI. We observed stronger decreases in brain volumes in patients with antibodies against Abeta, as compared to control patients within the initial year of observation. Together with the initial neuropathology findings, this decrease may reflect lowered beta-amyloid plaque load combined with reduced inflammation and reduced astrogliosis. In continued follow-up during the second and third years following Abeta vaccination, we found striking recoveries of hippocampal volumes in the patients with antibodies against Abeta: After the end of the second year, volumes essentially had returned back to baseline volumes measured before the start of the clinical trial; followed by stable vdlumes over the third year. In contrast, the hippocampal volumes in patients without antibodies against Abeta continued to decrease at the expected rate of 3% per each year. These changes in volumes were correlated with antibody titers and cognitive performance. The therapeutic mechanism of immunotherapy against beta-amyloid may be biphasic with an initial phase of beta-amyloid plaque removal combined with concurrent decreases in inflammation and astrogliosis, followed by a second phase of structural recovery, regeneration and restoration of function in the absence of beta-amyloid-related toxicity.

S6.1

Identifying and validating novel analgesic drug targets

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A feature of both inflammatory and neuropathic pain is the induction either in immune cells or in neurons, of very many genes. Expression profile analyses performed with high-density oligonucleotide microarrays have revealed many hundreds of genes whose transcripts are dynamically either up- or down regulated in the peripheral or central nervous system in multiple pain-related rodent models. The challenge is how to identify which of the induced genes are potential targets for the development of new analgesics. We find that only 10% of all transcripts identified from a replicate array analysis as changing in three neuropathic pain models in the dorsal root ganglion or dorsal horn are common to all the models. We have analyzed this subset for novel potential analgesic target candidates using gain and loss of function strategies in vivo and in vitro to try establish how they contribute to increased pain sensitivity and if blcoking their activity produces analgesia. A single nucleotide polymorphism analysis of the candidates in patients with chronic pain was also performed to establish validity of the targets in humans. Several candidates that have emerged from this approach will be presented.

S6.2

New Targets for the Control of Chronic Pain: where should we look

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We have used a combination of molecular, physiological and behavioural approaches to analyze the interactions between descending pathways from the brainstem and sensory nociceptive neurons. We will present the case that chronic pain is in part be the result of a malfunction of dynamic central mechanisms that normally control pain experience. The focus is on brainstem-spinal loops that increase the flow of nociceptive signals through the dorsal horn. Destruction of the lamina I pathway or of descending pathways that release 5-HT can attenuate neuropathic pain. Blocking the actions of serotonin at the 5-HT3 receptor or specific destruction of the serotonergic pathway reproduces many of the physiological and behavioural effects of ablating lamina I neurons. We have analysed gene expression in sensory neurons in neuropathic pain states after ablation of 5-HT locally within the spinal cord. This has indicated that gene expression in DRG neurons is influenced by the central nervous system. In other words it appears that activity in the primary afferent, lamina I projection neuron and descending facilitatory pathways are all necessary for the full expression and maintenance of neuropathic pain.

S6.3

Analgesic actions of non-opioid neuropeptide receptor agonists

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Normal physiological (acute) pain is an early warning system that helps to prevent or minimize tissue damage. Effective analgesic drugs for acute pain are readily available. These include anti-inflammatory drugs that target cyclooxygenase, and opioid analgesic drugs that target opioid receptors. With pathological (chronic) pain, however, new targets are required. An important clue is the discovery that tissue or nerve injury causes dramatic alterations in the gene expression of neuropeptides and neuropeptide receptors along pain transmission pathways. An important example of this "plasticity" involves neuropeptide Y and the NPY Y1 receptor. Both are highly expressed at key sites of pain transmission, and injury dramatically alters their concentration in sensory neurons and in the dorsal horn of the spinal cord. These and other neuroanatomical findings suggest that non-opioid neuropeptide receptors modulate the intensity of pathological pain. This presentation reviews the behavioral pharmacology of neuropeptides in models of acute pain, and then discusses more recent findings in models of inflammatory and neuropathic pain.

S6.4

Annexin 1, anti-inflammatory drugs and the neuroendocrine-immune interface.

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Annexin A1 (Anx-A1; lipocortin 1), a 37 Kd member of the annexin super-family of proteins, is of specific interest to pharmacologists because it has been established using immuno-neutralisation, antisense and transgenic strategies that it mediates several glucocorticoid (GC) actions. GCs induce the synthesis and release of Anx-A1 in many tissues and cells including components of the immune and neuro-endocrine system. The protein acts in a paracrine or autocrine fashion on its target cells, predominantly through G-protein coupled receptors of the FPR family, to produce inhibitory effects on inflammatory mediator release, neutrophil chemotaxis and many other important aspects of the innate inflammatory response. Within the adaptive immune system, Anx-A1 regulates the strength of T-cell signalling. Here, glucocorticoids down-regulate the synthesis of the protein thereby changing the Th1 - Th2 balance. In the neuroendocrine system the GC-induced release of the protein from folliculostellate cells of the anterior pituitary gland is crucial in the feedback control of ACTH and other hormone secretion.

Key words: Glucocorticoids, formylpeptide, T-cells, ACTH

Funded by the Wellcome Trust.

S6.5

A use-dependent blocker of Cav2.2 channel for neuropathic pain

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Previous studies have identified N-type voltage-dependent Ca^{2+} channels (Cav2.2) as a key molecule in pain signal transmission in the spinal cord. Clinical data with Cav2.2-specific peptide blocker have validated Cav2.2 as a new target for neuropathic pain. ONO-2921 is a novel, orally-active Cav2.2 blocker, designed for the treatment of neuropathic pain. Oral administration of ONO-2921 exhibited anti-hyperalgesic and anti-allodynic effects on the rat chronic constriction injury model of sciatic nerve without apparent effects on acute pain models at the effective doses. Electrophysiological studies using recombinant Cav channels expressed in HEK cells and Xenopus oocytes revealed that the inhibitory effects of ONO-2921 were selective for both Cav2.2 and Cav2.3 (R-type) channels. The inhibition of Cav2.2 and Cav2.3 channels was use-dependent and parallel to the cumulative channel open time. A hyperpolarizing shift in the steady-state inactivation curve caused an increasing blocking potency at depolarized membrane potentials. These results suggest that the use- and state-dependent blockade of both N- and R-type Ca^{2+} channels underlies the analgesic effect of ONO-2921 in the neuropathic pain.

S7.1

Modern GI pharmacology: From gene expression to gene therapy & new molecules. Introductory remarks.

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The goal of this symposium is to review new trends and the most recent developments in 'molecular pharmacology' with focus on ulcerative and inflammatory diseases of the gastrointestinal (GI) tract. Traditionally, GI pharmacology was descriptive & phenomenological for much longer period of time than other branches of pharmacology. The first discoveries in GI pharmacology that were related to endogenous molecules originate from the 1970s, i.e., the first specific histamine H2-receptor antagonits (e.g., cimetidine, ranitidine), the first cyto/gastroprotective agents related to endogenous prostaglandins, antioxidant sulfhydryls & phospholipids. The endogenously related gastroprotective & antiulcer compunds have been recently extended by NO-releasing or mimetic drugs & growth factors, e.g., EGF, HGF, bFGF, PDGF, VEGF. TRH, CRF & dopamine-related drugs represent a special category of novel pharmacologic agents. These may act centrally and/or peripherally, & they may exert not only mucosal protective actions but seem to correct motility disorders which play a role not only in gastro-esophageal & duodenal diseases, but also in IBD & IBS. The remaining challenges are related to NSAIDs which inhibit a specific form of cyclo-oxygenase & H. pylori which may cause not only gastritis & ulcer, but also gastric cancer: unfortunately, there are no specific agents which counteract the GI-damaging actions of these etiologic factors. Advances & new data on gene expression & gene therapy, however, may soon reveal novel molecular targets that may lead to novel GI therapeutic agents in the upper & lower GI tract.

S7.2

Cathelicidin: a molecule for antimicrobial or for ulcer healing in the stomach

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Objective: We investigated whether cathelicidin contributes to gastric ulcer healing. Methods: Gastric ulcers were induced in rats and the expression of cathelicidin was determined by RT-PCR and Western blot. Overexpression of cathelicidin was achieved by plasmid transfection. Proliferative cells and microvessels in gastric tissue were measured. The direct action of cathelicidin on cell proliferation and its signaling pathway in cultured gastric epithelial cells (RGM-1) were determined. Results: Ulcer induction increased cathelicidin expression in the gastric mucosa. Overexpressing this peptide promoted ulcer healing by increasing cell proliferation and angiogenesis. Cathelicidin directly stimulated RGM-1 cell proliferation through a MMP-, EGFR-, and MEK- dependent pathway. TGF knockdown in RGM-1 cells nullified the mitogenic signals evoked by cathelicidin. Conclusion: Cathelicidin exhibits ulcer healing activity through a TGF-dependent transactivation of EGFR to induce proliferation of gastric epithelial cells.

Key words: Cathelicidin, gastric ulcer, proliferation, EGFR

Acknowledgments: CRCG grant from the University of Hong Kong and the CERG grant from the Hong Kong Research Grants Council

S7.3

Current Topics of Gastric Secretion, Mucosal Integrity and H. pylori

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Recent advances in gene technology have succeeded to generate various gene deficient (knockout) mice. In M3R-KO mice, carbachol, histamine and gastrin stimulated gastric acid secretion as in wild-type mice. Carbachol-stimulated acid secretion was significantly inhibited by famotidine and pirenzepine. In M1R-KO mice, carbachol, histamine and gastrin significantly stimulated the acid secretion as in wild-type mice. Pirenzepine significantly inhibited the carbachol-stimulated acid secretion in M1R-KO mice. In H2R-KO mice, carbachol significantly stimulated acid secretion, yet histamine and gastrin had no or little effect on acid secretion. In the gastric mucosa with hyperplasia, numerous enlarged cysts and a marked expression of TGF-α were observed. In HDC KO mice, both carbachol and gastrin had little impact on acid secretion. These agents, however, synergistically stimulated the acid secretion when they were given together with exogenous histamine. H. pylori infection induced intestinal-type gastric adenocarcinoma in M. gerbils after infection. We found that the eradication (with PPI and antibiotic) timing plays an important role in prevention of H. pylori-associated mucosal changes.

S7.4

New CRF Antagonists: A New Approach to IBS

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Clinical investigations support the notion that stress contributes to visceral hypersensitivity of the gut and experimental models have been developed that recapture features observed in irritable bowel syndrome (IBS) with regard to stress-related hyperalgesia to colorectal distention (CRD), gender differences and comorbidity with anxiety/depression. Knowledge on brain distribution of corticotropin-releasing factor (CRF) ligands, the cloning of CRF1 and CRF2 receptors and the development of selective CRF antagonists, have allowed us to establish the role of brain CRF signaling in the gut response to stress. Pharmacologic approaches support the notion that the activation of brain CRF1 receptor contributes to the stimulation of colonic motor function, diarrhea, and hyperalgesia induced by various exteroceptive or interoceptive stressors in rats. In contrast CRF2 antagonists have no effect and CRF2 agonists are analgesic. CRF1 antagonists act by preventing stress-related activation of locus coeruleus neurons, sacral outflow and enteric cholinergic and mast stimulation in the colon. CRF1 antagonists may provide a novel option for IBS treatment.

Key words: CRF, CRF1 receptor, gut function, visceral pain

S7.5

TRPV1 capsaicin receptors in the GI mucosal damage and protection in human healthy subjects and in patients with different GI disorders

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Aims were to review: 1. the immundistribution of TRPV1, CGRP and SP in the gastric and colon mucosa of healthy subjects and in patients with different GI disorders; 2. the effects of capsaicin on a. the gastric transmucosal potential difference (GTPD) with and without topical application of ethanol, capsaicin and of ethanol plus capsaicin; b. the changes in the "parietal" and "non-parietal" components of gastric secretion, gastric emptying; c. indomethacin-induced gastric microbleedings; 3. the hormonal regulation during glucose loading test, with or without application of 400 µg (ED_{50}) capsaicin. Results: 1.The TRPV1, CGRP and SP were immunhistochemically detected in different distribution in the GI mucosa of healthy human subjects and of patients; 2. The capsaicin dose-dependently increased: a. the GTPD, the ethanol-induced decrease of GTPD; b. the gastric emptying, "non-parietal" component of gastric secretion and prevented the gastric microbleedigs (c). 3. Capsaicin enhanced the glucose absorption and glucagon release. Conclusion: The TRPV1 receptors were detected in the human GI mucosa and the small doses of capsaicin take place in the GI mucosal protection (Grant: RET-08/2005).

S8.1

5-HT1 and 5-ht5A/5B receptors mediate cardiac sympatho-inhibition in pithed rats.

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Continuous intravenous (i.v.) infusions of 5-hydroxytryptamine (5-HT) inhibit the tachycardic responses to preganglionic sympathetic stimulation in pithed rats. We have now identified the pharmacological profile of this response. 5-HT-induced cardiac sympatho-inhibition remained unaltered after i.v. saline, WAY100635 plus GR127935, ritanserin, tropisetron, LY215840 or a cocktail of drugs (consisting of yohimbine, prazosin, ritanserin, GR127935, WAY100635 and indomethacin), but was abolished by methiothepin. Moreover, continuous i.v. infusions of the agonists 5-carboxamidotryptamine (5-CT), CP93,129, sumatriptan, PNU-142633 and ergotamine mimicked the above sympatho-inhibition to 5-HT. In contrast, the agonists indorenate and LY344864 were inactive. Interestingly, 5-CT-induced cardiac sympatho inhibition was abolished by methiothepin, the cocktail of antagonists/inhibitors, GR127935 or the combination of SB224289 plus BRL15572. Therefore, 5-HT-induced cardiac sympatho-inhibition seems to be mediated by 5-$HT_{1B/1D}$ receptors and methiothepin-sensitive putative 5ht5A/5B receptors.

Key words: 5-ht5A/5B receptors, sympatho-inhibition, tachycardia.

Acknowledgement: We thank CONACyT (Mexico) for their support

S8.2

The Forgotten 5-HT Receptors: 5-HT_{1E}, 5-HT_{1F}, and 5-HT_{5A}. An Update and Overview

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Central and peripheral systems that use serotonin (5-hydroxytryptamine, 5-HT) have proven to be good targets for the development of therapeutic agents, and during the 1990s cloning studies revealed a large family of 5-HT receptor subtypes, giving promise that additional serotonergic drug targets might be forthcoming. However, remarkably little has been learned about some of these receptors. Three of these, 5-HT_{1F}, 5-HT_{1E} and 5-HT_{5A} have been chosen to summarize our current knowledge and to highlight the gaps in our understanding of their physiological roles and potential as drug targets. These three receptors appear to be primarily localized to the CNS, within regions that might make them interesting as drug targets. The 5-HT_{1F} receptor was discovered by cloning in 1992-93, but nothing is known about possible physiological roles, except that its agonists are active in animal models for testing anti-migraine drugs. The 5-HT_{1E} is even more of an enigma. The human clone was revealed in 1992, but it was 2004 before it was published that this receptor does not exist in rodents. The 5-HT_{5A} clones were reported in 1992-94. However, little has been learned beyond its distribution within the brain.

Key words: 5-HT_{1E}, 5-HT_{1F}, 5-HT_{5A}, serotonin

S8.3

5-HT2C receptor constitutive activity regulates in vivo dopamine release

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Serotonin2C receptor (5-HT2C R) constitutive activity (CA) participates in the tonic/phasic inhibitory controls of mesoaccumbens dopamine (DA) pathway in vivo. Here, we furthered this issue by assessing the contribution of ventral tegmental area (VTA) and nucleus accumbens (NAc) 5-HT2C Rs in the control of NAc DA release. Experiments were performed using in vivo microdialysis coupled with HPLC-ECD in halothane-anesthetized rats given peripheral and/or intracranial microinjections of selective 5-HT2C R ligands (SB 242084, SB 243213: antagonists; SB 206553: inverse agonist; Ro 60-0175: agonist). Intra-VTA injection of SB 242084 or SB 243213 (0.1-0.5 µg/0.2 µl) and intra-NAc infusion of SB 242084 (0.1-1 µM) significantly blocked the decrease in accumbal DA outflow induced by the intraperitoneal (i.p.) injection of 3 mg/kg Ro 60-0175. The increase in DA outflow induced by SB 206553 (5 mg/kg, i.p.) was blocked by the intra-NAc infusion of SB 242084, but unaltered by its intra-VTA injection. These results show that both VTA and NAc 5-HT2C Rs participate in the inhibitory control exerted by central 5-HT2C Rs on accumbal DA release, and that the NAc may serve as a major site for the effect of 5-HT2C R CA.

S8.4

The raphe neurocircuitry: Not just for serotonin anymore

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The dorsal (DR) and median raphe (MR) provide 5-HT input to forebrain areas that have been implicated in mediating stress responses and in the etiology and treatment of stress related mood disorders. Previous research has primarily focused on the 5-HT neurotransmitter system within the raphe. Glutamate and GABA are known to provide primary excitatory and inhibitory synaptic input to the dorsal and median raphe. The frequency of the glutamatergic and GABAergic synaptic activity was different in the MR and DR as recorded in the raphe brain slice preparation by visualized whole cell voltage-clamp techniques. 5-HT1B receptor activation selectively inhibited glutamatergic or GABAergic activity in the DR and MR. CRF receptor activation increased GABAergic activity only in the DR. Swim stress increased glutamatergic synaptic activity and blocked the CRF increase in GABAergic activity. These results disclose new mechanisms for the differential regulation of DR and MR raphe neuronal activity by both glutamatergic and GABAergic synaptic input. These mechanisms may underlie differences seen in DR and MR regulation of stress responses and the etiology and treatment of mood disorders such as anxiety

S8.5

5-HT receptor diversity: past and present

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Serotonin (5-HT, 5-hydroxytryptamine) acts via at least 13 G protein-coupled receptors and a (presumably a family) ligand-gated ion channel(s). 5-HT receptors form 7 distinct classes (5-HT_1 to 5-HT_7) based on structural and operational features. Such diversity underscores the physiological importance of 5-HT, but further diversity exits. The challenge for 5-HT research is to define what makes this incredible diversity relevant. Much progress was made by realizing that 5-HT is the least conservative monoamine transmitter and the cloning of its many receptors. Coupled with the actions of an extremely efficient uptake system, these re-

ceptor subtypes provide almost limitless signaling. The complexity of the system encompasses post-translational modifications: alternate splicing and RNA editing, oligomerization / heteromerization increase the number of receptor complexes, and multiple G proteins suggest receptor trafficking, allowing cross talk within or between receptor families. Whether all these possibilities are physiologically and/or pathologically relevant remains to be established and discussed. The prize for unraveling this complexity is the development of innovative drugs for a range of diseases.

S8.6

Pharmacological treatment of Pulmonary Hypertension: mechanism relevance to 5-Hydroxytraptamine, Receptors and Transporters

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There is critical relevance between 5-HT and pulmonary hypertension (PH). Further investigation of receptor and transporter mechanism using chronic "monocrotaline" rats, cultured pulmonary artery smooth muscle cells (PASMC) and liposomal transfection to introduce ERK1/2 ODNs into cultured rat PASMCs shown that selective serotonin reuptake inhibitor fluoxetine and sertraline concentration-dependently inhibited MCT-induced PH in rats and the proliferation of PASMCs induced by 5-HT. 5-HT_{1B} antagonist rather than 5-HT_{1D} antagonist inhibited 5-HT- and 5-$HT_{1B/1D}$-induced proliferation of PASMC. Meanwhile, antisense ODN to ERK1/2 inhibited 5-HT-induced proliferation of PASMCs. 5-HT_{1B} receptor and 5-HTT mediated mitogenesis of PASMCs by 5-HT and the intracellular signal transduction of 5-HT in PASMCs is dependent on ERKs signal pathway. PH compromised complicated pathology i.e. pulmonary vasoconstriction, vascular remodeling, inflammation and micro-thrombosis, in which multiple factors was involved. 5-HT_{1B} receptor and 5-HTT mechanism are of importance induce PH and both might be novel therapeutic targets.

Key words: Pulmonary Hypertension.

Supported by National Natural Science Foundation, China 30271510, 30

S9.1

Therapeutic antibodies: past, present and future perspectives

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Monoclonal antibodies (mAbs) are currently the predominant type of protein therapeutic in clinical study, with more than 150 products currently in studies sponsored by companies located worldwide. An understanding of past and present development trends and knowledge of benchmark data such as success rates and clinical phase times are valuable for companies developing these products for approval in the future. Data for therapeutic mAbs were collected by survey of pharmaceutical and biotechnology firms and from public documents (e.g., press releases, annual reports). The mAb data set contained 355 therapeutic products that entered clinical study sponsored by more than 100 commercial firms. Analysis of the current data set indicates trends toward the study of human mAbs and mAb fragments. In addition, results verify our previous findings that approval success rates for chimeric and humanized mAbs are consistently in the 18-29% range.

Key words: monoclonal antibody success rates

S9.2

Discovery and Development of Human Antibody Based Therapeutics

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S9.3

Safety of monoclonal antibodies to TNF alpha in the treatment of arthritis

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Methods: Review of clinical trial reports, post-marketing safety reports and patient registries. Results: Two monoclonal antibodies to TNF alpha are currently approved for use in the treatment of rheumatoid arthritis (RA), psoriatic arthritis (PsA) and ankylosing spondylitis (AS). Well done clinical trials have shown both infliximab (Remicade) and adalimumab (Humira) to be effective in the treatment of these diseases and they have become the state of the art therapies for patients who fail to respond to traditional disease modifying medications. Although these medications are very efficacious, it has become clear that there are safety concerns with both agents which has been demonstrated in the clinical trials, post-marketing safety reports and patient registries. These concerns include serious infections, opportunistic infections (including tuberculosis), cytopenias, lymphomas, hepatotoxicty, autoimuunity, demyelinating syndromes and infusion/injection reactions. This paper will discuss these safety concerns in depth

Conclusion: The risk:benefit ratio highly favors the use of monoclonal antibodies to TNF alpha in the treatment of RA, PsA and AS.

Key words: infliximab, adalimumab, anti-TNF antibodies.

S9.4

Targeting on ErbB3 for Cancer Therapy

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Receptor like protein tyrosine kinase ErbB2 serves as a co-receptor for ErbB3 and ErbB4 in neuregulin-1-mediated ErbB2/ErbB3 or ErbB2/ErbB4 heterodimer formation. In a number of adenocarcinoma tumor lines, ErbB2 is over-expressed resulting in ErbB2 homodimer formation and protein phosphorylation. ErbB2 has thus been associated with cancer growth, and therefore is used as a target for cancer therapy. However, our data indicated that over-expression of ErbB2 alone in NIH-3T3 cells suppressed cell growth, which was rescued by co-expression of ErbB3 with ErbB2, while over-expression of ErbB3 alone has no effect on cell growth. Data from protein chemistry studies indcated that expression of ErbB2 and ErbB3 results in the formation of ligand-independent heterodimers that are preferred over ErbB2 homodimers in cells. Receptor phosphorylation was activated dependent on dimer formation, and only trans-phosphorylation was observed between dimer partners. This finding is coincident with clinical observations that over-expression of ErbB2 is frequently associated with a higher-level expression of ErbB3. These results indicated that while ErbB2 is targeted for drug development, ErbB3 is a new target for cancer therapy. Based on this new notion, we developed several ErbB3 cancer vaccines, which showed positive efficacy in suppression of tumor growth in a mouse model.

S9.5

Introduction: Outlook of an Emerging Immunopharmacology Field.

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Immunotherapies are the largest group of agents that are either exiting pharmaceutical pipelines or under development. Antibodies represent a major part of this emerging field. A brief introduction into the history and fundamentals of antibody-related therapy and diagnostics will be presented that includes: Recombinant antibody fragments, antibody conjugates, vaccines and native acquired immunoprotection, intellectual property and antibody production methods, and global financial impact. The overview will provide the backdrop for specific aspects to be presented in the symposium on antibody-based therapies and diagnostics

Key words: Antibodies, immunotherapy, diagnostics

Acknowledgement: Supported in part by the National Cancer Institutes (R43 CA108222-01).

S10.1

Travelling back in time: Evolutionary aspects in G protein-coupled receptor research

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The common seven-transmembrane-domain architecture of G-protein-coupled receptors (GPCR) is maintained, in part, by amino acid motifs and highly conserved residues which are used to categorize GPCR into several families. Most members of the rhodopsin-like family of GPCR possess the highly conserved DRY and NPXXY motifs. The presence of these sequence signatures in worm and vertebrate GPCR suggests more than 700 million years of evolution of the rhodopsin-like family. Mining these evolutionary data as a source of structural information is helpful in understanding the functional relevance of individual GPCR, in interpreting naturally occurring GPCR mutations in patients and in guiding GPCR model generation and mutagenesis studies. As more than 99% of all species that ever lived on earth are extinct, most information about receptor repertoires and the structural basis of adaptive processes, that involve GPCR, appears to be lost. However, recent success in sequencing and functionally expressing GPCR from extinct Pleistocene species opens the possibility of studying ancient signalling pathways.

S10.2

Structure and organization of G protein-coupled receptors

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It is now widely accepted that G protein-coupled receptors (GPCRs) can exists as dimers or possibly as higher-order oligomers. Using combinations of co-immunoprecipitation of fragments of the alpha 1β adrenoceptor and both 2 and 3 coloured fluorescence resonance energy we demonstraterd that this receptor forms oligomers with key symmetrical interfaces provided by transmembrane domains I and IV. It has also become clear that certains pairs of GPCRs may form hetero-dimers/ologimers and that this may regulate receptor function and pharmacology. Co-expressed human CB1 cannabinoid and orexin-1 receptors form hetero-dimers and the cellular distribution of the orexin-1 receptor is altered by the presence of the CB1 receptor. Antagonists at each receptor alter the cellular distribution of the CB1/orexin-1 heterodimer and CB1 antagonists reduce the potency of orexin-A to activate downstream signals. This may be relevant to the clinical effectiveness of the CB1 receptor antagonist/inverse agonist Rimonabant.

S10.3

GPCR Allosterism: A novel approach to drug selectivity.

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G protein-coupled receptors (GPCRs) represent the major targets for approximately 50% of all medicines. Although most drugs act via the binding site for the endogenous agonist (orthosteric site), it is now recognized that GPCRs can possess allosteric sites that modulate receptor activity; targeting such sites can potentially lead to greater selectivity for GPCRs that exhibit high sequence homology within the orthosteric site across subtypes. However, the detection, quantification and validation of allosteric drug effects represent a significant challenge for drug discovery. This is because allosteric modulators can affect orthosteric binding affinity and/or signaling efficacy in a manner that is totally orthosteric-ligand-dependent; some modulators can also demonstrate agonist activity in their own right. These different properties of allosteric modulators have all been observed in studies of the muscarinic actetylcholine GPCRs. Most recently, we have used mutagenesis and 3D homology modeling to map a putative allosteric site on the M2 muscarinic receptor, and have identified a key role for flexibility of the second extracellular loop in the binding of both orthosteric and allosteric ligands.

S10.4

Real-Time Measurement of GPCR-Mediated Signaling Events

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Cellular signaling via G-protein-coupled receptors presumably occurs in a temporally and spatially organized manner. However, techniques to monitor this spatial and temporal patterning have so far barely existed. We have developed a series fluorescent methods that permit the analysis of ligand binding, receptor activation, G-protein activation, and the generation of the second messengers cAMP and cGMP. Our data show that ligand binding, studied with PTH as an example, occurs as a biphasic process and that the second phase co-incides with a conformational change in the receptor. This conformational change presumably reflects receptor activation and is much faster than previously thought (millisecond to second range). It depends both on the receptor and on the type of ligand. Receptor/G-protein coupling is similarly fast. In contrast, G-protein activation is significantly slower than receptor activation (hundreds of milliseconds) and appears to by temporally tightly linked to effector activation such as opening of the GIRK K-channel. Increases in second messenger concentrations occur over seconds to minutes. There are complex interactions between the levels of cAMP, cGMP and calcium.

S10.5

Crosstalk in G protein-coupled receptors: Changes at the transmembrane homodimer interface determine activation

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Functional crosstalk between G protein-coupled receptors in a homo- or heterodimeric assembly likely involves conformational changes at the dimer interface, but the nature of this interface is not yet established, and the dynamic changes have not yet been identified. We have mapped the homodimer interface in the dopamine D2 receptor over the entire length of the fourth transmembrane segment (TM4) by crosslinking of substituted cysteines. Their susceptibilities to crosslinking are differentially altered by the presence of agonists and inverse agonists. The TM4 dimer interface in the inverse agonist-bound conformation is consistent with the dimer of the inactive form of rhodopsin modeled with constraints from atomic force microscopy. Crosslinking of a different set of cysteines in TM4 was slowed by inverse agonists and accelerated in the presence of agonists; crosslinking of this latter set locks the receptor in an active state. Thus, a conformational change at the TM4 dimer interface is part of the receptor activation mechanism.

S10.6

Novel functions of human receptor activity-modifying proteins (RAMPs) during cellular trafficking of calcitonin receptor-like receptor (CRLR)

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RAMP2 and -3 enable CRLR to function as an adrenomedullin (AM) receptor (CRLR/RAMP2 or -3). We examined the functions of the transmembrane (TM) domain and cytoplasmic C-terminal tails (C-tails) of RAMP2 and -3 by cotransfecting their various mutants and chimeras into HEK-293 cells stably expressing human CRLR. FACS analysis revealed that substituting a Thr-Val sequence in the RAMP3 TM with the corresponding region (Ile-Pro) from RAMP2 significantly enhances AM-induced internalization of CRLR, suggesting the RAMP2 sequence participates in the positive regulation of CRLR internalization. Deletion of the C-tail from RAMP2 disrupted CRLR transport from the endoplasmic reticulum to the cell surface, markedly reducing ^{125}I-AM binding and evoked cAMP accumulation. Deletion of the C-tail from RAMP3 markedly enhanced CRLR internalization, though there was no change in agonist affinity. The highly conserved Ser-Lys sequence within RAMP C-tails is invdlved in the cellular trafficking of the two AM receptors, but deleting the C-tails from RAMPs had no effect on lysosomal sorting of CRLR. Thus, the respective C-tails of RAMP2 and -3 differentially affect CRLR surface delivery and internalization.

S11.1

Pathophysiology of Drug-Induced Torsades de Pointes and Strategies for Non-clinical Detection of Cardiotoxic Drugs

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Unwanted adverse cardiac arrhythmias associated with drugs poses a significant challenge to the clinician who is judging the benefit of therapy against the risk of potential toxicity. Drug induced delay in cardiac ventricular repolarization (CVR) is recognized as a substrate for a potentially critical arrhythmia, torsades de pointes (TdP). Although the cellular events that underlie this drug induced phenomena have been postulated, clear evidence that these mechanisms are operative in humans has not been established. In particular, the incidence of TdP in the clinical population can be extremely small (1:120,000); thus the link between drug-induced delay in CVR and TdP is not fully understood. The importance of understanding this link is that it may allow us to distinguish proarrhythmic from non-proarrhythmic drugs; even for those agents that exhibit the propensity to delay CVR. This presentation will provide the background to our understanding of the electrophysiologic basis for drug-induced TdP and the standard assays and strategies used to identify potentially proarrhythmic drugs.

S11.2

Emerging Non-clinical Models and Strategies for Detecting Drugs with Potential to Elicit Torsades de Pointes

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There seems to be a dissociation between the risk of QT interval prolongation and the proarrhythmic risk. In vitro and in vivo proarrhythmia models will be reviewed along with their particular merits and shortcomings. These models use electrophysiological markers such as dispersion of repolarization, action potential duration as well as triangulation, instability, reverse use-dependence, and the incidence of early after-depolarizations to predict the proarrhythmic risk. The variables used by each model to predict the torsadogenic propensity of a drug has been reported to be concordant with clinical outcome, although data should be interpreted cautiously since no models have been independently assessed. Furthermore, mechanisms other than direct hERG inhibition may contribute to drug-induced QT interval prolongation / proarrhythmia. These include activity at other cardiac ion channels, inhibition of hERG transcription / trafficking, influence of autonomic tone, and cardiac tissue accumulation. An enhanced understanding and validation of the key proarrhythmic mechanisms may provide a rational basis for drug progression into clinical development particularly in areas of unmet medical need.

S11.3

Clinical methoddologies to assess QT Liability: From Early Human Studies to Post Market Surveillance and Pharmacoepidemiology

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The focus of the clinical assessment of proarrhythmic liability is currently measurements of QT interval prolongation. The ICH E14 identifies the outcome of the 'thorough QT study' as critical for the level of ECG evaluation that should be performed during later stages of clinical development. This study is typically conducted in healthy volunteers (HV), using high doses, includes a positive control drug and can detect effects that are clearly smaller (4-5 ms) than clinically relevant effects. A careful QT assessment should however be conducted in earlier studies, as well, including the first-dose-in-man study. Unequalled exposure is sometimes achieved in these studies, and a fist estimate of the QT effect size can be obtained, which may impact the design of the thorough QT study and support further development decisions. Some drugs, such as neuroleptics, cannot be readily tested in HVs, based on tolerability, and alternative approaches using the targeted patient population must in these cases be undertaken. The correct identification of drugs with proarrhythmic liability is achievable only through an integrated risk assessment, utilizing both non-clinical and clinical data.

S11.4

Benefit versus Risk: Can Potentially Proarrhythmic Drugs Be Brought to the Marketplace

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In an ideal world, new candidate drugs would not interact with hERG channels, prolong cardiac action potentials in vitro, nor increase QT intervals, or be associated with arrhythmias in vivo. The real world is otherwise and today many promising new drugs are approaching clinical development and even registration with one or more signals of potential arrhythmia hazard. Under recently agreed ICH guidance 'Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmia Potential for Non-Antiarrhythmic Drugs' (ICH E14) virtually all drugs must undergo a 'thorough QT/QTc study' in humans, the results of which if negative will absolve a new drug of any lingering preclinical arrhythmia hazard signals. However, ICH E14 states that 'substantial' QT/QTc prolongation in humans, with or without documented arrhythmias, could be a basis for non-approval and/or discontinuation of clinical development, additional clinical studies, warnings in the product label, particularly when the drug offers no compelling advantage over available therapy, and available therapy appears to meet the needs of most patients (insufficient benefit). Failure to conduct an adequate clinical QT/QTc study will justify delay or denial of marketing authorization (unstudied risk). These concepts are based upon conservative assumptions of relationships between interactions of drugs at specific ion channels, effects upon cardiac action potentials and QT interval and potentially fatal arrhythmias. Today, scientists and cardiologists recognize that these relationships are not absolute, that not all drugs that interact with the hERG channel, and most recently that not all drugs that prolong QT interval pose the same or any arrhythmia hazard. This presentation will discuss how to bring potentially proarrhythmic drugs can be brought to the marketplace in light of recent regulatory positions and emerging scientific understandings.

S12.1

Molecular and biochemical aspects of chloroquine resistant malaria

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The spread of chloroquine-resistant Plasmodium falciparum strains has dashed hopes of global malaria eradication and, due to a paucity of other affordable drugs, has complicated the clinical management of malaria in endemic areas. Chloroquine, which targets the intraerythrocytic stages of P. falciparum, accumulates to millimolar concentrations within the parasite's acidic food vacuole where the drug is believed to interfere with endogenous heme detoxification processes. Resistance to the antimalarial drug chloroquine has been linked with polymorphisms within a gene termed pfcrt in the human malarial parasite Plasmodium falciparum, yet the mechanism by which this gene confers the reduced drug accumulation phenotype associated with resistance is largely unknown. To better characterize chloroquine movement in and out of P. falciparum-infected erythrocytes, we have investigated the kinetics of chloroquine accumulation and efflux. Our data suggest that pfcrt is directly or indirectly involved in carrier-mediated chloroquine efflux from resistant cells. Blocking this carrier might enable chloroquine to be re-introduced as an antimalarial drug.

S12.2

A 4-Aminoquinoline Antimalarial for the 21st Century

Dr Paul. M. O'Neill; Departments of Chemistry and of Pharmacology; The University of Liverpool Liverpool L69 3BX; United Kingdom

Amodiaquine (AQ) is a 4-aminoquinoline antimalarial that can cause adverse side effects including agranulocytosis and liver damage. The observed drug toxicity is believed to involve the formation of an electrophilic metabolite, amodiaquine quinoneimine, which can bind to cellular macromolecules and initiate hypersensitivity reactions. We proposed that interchange of the 3' hydroxyl and the 4' Mannich side-chain of amodiaquine would provide a new series of analogues that cannot form toxic quinoneimine metabolites via metabolic activation. By a simple three-step synthetic procedure, ten isomeric amodiaquine analogues were prepared and subsequently examined in vitro and in vivo against chloroquine resistant plasmodia. Isoquine, the direct isomer of amodiaquine was selected as the initial development compound. The talk will describe how further lead optimisation was achieved, to provide a candidate, NTB Isoquine, that has a simplified metabolic profile and improved antimalarial activity. The presentation will also describe studies conducted to elucidate the molecular mechanism of action of this novel antimalarial drug.

S12.3

Antimalarial Drugs Targeting the Unusual Mitochondrion and Plastid of Parasites

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Malaria parasites possess two separate maternally transmitted cytoplasmic genomes: a 6 kb mitochondrial DNA and a 35 kb chloroplast DNA. The presence of these unusual genomes and the functions they serve in the highly derivatized organelles provide opportunities for developing drugs that selectively interfere with these functions. Atovaquone, a hydroxynaphthoquinone, interferes with mitochondrial electron transport. Detailed studies have revealed the molecular basis for the selective activity of this antimalarial drug as well as the basis for resistance development. New compounds that also target parasite mitochondrial electron transport are under development and offer much promise. Other mitochondrial functions such as dihydroorotate dehydrogenase (pyrimidine biosynthesis pathway) and mitochondrial protein synthesis are also being examined as targets for antimalarial drug development. The discovery of a chloroplast remnant has created much excitement for the prospects of developing antimalarial drugs. Fatty acid and isoprenoid synthesis by the plastids are attractive targets being explored.

S12.4

Artemisinin Combination Therapy- not the magic bullet

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The research aims to rationalise the usage of artemisinin based combination (ACT) for antimalarial chemotherapy. The great success of ACT with mefloquine in South East Asia has suggested other combinations, with amodiaquine, lumefantrine, sulfadoxine-pyrimethamine, chloroquine and chlorproguanil-dapsone, in Africa. All these combinations lack what was initially the requirement, similar pharmacokinetics of both co-drugs, allowing selection of resistance to the co-drug. In addition, for many of the co-drugs there is already high or patchy resistance, which will be amplified when used as first-line treatment in endemic countries. In this paper we present a mathematical basis of a pharmacokinetic-pharmacodynamic model of ACT and a model of selection of resistance. Combining these we predict the rate of selection of resistance to the co-drugs under varying levels of initial resistance prevalence, transmission and population coverage. We find that the limits specified by the WHO for ACT are somewhat lenient, and that a specific evaluation is required for each setting.

S12.5

Study on the action mode of qinghaosu (artemisinin) —an against malaria magic bullet from nature

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Qinghaosu (Artemisinin) isolated from Chinese traditional medicine qinghao (Artemisia annua. L.) is a new generation of antimalarial compound. Recently we have reported the formation of carbon-centered free radicals in the reaction of qinghaosu and its derivatives with ferrous ion in a condition, which mimicked the environment in red cell. Herewith the formation of these free radicals, their reaction with DNA, nucleoside, nucleotide, amino acid and peptide and the plausible relationship with anti-malarial and anti-schistosomal activity will be presented. A brief introduction about the discovery, structure determination and other early chemistry study on qinghaosu will also be addressed.

Key words: qinghaosu, antimalarial activity, mechanism, free radical,

S13.1

Molecular Basis of Peripheral Nociceptor Sensitization

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S13.2

Sensitization of TRPV1 through G-protein-coupled metabotropic receptors

Section of Cell Signaling, Okazaki Institute for Integrative Bioscience, and Department of Physiological Sciences, The Graduate University for Advanced Studies Makoto Tominaga

One important aspect of TRPV1 regulation concerns the mechanisms by which the inflammatory mediators in damaged tissues sensitize TRPV1. TRPV1 can be phosphorylated by several kinases including PKA, PKC, Ca^{2+}/CaM-dependent kinase II or Src kinase. There has been extensive work demonstrating that activation of a PKA-dependent pathway by inflammatory mediators influences capsaicin- or heat-mediated actions in sensory neurons. PKG-dependent phosphorylation of TRPV1 occurs downstream of activation of Gq-coupled receptors by several inflammatory mediators including ATP, bradykinin, prostaglandins and trypsin or tryptase. PKG-dependent phosphorylation of TRPV1 caused not only potentiation of capsaicin- or proton-evoked responses but also reduced the temperature threshold for TRPV1 activation so that normal body temperature were capable of activating TRPV1, thereby leading to the sensation of pain. Direct phosphorylation of TRPV1 by PKC was proven using, and two target Ser residues were identified. Phosphorylation of TRPV1 by different kinases seems to control TRPV1 activity through the dynamic balance between the phosphorylation and dephosphorylation.

Key words: inflammation, TRPV1, phosphorylation.

S13.3

Protease-Activated Receptors and Inflammation

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Proteases have been considered for decades merely as degradative enzymes. However, the discovery of receptors specifically activated by proteases: the Protease-Activated Receptors (PARs) have highlighted a "hormone-like" signalling role for proteases. Particularly, an important function for proteases, through PAR activation, has been established during inflammatory processes. Proteases from the coagulation cascade, damaged cells, inflammatory cells or even from pathogens have been shown to interact with PARs, thereby participating to inflammatory response. Proteases as well as selective agonists of PARs induces all the hallmarks of inflammation in tissues as different as gut, airways, skin, or joints. Because of the poor availability of PAR antagonists, gene-deficiency approach has determined a major role for at least 2 members of the PAR family: PAR-1 and PAR-2, in different animal models of chronic inflammation or infection. More recently, studies using human tissues further showed that proteases and PARs are major mediators of inflammation in chronic inflammatory diseases such as inflammatory bowel disease. Proteases and PARs appear as important mediators of inflammatory sensitization.

S13.4

G protein-coupled signal transduction in synoviocytes of immune arthritis

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G proteins are partners of G-protein-coupled receptors (GPCRs). GPCRs catalyze guanine nucleotide exchange on Gαsubunits, enabling both activated Gαand Gβγ subunits to target downstream effector. Diverse extracellular signals regulate receptors to modulate cellular physiology. GPCRs signaling via heterotrimeric G proteins is attenuated rapidly by G protein-coupled receptor kinase (GRK). GPCRs phosphorylation is to promote the binding of arrestin proteins which block interactions of receptors and G-proteins. Regulators of Gprotein signaling are GTPase activating proteins that attenuate signaling by G proteins. G proteins-AC-cAMP signal transduction play a crucial role in pathogenesis of immune arthritis. Gαs mRNA, protein express and function were decreased, and Gαi mRNA, protein express and function of were increased in synoviocytes of rats with immune arthritis. The "cross-talk" was found between MAPK signal transduction and G proteins associated signal transduction. Activation of MAPKs was regulated by Gαi and Gαs signal transduction pathway. G proteins transmembrane signal pathway became new target for treatment of arthritis arthritis.

Key words: G protein, MAPK signal transduction, arthritis

S13.5

Modulation of oxidants signaling as a new therapeutic approach of obstructive pulmonary disease.

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Chronic obstructive pulmonary disease (COPD) is a major public health problem that is related to cigarette smoke exposure. COPD is characterized by non reversible airflow obstruction, secondary to airways and lung parenchyma inflammation and remodeling. An increased airway smooth muscle mass and mucus hypersecretion are characteristic features of airways remodeling whereas a proteases/antiproteases imbalance is characteristic of lung remodeling (also know as emphysema). Heme oxygenase (HO) and NADPH oxidase (NOX) are anti and pro-oxidant proteins respectively, that are involved in the control of smooth muscle proliferation, mucus protein expression and proteases/antiproteases balance, via oxidants signaling and activation of mitogen activated protein kinases. We have shown that a decreased HO expression, secondary to a promoter polymorphism in HO-1 gene, is associated with an accelerated decline in lung function in smokers, and that experimental up regulation of HO and down regulation of NOX proteins prevent airway and lung remodeling after cigarette smoke exposure in vivo and in vitro. Therefore, modulation of oxidants signaling by acting on HO and/or NOX could be proposed as a new therapeutic approach of COPD.

S14.1

Oxidative stress in pulmonary hypertension- a possible new therapeutic target

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Significant oxidative stress exists in the lungs of patients with pulmonary hypertension, and experimental models of pulmonary hypertension have also been shown to be associated with increased generation of reactive oxidants. Evidence is also accumulating that anti-oxidant defences are impaired in pulmonary hypertension. Several molecular targets have been identified that are affected by superoxide, leading to alterations in cell regulation that could be important in the progression of the disease. It is likely that a degenerative cycle is set up whereby oxidative stress impairs the normal ability of the pulmonary vasculature to defend against the pro-oxidant environment, thus accelerating oxidative cellular damage. A key target is tha pulmonary vasular endothelium and oxidative stress is emerging as a major cause of the endothelial dysfunction that is characteristic of pulmonary hypertension. Experimental studies have suggested a number of drug targets that could provide novel therapy for the treatment of pulmonary hypertension by correction of oxidative stress and its consequences.

S14.2

From nitric oxide to phosphodiesterase inhibitors in treatment of pulmonary hypertension

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Inhaled nitric oxide (NO) has been applied in the treatment of some patient groups suffering from pulmonary hypertension. Inhaled NO is, however, hampered by expensive delivery devices and possibly toxic side effects. Alternatives include NO donors, decreased degradation of endogenous NO with superoxide dismutase mimetics, or increased cyclic GMP with direct activators of guanylyl cyclase, or inhibition of phosphodiesterase type 5 (e.g. sildenafil). In a series of studies in the chronic hypoxic rat, we addressed the role of these treatments on endothelium-dependent vasodilatation and pulmonary arterial remodelling as well as right ventricular hypertrophy. L-arginine supplementation had no effect, while an NO donor, molsidomine, a superoxide mimetic, tempol, and sildenafil reduced right ventricular systolic pressure and hypertrophy. Molsidomine reduced muscularized pulmonary small arteries (< 50 μm), while sildenafil had no effect but improved endothelium-dependent vasodilatation. These studies of the NO pathway suggest both prevention of structural remodelling and impairment of vasodilatation can contribute to reduction of pulmonary pressure and right ventricular hypertrophy in pulmonary hypertension.

S14.3

Use of prostacyclin and prostacyclin-analogues in the treatment of pulmonary hypertension

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Severe pulmonary arterial hypertension (PAH) is a progressive disease of various origins. In most cases increasing right ventricular load subsequent to high pulmonary vascular resistance leads to right ventricular decompensation. Intravenous prostacyclin was the first treatment for patients suffering from primary pulmonary hypertension to show improvements in functional capacity and survival in controlled clinical trials. However, this therapy requires permanent i.v. administration of the drug via indwelling catheters associated, bearing the risk of line-infections and right heart decompensation upon accidental discontinuation of the therapy. Administration of prostacyclin analogues via the oral (Beraprost), subcutaneous (Treprostinil), or inhaled route (Iloprost, Treprostiril) has been proposed to combine the proven beneficial effects of prostanoids as a treatment for severe pulmonary hypertension, while sparing out the disadvantages associated with the intravenous administration. The topic of this presentation will be to review the currently available prostanoid therapies and to provide information about the ongoing and future developments in this therapeutic area.

S14.4

Prostanoid signaling and receptor desensitization

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Prostacyclin (PGI2) is the major product of the cyclooxygenases (COX) in vascular endothelium and mediates potent anti-platelet, vasodilator, and anti-inflammatory actions by a prostacyclin receptor (IP). This receptor is a member of the G protein coupled receptor (GPCR) superfamily and is coupled to adenylate cyclase (AC) and phospholipase C (PLC). The prostanoid receptors are classified into DP, IP, EP (EP 1-4), FP and TP receptors with different affinities to agonists and differences in signal transduction. The IP, EP2, EP4 and DP receptors are coupled to stimulation of adenylate cyclase, the TP, EP1 and FP receptors are coupled to Ca^{2+} mobilization, and the EP3 receptor related signaling results in inhibition of adenylate cyclase. Agonist binding to the IP receptor leads to activation of the proteinkinase A by cyclic adenosinmonophosphat (cAMP). The tolerance development of the lung vasodilatory response to continuously infused prostacyclin is a clinical problem which can be avoided by repetitve inhalation of the prostanoids.

S15.1

GTP Cyclohydrolase I Regulation of Endothelial Function in Vascular Disease

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GTP cyclohydrolase I (GTPCHI) is the rate-limiting enzyme for de novo biosynthesis of tetrahydrobiopterin (BH4), an essential cofactor for all three nitric oxide synthases (NOS). Recent studies have shown that vascular BH4 is prone to oxidative degradation in vascular disease states including hypertension and diabetes. However, the molecular and cellular mechanisms underlying upstream regulation of BH4 synthesis by GTPCHI on endothelial dysfunction in vascular disease are incompletely understood. Our current studies have focused on in vivo studies of GTPCHI regulation of endothelial function and vascular injury induced by excessive oxidative stress in hypertension and diabetes. Our approaches include the use of transgenic mice with endothelial-specific overexpression of GTPCHI (Tg-GTPCH), hph-1 mice that are partially deficient of GTPCHI and BH4 as well as gene transfer of human GTPCHI to diseased blood vessels. Our experimental observations suggest that down-regulation of GTPCHI may play an important role on endothelial dysfunction and vascular injury. These findings may provide a mechanistic basis for targeting endogeneous GTPCHI as a new therapeuitc strategy for vascular disease.

S15.2

Gene Transfer to Blood Vessels: A Research Tool and Possible Therapy

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We have transferred genes to blood vessels or liver to alter vascular function. Hypertension is associated with oxidative stress, which (by inhibiting NO-mediated vasodilation) may contribute to vasoconstriction and hypertension. After gene transfer to liver of extracellular superoxide dismutase (ecSOD), the transgene releases ecSOD into blood, and the protein binds to blood vessels. Gene transfer of ecSOD improves endothelial function and reduces arterial pressure in hypertensive rats. We studied the vascular biology of ecSOD by removing its heparin-binding domain (HBD), which mediates binding of ecSOD to the outer membrane of cells. Gene transfer of ecSOD without the HBD failed to improve endothelial function or reduce arterial pressure during hypertension, even though enzyme activity was normal. A gene variant (ecSOD R213G) in the HBD in 3-6% of humans is associated with increased risk of cardiovascular disease. Gene transfer of ecSOD R213G failed to improve endothelial function or reduce arterial pressure during hypertension. There are almost 500 proteins that have an HBD. The approach that was used to study ecSOD R213G may be used to study many other proteins.

S15.3

Vascular Protective Effects of Endothelial Progenitor Cells

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Discovery of endothelial progenitor cells (EPCs) and their ability to repair injured endothelium and stimulate angiogenesis initiated intensive search for novel therapeutic approaches in prevention and treatment of cardiovascular disease. Harnessing full therapeutic potential of EPCs require better understanding of their biology. Our studies provide evidence demonstrating that EPCs are resistant to oxidative stress enabling them to perform complex regenerative program under unfavorable conditions of ischemia/reperfusion. We identified high expression and enzymatic activity of manganese superoxide dismutase (MnSOD) in cultured EPCs as major mechanism underlying their tolerance of oxidative stress. Our in vivo studies demonstrated that autologous transplantation of EPCs accelerated morphological and functional recovery of injured carotid artery endothelium. Both endothelial repair and endothelium-dependent relaxations to acetylcholine were significantly improved after vascular injury in rabbits treated with EPCs. These findings suggest that over-expression of MnSOD may enhance regenerative potential of EPCs and improve their therapeutic effect.

S15.4

Tachyphylaxis To Angiotensin II Is Prevented By Caveolae Disruption And Inhibition Of Receptor Internalization In Rat Aorta

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Most of the actions of angiotensin II, the major player of the renin-angiotensin system, have been attributed to stimulation of the angiotensin II type I (AT_1) receptor. We have previously observed that angiotensin II fails to induce reproducible contractile responses in rat aorta upon repetitive stimulation, a phenomenon called tachyphylaxis. This phenomenon was prevented by methyl-β-cyclodextrin, an agent that depletes cholesterol from the membrane disrupting caveolae, small invaginations at the plasma membrane. We hypothesize that caveolae are involved in AT_1 receptor internalization leadng to the tachyphylactic response to angiotensin II in rat aorta. To test our hypothesis, tension recording, immunohistochemistry and electron microscopy were performed. Prevention of the tachyphylactic contractions to angiotensin II by methyl-β-cyclodextrin was associated with AT_1 receptor co-localization with the caveolae marker cavedin-1 at the plasma membrane. When tachyphylaxis to angiotensin II was observed, no AT_1 receptor signal at the plasma membrane was observed. The contractile responses to angiotensin II were abolished by an AT_1 receptor antagonist. Phenylephrine-induced contraction was reproducible and unaffected by methyl-β-cyclodextrin treatment, indicating selective effects to angiotensin II. Rat aortic smooth muscle cell caveolae was disrupted by methyl-β-cyclodextrin. These data indicate that caveolae disruption by methyl-β-cyclodextrin prevents AT_1 receptor internalization and angiotensin II-induced tachyphylaxis.

S15.5

Redox Modulation of Vascular Phenotype

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The endothelium plays an important role in the maintenance of vascular homeostasis, in large part, due to its production production of nitric oxide (NO). Vascular diseases including hypertension, diabetes and atherosclerosis are characterized by impaired endothelium-derived NO bioactivity that may contribute to clinical cardiovascular events. Considerable evidence indicates that impaired vascular NO bioactivity is due, in part, to excess vascular oxidative stress. Five isoforms of NAD(P)H oxidase (Nox) have been identified with many expressed in the vasculature and they appear to be important sources of oxidative stress that modulate both NO bioactivity and endothelial cell phenotype. We have found that endothelial cells are characterized by expression of Nox isoforms 4 and 2, with the former most abundant. Experiments using small interfering RNA (siRNA) and adenoviral overexpression have indicated that Nox4 regulates endothelial cell growth. In keeping with this role of Nox isoforms on vascular cell growth, we found that Nox2 is important in the vascular response to balloon injury. This presentation will discuss the role of Nox isoforms and NO on vascular cell phenotype.

Lecture 2

Annexin 1, anti-inflammatory drugs and the neuroendocrine-immune interface.

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Annexin A1 (Anx-A1; lipocortin 1), a 37 Kd member of the annexin super-family of proteins, is of specific interest to pharmacologists because it has been established using immuno-neutralisation, antisense and transgenic strategies that it mediates several glucocorticoid (GC) actions. GCs induce the synthesis and release of Anx-A1 in many tissues and cells including components of the immune and neuro-endocrine system. The protein acts in a paracrine or autocrine fashion on its target cells, predominantly through G-protein coupled receptors of the FPR family, to produce inhibitory effects on inflammatory mediator release, neutrophil chemotaxis and many other important aspects of the innate inflammatory response. Within the adaptive immune system, Anx-A1 regulates the strength of T-cell signalling. Here, glucocorticoids down-regulate the synthesis of the protein thereby changing the Th1—Th2 balance. In the neuroendocrine system the GC-induced release of the protein from folliculostellate cells of the anterior pituitary gland is

crucial in the feedback control of ACTH and other hormone secretion.

Key words: Glucocorticoids, formylpeptide, T-cells, ACTH
Funded by the Wellcome Trust.

L5

Discovery of Ghrelin: Its Structure and Physiological Significance

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A complex network of cell-cell communication system by peptide hormones works for maintaining the mammalian homeostatic balance. To further clarify the intricate mechanisms of the regulation, it is important to discover unidentified bioactive peptides. For this purpose, we discovered novel bioactive peptides such as neuromedins, three natriuretic peptides (ANP, BNP, CNP), and adrenomedullin by using our own methods. Moreover, in 1999, we discovered an endogenous ligand for GHS-R, an orphan GPCR, from rat stomach, and named this novel GH-releasing peptide "ghrelin". Ghrelin is a 28-amino acid peptide with a marvelous structure modified by fatty acid, n-octanoic acid, which is essential to its activity. Ghrelin potently induces GH release both in rats and humans. Ghrelin is primarily produced in distinct endocrine cells, X/A-like cells, in the stomach. Ghrelin-producing neurons are also present in the hypothalamic arcuate nucleus. Beside the stimulatory effect of GH release, ghrelin is also involved in the stimulation of feeding, and the regulation of energy metabolism and cardiovascular functions. Thus, ghrelin has multifaceted roles in central and peripheral homeostatic systems.

L6

Pharmacogenomics: Basic and Clinical Research

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Pharmacogenomics is the study of the role of inheritance in variation in the drug response phenotype -- ranging from adverse drug reactions at one end of the spectrum to lack of therapeutic efficacy at the other. Pharmacogenomics is both a translational discipline -- with increasing examples of the striking clinical relevance of inherited variation in drug response -- as well as a basic scientific discipline that provides insight into mechanisms by which genetic polymorphisms can alter function. This presentation will use phase II (conjugating) drug-metabolizing enzymes to illustrate examples of both the translational relevance of, and mechanistic insights gained through pharmacogenomics.

L7

Ca^{2+} Sensitizers: Characteristics, Classification and Potential Clinical Relevance

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Cardiotonic agents are inevitable for the treatment of contractile dysfunction in acute heart failure and in aggravating phase of chronic heart failure. These agents act at different steps of cardiac E-C coupling through upstream, central and downstream mechanisms. Currently available agents (digitalis, catecholamines and PDE III inhibitors) act via upstream mechanisms by increasing Ca^{2+} mobilization. These agents possess high risks of Ca^{2+} overload to result in cardiac arrhythmias, cell injury and ultimate cell death. In addition, they have energetic disadvantage requiring activation energy and stimulating metabolism. Furthermore, they lose the effectiveness under pathological conditions, including acidosis, stunned myocardium and chronic heart failure. Ca^{2+} sensitizers that act via central and downstream mechanisms by means of an increase in Ca^{2+} binding affinity of troponin C, thin filament regulation of actin-myosin interaction and/or direct activation of crossbridge cycling have high potential to replace the existing agents, but ideal agents are not yet clinically available. Ca^{2+} sensitizers under basic research are classified into three groups, which are differentially affected by acidosis.

L8

Modulation of cytochrome P_{450} activity by reactive species

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Many pathological conditions trigger the release of pro-inflammatory cytokines that activate protein tyrosine kinases, protein kinase C and extracellular signal-related kinases, cause of an early reversible post-translational decrease in cytochrome P_{450} (P_{450}) activity, effects mediated by reactive oxygen species (ROS) and nitric oxide (NO.). In parallel, ROS and NO can reduce NADPH P_{450} reductase activity, effect that may contribute to the post-translational reduction in P_{450} activity. Pro-inflammatory cytokines also trigger a pre-transcriptional down-regulation of P_{450} genes and proteins, involving transcription factors such as hepatic nuclear factor-4, NF-κB, and CCAAT enhancer binding protein, and c-myc, factors that are activated by ROS. Hydrogen peroxide (H_2O_2) regulates the translocation of nuclear receptors, and oxidizes cysteine residues in the DNA binding domains of transcription factors, resulting in the reduction of the expression of CYP1A, CYP2B, CYP2C and CYP3A isoforms. During hypoxia, ROS activate hypoxia inducible factor-1α that increases CYP3A expression. Understanding how and why ROS and NO regulate P_{450} activity will be helpful to comprehend the multiple roles of the P_{450}.

L9

Why Pharmacology Teaching and Research are Inextricably Linked

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Pharmacology is the scientific study in humans and/or animals of the mechanisms and sites of action of chemicals that may have a therapeutic benefit. Pharmacology was originally regarded as applied physiology and taught to students as such. This resulted in drugs being classified according to the acute changes they caused in physiological parameters such as blood pressure or heart contractility. However, current molecular studies of the pharmacological effects of drugs during chronic administration have revealed additional, more complex mechanisms of action. Examples of drugs whose mechanisms of action have been revised from that derived from acute administration include: angiotensin converting enzyme inhibitors; statins; and biogenic amine uptake inhibitors. Ph.D. students need to be taught in vivo techniques for investigating molecular and cellular effects of drugs during chronic administration. Additionally, transgenic rodent models of complex degenerative diseases are needed as degenerative diseases will become more frequent with increased ageing of humans. This means that Departments of Pharmacology need both experienced teachers and modern equipment to study novel drugs for complex diseases.

S16.1

Genetic approaches to study opioid receptor function

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Mu, delta and kappa opioid receptors control responses to pain and stress, and play a central role in modulating emotional and addictive behaviors. To elucidate the role of each opioid receptor in these responses, we have created mice lacking the mu, delta or kappa receptor gene (see Kieffer and Gaveriaux-Ruff, Progr Neurobiol 2002,). We have found that the reinforcing properties of both opioid (Matthes et al. Nature 1996) and non-opioid drugs of abuse are abolished in the mu knockout mice (see Contet et al. Curr Opin Neurobiol 2004), and that maternal attachment is impaired (Moles et al. Science 2004). This receptor type therefore represents a key molecular switch in the initiation of addictive behaviors, and is also implicated in modulating physiological reward. We have discovered increased emotional reactivity in the delta knockout mice (Filliol et al 2000, Nat Genet 25, 195). Finally, mu, delta and kappa receptor-deficient mice exhibit distinct phenotypes in nociceptive assays, highlighting the specific roles of each opioid receptor in regulating pain. Future studies will explore receptor dynamics in vivo and neural circuits involved in opioid-controlled behaviors.

S16.2

Suppression of heroin-induced psychic dependence by low frequency (2 Hz) electroacupuncture stimulation

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Heroin constituts one of the major drugs of abuse in the world, especially in China. Compared to the physical dependence, the psychic dependence is much more difficult to deal with and plays much more important role in drug relapse. In a rat model of morphine-induced conditioned place preference (CPP) we have shown that electroacupuncture (EA) of 2 Hz was significantly more effective than 100 Hz in decreasing the CPP. In abstinent heoine addicts the degree of heroin craving as measured by visual analog scale (VAS) was suppressed by TENS of 2 Hz rather than 100 Hz. While VAS is a subjective index, we thought to use functional megnetic resonance imaging (fMRI) for the assessment of the objective changes occurred simulataneously with video- induced craving. Video cue did induce characteristic changes in BOLD signals, especially in limbic systems such as anterior cingulate cortex (ACC), amygdala (AMY), etc. These changes could be markedly suppressed by 2 Hz TENS for 30 min, but not by 100 Hz, a result highly correspondant with those obtained in VAS study.

Key words: heroin, craving, electroacupuncture, fMRI.
Supported by Basic Research Program of China 2003CB515407

S16.3

Medications Development for Treating Drug Abuse

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The reinforcing effects of opiates and many other drugs of abuse result from these drugs increasing dopamine levels in the nucleus accumbens. Therefore, long-acting medications that decrease dopamine levels may be potential pharmacotherapeutics to treat drug abuse. Mu opioid antagonists, such as naltrexone, and kappa opioid agonists, such as U50,488, have been shown by microdialysis to decrease dopamine release in the nucleus accumbens. Benzomorphans, such as cyclazocine and ethylketocyclazocine, and some morphinans, such as cyclorphan, are kappa agonists with mixed agonist and antagonist activity at mu opioid receptors. The pharmacological properties of novel benzomorphans and morphinans have been characterized in receptor binding and $^{[35S]}$GTPgammaS binding assays to determine their efficacy. Some compounds produced long-acting antinociception in mice, and reduced cocaine self-administration in non-human primates, suggesting that they or related compounds may be effective in treating drug abuse.

Key words: drug abuse, medications, opioid, medicinal chemistry
Acknowledgements: We thank Drs. John Neumeyer and Mark Wentland for the synthesis of the compounds.

S16.4

Opioids and the dynamics of addiction

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Different stages in the addiction course can be delineated: the initation, maintenance, withdrawal and relapse phase. Endogenous opioids have been implicated in these different stages. Opioid systems, particularly those present in the ventral tegmental area, play a modulatory role in drug reinforcement and may therefore be important for initiation of substance dependence and the individual susceptibility for development of drug addiction. Endogenous opioids in the limbic system change with the dynamics of daily drug intake; their release was increased before scheduled intake. This has been linked to the desire and need for the drug, and may thus be related to craving and/or dysphoria present prior to drug taking. The role of endogenous opioids in craving and also relapse was further substantiated by studies with cocaine in rats (place preference and reinstatement procedures) and with alcohol drinking in monkeys and is in agreement with clinical studies showing a beneficial effect of the opioid antagonist naltrexone in detoxified alcohol addicts.

Key words: opioids, addiction, self-administration, endorphins.

S16.5

Mu and Kappa Opioid Receptors and the Addictions: Rewarding and Countermodulatory Effects and Implications for Functional Polymorphisms

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The mu opioid receptor (MOPr) system has been shown to be directly involved in the rewarding effects of opiates, but also alcohol and cocaine. We have shown that components and function of MOPr system may be altered by chronic exposure to cocaine and alcohol, as well as opiates. In contrast, we and others have shown that kappa opioid receptor (KOPr) system, with its endogenous ligand dynorphin, acts in a countermodulatory mode, including suppression of dopamine tone and drug-induced dopamine surges. Our laboratory has shown that the MOPr and KOPr systems directly modulate the stress responsive hypothalamic-pituitary-adrenal axis, which, in turn, has been shown to be altered in human opiate, cocaine and alcohol addicts. We have identified variants of the MOPr gene, including a variant which we found is functional, and is associated with opiate and alcohol addictions. We have also identified multiple variants and haplotypes of the KOPr gene, and identified a specific variant and a haplotype associated with opiate addiction. We have also identified functional variants of the prodynorphin gene, which are associated with alcohol/cocaine dependency.

Key words: MOPr, KOPr, genetics, addictions
Supported by NIH-NIDA.

S16.6

Nociceptin/Orphanin FQ and Central Dopamine Neurotransmission

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Dopamine (DA) pathways from ventral tegmental area (VTA) to nucleus accumbens (Ac) and substantia nigra (SN) to striatum (Str) are implicated in the behavioral reinforcement and habitual behaviors induced by drugs of abuse. The mesolimbic and nigro-striatal pathways are subject to damage by selected neurotoxins. Methamphetamine (METH) causes profound and long-lasting damage to DA neuron terminals in Str, less damage in Ac and only modest loss of DA neurons in SN, while MPTP, a toxin producing symptoms resembling Parkinson's disease, additionally causes loss of >60% DA cells in SN. The opioid-like peptide nociceptin/orphanin FQ and its receptor (NOP-r) are expressed in both SN and VTA; activation of NOP-r reduces DA release from these neurons. Increased N/OFQ expression has been observed following injury to DA neurons. Deletion of the N/OFQ gene in mice significantly protects against MPTP neurotoxicity. Potential roles of N/OFQ and other opioids in regulation of DA pathways, and the potential use of antagonists of N/OFQ in the treatment of motor disorders and DA neuron toxicity induced by abused drugs will be discussed.

Key words: nociceptin, dopamine, injury;
supported by US National Institute on Drug Abuse

S17.1

Effects of Ganoderma lucidum polysaccharides on proliferation and cytotoxicity of cytokine-induced killer cells and its mechanism

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Ganoderma lucidum polysaccharides have shown immune modulating effects and anti-tumor activity. In this study, the effects of Ganoderma lucidum polysaccharides (GL-PS) with molecular weight of 584,900 and the ratio of polysaccharides to peptides is 93.61:6.49% on the proliferation and the anti-tumor activity of cytokine induced killer (CIK) cells were investigated in mice. CIK cells were prepared by using the standard protocol as a positive control. Experimental groups also underwent the standard protocol, except that GL-PS (400 mg/L or 100 mg/L) was added and the dose of anti-CD3 and interleukin-2 they received was reduced by 50% and 75%, respectively. The results suggested that GL-PS (400 or 100 mg/ml) promoting CIK cells proliferation and cytotoxicity were relevant to enhancing IL-2, TNF production, protein and mRNA expression of granzyme B and perforin in CIK cells through synergizing cytokines in decreasing doses of IL-2 and anti-CD3 by 75 and 50%. The activity of GL-PS could mostly be blocked by anti-CR3. These results confirmed that GL-PS was shown to be a promising immune potentiator. The effect of GL-PS on CIK cells is possibly mediated primarily through complement receptor type 3.

S17.2

Fingerprint Profiles of Myrrh and Frankincense from Africa

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Commiphora myrrha (Nees) Engl. occurrs in dry and arid regions of eastern Africa and yields the medicinal and aromatic resin myrrh. African frankincense is produced by Boswellia species, B. carterii and B. frereana occurring in Somalia and B. rivae, B. neglecta and B. papyrifera from Ethiopia. These resins are widely traded in commerce for use as incense, their essential oils as fixatives for perfumes and for aromatherapy and the extracts as analgesic and anti-inflammatory agents. China is among the biggest buyers of myrrh and frankincense from Africa. The six principal characteristic compounds of myrrh have been identified by our group as furanoeudesma-1,3-diene, lindestrene, furanodiene, 2-methoxyfuranodiene, 2-acetoxyfuranodiene and isofuranogermacrene. Other compounds reported from myrrh before originate from adulterants.

Due to the close physical similarity of the resins of different species, it has become increasingly important to develop analytical tools that would aid in distinguishing one type of resin from the other. To this effect, using Nuclear Magnetic Resonance Spectroscopy and other techniques we have developed finger print profiles to distinguish one type of resin from the other.

S17.3

Effects of Kai-Xin-San, a traditional Chinese medicine, and its four herbal components on learning and memory.

Hroshi Saito, Nanae Itokazu. Musashino University, Faculty of Pharmacy

Senile dementia disease characterized by memory dysfunction and Alzheimer disease, are becoming more frequent in the aged populations. Modern medicine does not offer medical treatments to the amnesia and dementia. However, in the traditional Chinese medicines, several crude drugs created already w thousand years ago, were thought to benefit the brain functions and to improve memory abilities. Kai-Xin-San, has been used since the Tang dynasty, and it has bee applied in numerous compositions targeting senile dementia. Even in Japan, Kai-Xin-San has been used since the Heian dynasty. Kai-Xin-San contains ginseng (panax ginseng C.A. Meyer), polygala (Polygala tenaifolia Wildenon), acorus (Acorus graminus Soland), and hoelen (Poria cocos Wolf), at a ratio of 1:1:2.5:5.0 (dry weight). The traditional medicine suggested that Kai-Xin-San and four herbal components on memory dysfunctions using several behavioral animal models, as well as electrophysiological models of memory formation (short- and long-term potentiation) and hippocampal neuronal cell culture. Kai-Xin-San has protective effects against ischemia, ameliorated impairment of memory acquisitions induced by alcohol, enhanced recovery of memory functions of amygdale-lesioned mice, improved aging process in senile dementic animal model, and facilitated the hippocampul LTP-induction. Taken together, the results suggest that Kai-Xin-San directly affected the hippocampal synaptic transmission, which might be the major mechanism accounting for its effects on learning and memory. In conclusion, our results offer a new experimental proof for clinical effectiveness of Kai-Xin-San in the treatment of dementic brain disorders.

S17.4

Sponge-Derived Fungi - a Prolific Source for New Natural Products

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Marine natural products continue to draw attention from researchers in academia and industry alike due to their structural uniqueness and their pronounced biological activities. So far over 10,000 different natural products have been isolated mostly from marine invertebrates such as sponges, tunicates, molluscs and others. In recent years the focus of marine natural products chemistry is shifting more and more towards microorganisms which are also prolific sources of interesting new metabolites but in sharp contradiction to most marine macroorganisms can be cultivated in vitro through biotechnological means. Besides bacteria marine derived fungi have attracted considerable attention in recent years. Especially sponges have been shown to harbor fungi even though the true nature of this association is not understood at present. Examples of new, bioactive natural compounds recently isolated from sponge-derived fungi will be presented in this overview.

S18.1

Presynaptic autoreceptors: location and function

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The discovery that the cytoplasmic membrane of presynaptic nerve terminals possess receptors that modulate the release of neurotransmitters was made more than 30 years ago. This new concept in neurotransmission represents a clear departure from the traditional view that neuronal communication was unidirectional, i.e. from the nerve terminal to the postsynaptic receptors, because with presynaptic receptors the transfer of information occurs in the opposite direction: from the synaptic cleft to the nerve terminals which release the neurotransmitter.

The term autoreceptor is employed to describe the presynaptic receptors which are acted upon by the endogenous transmitter of the neurone, triggering a regulatory feedback loop through which the transmitter can modulate its own release. The presynaptic inhibitory terminal autoreceptors were first described in peripheral noradrenergic neurons: these presynaptic autoreceptors were soon established to correspond to a novel subtype of adrenoceptor, the alpha-2 adrenoceptor which was shown to possess different pharmacological properties from the alpha-1 adrenoceptor. The evidence for the existence of terminal presynaptic autoreceptors which inhibit the release of the neurotransmitter was based on the following findings: 1) the calcium-dependent release of the neurotransmitter elicited by action potentials, was inhibited by receptor agonists; 2) receptor antagonists, on their own, enhanced the stimulation-evoked release of the transmitter, particularly at low and intermediate frequencies of nerve stimulation; 3) antagonists, blocked competitively the effects of receptor agonists on transmitter release. Evidence for this autoregulation of neuronal chemical signaling by presynaptic inhibitory autoreceptors was obtained under in vitro and in vivo experimental conditions, both in the peripheral and in the central nervous systems. In addition to the alpha-2A adrenoceptors modulating noradrenaline release through a negative feedback mechanism, presynaptic terminal autoreceptors now recognized include those for dopamine (D2/D3); acetylcholine (M2); serotonin (5-HT1D in humans and 5-HT1B in rodents); histamine (H3); GABA (GABA-B); and excitatory amino acid transmitters. Presynaptic terminal facilitatory autoreceptors exist for the modulation of acetylcholine release (nicotinic receptors), and also for noradrenaline (beta-2 receptors). Most neurons possess autoreceptors located not only on presynaptic terminals but also on their somata and dendrites, where they modulate the firing rate of the neurone. Activation of these inhibitory somatodendritic autoreceptors by agonists, reduces the firing rate of the neurone, while antagonists block the effects of the agonists. The term presynaptic heteroreceptors was introduced to identify a second category of presynaptic receptors that modulate transmitter release in response to chemical signals present in the synaptic cleft, but different from the neurons own transmitter. These presynaptic heteroreceptors are sensitive to cotransmitter neuropeptides, to transmitters released from adjacent terminals, or to locally produced or blood borne chemicals that either inhibit or facilitate the release of a neurotransmitter. For example noradrenergic nerve terminals possess facilitatory angiotensin-2 receptors and inhibitory opiate receptors. Acetylcholine, serotonin, and glutamate neurons possess alpha-2 terminal presynaptic inhibitory heteroreceptors. Presynaptic release-modulating receptors represent appropriate targets for pharmacological intervention by exogenous compounds acting as agonists, partial agonists or antagonists. Such compounds may be of therapeutic value by modifying transmitter release presynaptically and having fewer side effects than the well established approach of using agonist or antagonist drugs to stimulate or block postsynaptic receptors. Three marketed drugs act at least partly by selective stimulation or blockade of presynaptic release-modulating receptors: 1) the antidepressant mirtazapine, antagonist of alpha-2 adrenoceptors modulating the release of noradrenaline and 5-HT; 2) aripiprazole, approved by FDA in 2002, a central dopamine autoreceptor partial agonist for the treatment of schizophrenia. Aripiprazole does not elevate prolactin levels as most antipsychotics do; 3) sumatryptan and second generation tryptans for the treatment of migraine. The tryptans are selective 5-HT1D agonists which inhibit presynaptically the release of substance P and of CGRP.

S18.2

Presynaptic heteroreceptors

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The discovery that the presynaptic nerve terminals possess receptors whose activation by a transmitter released from another terminals or by a drug could modulate (inhibit or increase) the release of neurotransmitters from the nerve endings represented at that time a new concept in neurotransmission and has changed our way of thinking. Through activation of these receptors it is possible to modulate the $[Ca^{2+}]_o$-dependent release of different transmitters. Different endogenous ligands have effect on presynaptic heteroreceptors. Strong evidence is available that there is a functional interactions between neurons without synaptic contacts and they are specialized to function on a time scale of seconds (minutes) and a distance scale of hundreds of micrometers. The transmitter released into the extracellular space diffuses far away from release site and have tonic effect on nonsynaptic heterore-

ceptors and transporters of high affinity (versus low affinity receptors and transporters in the synapse). These receptors are the targets of drug action (Vizi, Pharmac. Rev. 52:63-89).

S18.3

Significance of alpha 2 adrenoceptor blockade in the treatment of depression and schizophrenia

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Antidepressant drugs (ADs) blocking the reuptake of noradrenaline (NA) and/or 5-hydroxytryptamine (5-HT) cause an acute autoreceptor-mediated feed-back inhibition of nerve activity and transmitter release in NA and/or 5-HT systems that gradually recedes due to autoreceptor desensitization. Adjunctive α_2 adrenoceptor blockade which antagonizes both noradrenergic α_2 autoreceptors and serotonergic α_2 heteroreceptors may thus enhance the release of both transmitters during acute as well as chronic AD treatment. Indeed, clinical use of adjunctive mirtazapine, a weak α_2 adrenoceptor antagonist, indicates a more rapid onset of action and improved efficacy of 5-HT reuptake inhibitors in depression. Employing adjunct α_2 antagonists with typical antipsychotic drugs (APDs), which contrary to clozapine lack potent α_2 antagonistic activity, markedly enhances their efficacy in treatment-resistant schizophrenia. Preclinical data propose that the increased efficacy is related to a clozapine-like enhanced prefrontal DA outflow and an associated facilitation of glutamatergic neurotransmission. Thus, α_2 adrenoceptor blockade appears as a common means to improve the clinical efficacy of both ADs and APDs.

S18.4

Subtype-specific functions of alpha2-adrenergic receptors - insights from transgenic mouse models

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Three subtypes of α_2-adrenergic-receptors have been identified, α_{2A}, α_{2B} and α_{2C}. Gene targeting in mice has led to the identification of specific roles for each of these receptors subtypes in adrenergic signalling in vivo. Surprisingly, all three α_2-receptors participate in presynaptic control of neurotransmitter release. In isolated tissues preparations, the α_{2A}-receptor is the major feedback regulator of noradrenaline release, but α_{2C}- and α_{2B}-receptors contribute to presynaptic control at the sympathetic nerve terminal. In vivo, α_{2A}- and α_{2C}-receptors differentially control the release of noradrenaline from sympathetic nerves (α_{2A}) and adrenaline from the adrenal gland (α_{2C}). Heterozygous deletion of α_{2A}-receptors did not significantly affect presynaptic control of transmitter release from adrenergic nerve terminl. In contrast, heterozygous α_{2C}-deficient mice showed enhanced urinary adrenaline excretion and were more susceptible to develop cardiac hypertrophy and failure after transverse aortic constriction. In the future, it will be important to translate these findings derived from genetic mouse models to humans.

Key words: alpha2-adrenergic receptor, sympathetic system, noradrenaline, knockout

S19.1

Integration of pharmacology teaching-does it work

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'Integration' as applied to pharmacology teaching may be interpreted in several ways. For example, integrated teaching to the different professions allied to medicine (INTERPROFESSIONAL TEACHING), integration of aspects of the discipline of pharmacology (INTEGRATIVE PHARMACOLOGY), integration of new and traditional teaching METHODS or integration of the CURRICULUM. Various drivers have created pressures to introduce integration into pharmacology teaching and each form of integration is associated with difficulties. In the UK, integration of the medical CURRICULUM has been required by the regulatory body (the General Medical Council) since its publication of 'Tomorrow's Doctors' in 1994 and has had a number of consequences for pharmacology teaching. These include a reduction in pharmacology teaching time (and possibly staffing) and an inadequacy, perceived by students as they enter the wards and by their clinical teachers, with regard to student's knowledge of the names and properties of common drugs. The extent of some of the changes following integration of the curriculum will be illustrated together with some of the innovations which have mitigated the problems encountered.

S19.2

A Pharmacology Teaching Model

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We have established a Pharmacology teaching model for seven-year program medical students in our university.

1. Compiling pharmacology textbook in English in 1997, 1999 and 2002 (3rd Ed), writing pharmacology experiment guideline in English in 2003 (4th Ed) and a review test book in English in 2003.

2. From 1985 till now, Pharmacology lectures have been given in English persistently by some professors. Exam papers are in English. Experiment reports have been written by students in English.

3. Using elicitation teaching method, preparing lessons collectively, and connecting pharmacology knowledge with clinic, directing students to write abstracts and reviews.

4. Inviting foreign professors to give lectures and seminars.

5. Offering medical English course for young faculty members and graduate students 1.5 hours per week for more than 25 years. The purpose of that is to train them giving Pharmacology lectures in English and to encourage them presenting papers in English when they attend academic meetings.

In 1999 Pharmacology text book in English achieved 1st grade award given by Tianjin Medical University. In 2000 Pharmacology teaching model for seven-year program medical students got 2rd grade aw

S19.3

Integrated Teaching in Devloping Countries

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Teaching approaches and models to train health care professionals, including in pharmacology, have changed significantly over the past decade. This includes a transition from the traditional teaching-based approaches to the learner-centred approaches and approaches to achieve integration of curriculum elements. Full teaching integration is reached via trans-disciplinary integration, while multi-disciplinary and inter-disciplinary approaches represent partial integration. Our recent investigations through questionnaires and personal communication, clearly show that teaching approaches in pharmacology have changed significant in developing countries during the past years. Driving factors include politics, economics, educational systems, changing needs in health care settings and globalization. The willingness and eagerness to integrate is clearly expressed by Institutions, although resistance to change exists. Integrated teaching was found to vary between these countries with respect to pace and level of integration. In conclusion developing countries have made strides towards integrated teaching, although satisfactory trans-disciplinary integration has in most cases not yet been achieved.

S19.4

Learning of medical pharmacology via relevant case problems.

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As a integral part of the traditional medical curriculum, pharmacology has always been considered a preclinical discipline in parallel with other traditional disciplines, such as anatomy, physiology and biochemistry. Like other basic sciences, pharmacology has traditionally been taught by faculties in the pharmacology department in a didactic manner with an examination-driven curriculum. Very often, teaching in pharmacology was over taught, boring, uncoordinated from other allied disciplines and lack of clinical relevance. In some schools, Clinical Pharmacology is introduced to overcome the perceived deficiency in "preclinical" pharmacology regarding its therapeutic relevance and application to medcine. Clinical pharmacology, if given, is unfortunately also taught in a didactic and problem-solving manner, not different from the basic pharmacology course. In recent years, education in pharmacology, which is primarily teacher-centered, disciplinary and content-oriented curriculum has been increasingly replaced by a more student-centered, integrative and process-oriented curriculum, which is usually case-oriented. Indeed, medical curricula which follow problem-based learning philosophy have increasingly emerged, albeit in varying forms, as a platform in which pharmacology is viewed as an integrated component in a holistic approach to medical education. In this problem-based learning (PBL) model, pharmacology is learned in a student-centered environment (self-directed learning), clinically relevant and case-oriented approach (case-based learning), usually in a small-group tutorial format. In PBL, pharmacology is learned in concert with other subject issues relevant to the case-problem in question (context-based learning), such as anatomy, physiology, pathology, microbiology, population health, behavior science, etc. (integration-oriented learning). Students learn via problem-evoked curiosity and motivation, in an environment which encourage free inquiries (in-

quiry-based learning) and intensive discussions in a cooperative rather than competitive atmosphere (cooperative learning). Teachers facilitate students' learning objectives rather than deliver pre-packed knowledge and dictate what they think students should learn. Based on the above two models, a change towards PBL curriculum appears to be beneficial in better preparing the medical students as life-long learners capable of coping with changes in knowledge and skills associated with the progressive and dynamic social/economic transformation in the Asia-Pacific regions. The use of PBL in pharmacology is more effective when it is carried out in smaller groups. PBL can also be conducted even in large classes, but it requires a well-trained and experienced PBL teacher to make the lecture more interesting and interactive involving student participation.

S20.1

Sarcoplasmic reticulum and membrane events in smooth musde cells

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The membrane excitability of smooth muscle cell (SMC) varies widely depending upon organs and the typical excitation-contraction (E-C) coupling is observed only in highly excitable SMCs. Roles of ryanodine receptor Ca^{2+} releasing channels (RyRs) in the regulation of cell functions including Ca^{2+} regulation are strikingly different between excitable and non-excitable SMCs. RyRs in excitable SMC may have substantial roles in the E-C coupling, whereas the contribution of Ca^{2+}-induced Ca^{2+} release (CICR) to the E-C coupling varies even in excitable SMCs from different organs. Moreover, the structural and molecular basis for the local Ca^{2+} regulation in a narrow space between junctional sarcoplasmic reticulum and plasma membrane is still a hot issue. The functional coupling between several major molecules in subcellular microdomain during E-C coupling was examined mainly in urinary bladder (UB) SMCs of the mice, in which the expression of key molecules, such as RyR, junctophilins, voltage-dependent Ca^{2+} channels, Na^{+}-Ca^{2+} exchangers, was genetically modulated. Evidence for the regulation of the molecular expression in the subcellular microdomains has also been accumulated in UBSMCs.

Key words: Ryanodine receptor, NN

S20.2

Role of Plasma membrane-SR (PM-SR) junctions in Smooth Musde Calcium Signaling

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Electron-microscopy of smooth muscle membranes reveals specialized junctional complexes between the PM and the superficial SR. These PM-SR junctions, which extend over about 300 to 400 um are characterized by a distance of 20 nm between the two membranes and are often bordered or perforated by caveolae. In the inferior vena-cava of the rabbit they occupy approximately 15% of the PM surface and concentrate the Na^{+}/Ca^{2+}-exchangers (NCX) 20 fold. The PM-SR junctions have been shown by Blaustein and collaborators to contain the low Na^{+}-affinity Na^{+}/K^{+}-ATPase (NKAa2) and in addition are thought to concentrate receptor activated non-specific cation channels (NSCC) containing TRPCs 1 and 6. The junctional SR membrane contains both SERCA and ryanodine receptors (RyR). Vaso-constricting agonists stimulate Na^{+} entry through NSCC, which elevates the $[Na^{+}]$ in the junctional space to levels sufficient to reverse the NCX and reload the SR with Ca^{2+} lost upon initial opening of IP3 receptors. We have developed a realistic quantitative model to show that Ca^{2+} entry from the extracellular space to the SR lumen through the PM-SR junctions is sufficient to support repetitive waves of regenerative SR Ca^{2+} release in response to stimulation with phenylephrine and endothelin I in vascular smooth muscle and with acetylcholine in bronchial smooth muscle. When the smooth musde is in its resting state the PM-SR junctions couple Ca^{2+} release by RYR to forward mode NCX to extrude Ca^{2+} from the cell.

S20.3

Store-operated Calcium Entry in Vascular Smooth Musde

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Several mechanisms regulate $[Ca^{2+}]i$ in vascular smooth musde cells (VSMC). Three main mechanisms mediate Ca^{2+} entry into VSMCs: voltage-operated (VOCC), receptor-operated (ROCC) and store-operated calcium channel (SOCC). VOCC are activated by depolarization. ROCC are coupled to receptors upon binding of specific agonists. SOCC is the non-selective cation channel that is activated when intracellular Ca^{2+} stores are depleted, thus serving as a housekeeping function to refill the stores.

This study examined relative contribution of ROCC and SOCC to contraction of rat carotid and renal arteries. ROCC is activated by phenylephrine, while SOCC is activated by SERCA inhibitors. In the presence of nifedipine, phenylephrine-induced ROCC-mediated contractions in both arteries were blocked by prazosin, while SOCC-mediated contraction was inhibited by lanthanum and 2-APB. 2-APB attenuated phenylephrine-induced Ca^{2+} entry via SOCC and ROCC in renal or carotid arteries. Ca^{2+} entry through SOCC is not directly coupled to VSMC contraction in renal arteries. Our results suggest that the relative contribution of SOCC to excitation-contraction coupling in VSMC depends on the types of arteries. (CUHK 4362/04M)

S20.4

Smooth Musde SR in Health and Disease

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S20.5

SR and vasomotion

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Vasomotion - the oscillation of tone - seen in most vascular segments may arise through a SR-dependent and a SR-independent mechanism. The SR-dependent vasomotion is a consequence of oscillatory release of Ca^{2+} from the SR, which through oscillating activation of an ion-channel in the cell membrane will lead to oscillations in membrane potential. The oscillation in membrane potential serves two purposes. It causes an oscillating influx of Ca^{2+} through L-type Ca^{2+}-channels which is important for oscillating tone development and it synchronizes the individual smooth muscle cells, so vasomotion occurs. The oscillation of membrane potential is cGMP dependent and we have suggested that it is mediated by a cGMP dependent Cl^{-} channel activated by release of Ca^{2+} from the SR. We describe a Ca^{2+} activated, cGMP dependent Cl^{-} channel with biophysical and pharmacological characteristics distinct from previously described Cl^{-} channels, which is a likely candidate. Based on siRNA induced knock-down of a gene candidate, we further suggest the molecular structure of the channel.

S20.6

'Quantal' Ca^{2+} release by at the cytoplasmic aspect of the IP3 receptor channel.

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Smooth muscle responds to IP3 receptor (IP3R) activation by a graded concentration-dependent ('quantal') Ca^{2+} release from the sarcoplasmic reticulum (SR). The mechanisms underlying quantal release have now been re-examined. The entire store was luminally continuous and Ca^{2+} could freely diffuse throughout; SR structure could not explain quantal release. Ca^{2+} release was apparently regulated by $[Ca^{2+}]$ within the SR. Ca^{2+} release velocity increased (accelerated) during release i.e. as SR $[Ca^{2+}]$ declined more release occurred. The acceleration determined the peak $[Ca^{2+}]c$, was attenuated with reduced SR $[Ca^{2+}]$ or increased cytoplasmic Ca^{2+} buffering. Positive feedback by released Ca^{2+} acting at the cytoplasmic aspect of IP3R (i.e. CICR) may explain the acceleration. When positive feedback was limited, quantal Ca^{2+} release was attenuated. The extent of positive feedback explains quantal release. During Ca^{2+} release, SR $[Ca^{2+}]$ and so unitary IP3R currents decline, positive feedback reduces and stops. With increasing [IP3], co-incidental activation of several neighbouring IP3Rs offsets the reduced IP3R current to renew positive feedback and Ca^{2+} release. Supported by the Wellcome Trust.

S21.1

Receptor closure - new frontiers for functional definition.

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France; chairman, NC-IUPHAR.

Pharmacology is at a historic turning point, because we know now nearly all the sequences of the receptors in the human genome. Thus virtually all the receptors coupled to G proteins (GPCRs) in the human genome have been published by sequence homology (Foord et al., 2005). The Nomenclature Committee of the International Union of Basic and Clinical Pharmacology (NC-IUPHAR) has announced the latest release of a major update to its receptor database http://www.iuphar-db.org/iuphar-rd/. The database contains information for 149 G protein-coupled receptors (GPCRs), encapsulating the conclusions of some 12 years of work by the 50 subcommittees. At this meeting, our classification of the nuclear receptors is presented by V Laudet. All of the voltage - gated ion channels are now classified (W Catterall). New directions for pharmacology include: the search for the function of orphan receptors (A Davenport), the role of receptor dimers (J-P Pin) and biologically active receptor polymorphisms (S Foord). NC-IUPHAR is thus addressing the key pharmacological problems of tomorrow and all pharmacologists are invited to participate.

The database is funded in part by the International Council for Science (ICSU), and UNESCO. Incyte Pharmaceuticals, Servier, GSK, and Wyeth contributed.

S21.2

Nuclear hormone receptors: evolutionary and pharmacological considerations for classification.

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Nuclear receptors are ligand-activated transcription factors that form a family of closely related molecules sharing a similar structure. In addition to receptors for known ligands (such as thyroid hormones, retinoic acids, steroids or fatty acid derivatives) the nuclear receptor superfamily contains members for which no ligands have been described, the so-called orphan receptors. It is still unclear whether these orphan receptors are real ligand-less orphans that are regulated by other mechanisms, like phosphorylation or protein-protein interaction, or if they are receptors to ligands, which are still to be discovered. The orphan receptors complicate the establishment of a pharmacology-based nomenclature. In addition, the situation is complicated by the fact that the liganded receptors are able to form heterodimers and that many different partially redundant receptor isoforms have been characterized. These problems will be discussed in an evolutionary context including the analysis of complete genomes and a phylogenetic analysis of the superfamily. This novel and more inclusive approach might provide a framework for a pharmacology-based nomenclature.

S21.3

Biologically Active Receptor Polymorphisms

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Humans are 99.9% similar. However, put another way in every 1,000 base pairs, there are approximately two differences between any two individuals. The majority of these differences are single nucleotide polymorphisms (SNPs). SNPs have been identified as the cause of thousands of known Mendelian diseases. The roles of SNPs in such diseases are often clear as they have been found to change an amino acid (non-synonymous, nsSNPs or coding, cSNPs) within a critical part of a protein. cSNPs are probably important in more complex diseases but it will be some time before their role is properly understood.

GlaxoSmithKline (GSK) are accumulating a significant amount of information on the variation between genes within human populations and between species. The human studies are being performed to determine the genetic basis of at least a dozen distinct and common diseases. GSK has genotyped selected SNPs in 2000 selected genes including almost all non-sensory GPCRs and within 12,000 individuals representing 12 major diseases (1). There are about 350 non-sensory GPCRs. They represent one of the largest and best characterised of all gene families and the family at which 40% of medicines are directed. It also has one of the highest ratios of nsSNPs/SNPs of any characterised gene family. The data obtained from 4 completed studies so far suggest that between 8 and 20 genes/2000 will show significant association with the disease in question with between 1 and 4 being GPCRs.

When a significant association is found it prompts an attempt to assign physiological and pharmacological significance to the SNP in question or those in linkage disequilibrium. Because GSK has significant data on gene expression, receptor modelling, inter human variation in drug responses and other parameters there is supporting data which can be viewed in the light of any hypothesis. Experimentation is the last resort - in silico studies can analyse the huge amounts of data available and provide background data on what variation is found within the human population and in the genomes of other mammals. By calculating the ratio between those nucleotide changes that lead to an amino acid change in a protein and those that do not it is possible to gain an estimate of the evolutionary selection pressure that the gene is under (the omega ratio = dN/dS). These two strands of research are related in that they determine variation (and so evolutionary trends) in the short (human) and long (inter species) term. These studies will provide the substrate data for an attempt to understand and interpret variation in human GPCRs.

S21.4

Structure, Function, and Classification of Voltage-Gated Ion Channels and Their Relatives.

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The superfamily of voltage-gated ion channels includes 10 voltage-gated sodium (Na_v), 10 calcium (Ca_v), and 40 potassium (K_v) channels, 32 transient receptor potential (TRP) channels, 10 cyclic nucleotide-modulated (HCN and CNG) channels, 8 calcium-activated potassium (K_{ca}) channels, 15 inwardly-rectifying potassium (K_{ir}) channels, and 15 two-pore-loop potassium (K_{2p}) channels. Their principal subunits share a common pore structure combined with transmembrane domains for voltage-dependent gating on the N-terminal side and intracellular domains for regulation by second messengers and interacting proteins on the C-terminal side. In most cases, the principal subunits are associated with one or more auxiliary subunits of different size, structure, and function. The recommended nomenclatures and the molecular relationships of these channel families will be presented. Common structural themes for voltage-sensing, pore-gating, ion conductance, and regulation by intracellular proteins will be discussed. These results highlight key similarities and differences in function of this diverse superfamily of ion channels.

S21.5

Regulation of vascular reactivity by established and novel GPG-receptors: emerging pharmacology of urotensin II in the human cardiovascular system.

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The vascular system is rich in G-protein-coupled receptors, particularly Class 1, that are activated by an eclectic range of chemical entities including peptides. These chemical messengers can function in blood vessels as directly acting constrictors, dilators or indirectly acting vasodilators. They are important contributors, especially in small arterioles, in setting peripheral resistance and blood pressure. During the last ten years over 50 receptors previously designated as 'orphans' have been paired with their cognate ligands. New transmitter systems are emerging with some displaying potent activity in the vascular system such as the vasodilator ghrelin or constrictors including apelin, motilin, neuromedin U and urotensin-II. In Class 2, all 20 receptors are activated by peptides. Those displaying vasoactivity all function as directly acting vasodilators including adrenomedullin, CGRP and VIP as well as emerging transmitters, the urocortins. Hypertension can persist despite combinations of current blood pressure lowering drugs, suggesting further transmitter systems wait to be discovered from the remaining orphan receptors that may provide new targets for novel therapies or diagnosis.

S21.6

G-protein coupled receptor dimers, homomers and heteromers

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G-protein coupled receptors have long been considered to be monomeric membrane proteins. While numerous recent studies have indicated that GPCRs can form multimeric complexes, the functional and pharmacological consequences of this phenomenon have remained elusive. With the discovery that the functional $GABA_B$ receptor is an obligate heterodimer, and the use of energy transfer technologies, it is now accepted that GPCRs can form hetero multimers. In some cases, specific properties of such heteromers not shared by their respective homomers have been reported. Although in most cases these properties have only been observed in heterologous expression systems, there are a few reports describing data consistent with such hetero multimeric GPCR complexes also existing in native tissues. The present presentation will illustrate well-documented examples of such native multimeric complexes, lists a number of recommendations for recognition and acceptance of such multimeric receptors, and finally defines a minimal rule for their nomenclature.

S22.1

MMP9 mediates angiogenic switch in early phases of carcinogenesis: implications for MMPI-based therapy

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Modifying the inflammatory response decreases tumor development and progression in animal models and human patients. However, it is not understood how leukocytes interact with the mammary epithelium during these events. We used genetic and in vivo imaging techniques to study the interaction between leukocytes and epithelial cancer cells during tumor progression by crossing transgenic tumor-prone mice with mice expressing enhanced green fluorescent protein under general and inflammatory cell-specific promoters and with mice lacking specific matrix metalloproteinases (MMPs). We visualized developing tumors in living, anaesthetized mice and found that they undergo an inflammatory switch. The leukocytes observed at the tumor-stroma interface were very motile. We modified the function of the leukocytes recruited by the tumors by crossing mice null for MMP9, which is expressed by inflammatory cells, into the tumor-prone background. We found that the vasculature in the tumors of MMP9 null mice has decreased density and integrity than in tumors of wild type mice. However, the absence of MMP9 allowed more early lesions to progress to tumors. This suggests that inflammatory cells may enhance tumor growth.

S22.2

Membrane-Anchored Metalloproteinases, Angiogenesis and Cancer

Stephen J. Weiss, University of Michigan

Cancer cells express tissue-invasive activity while simultaneously signaling endothelial cells to engage an angiogenic response. Despite decades of conjecture, the mechanisms by which matrix barriers are negotiated remain undefined as more than 500 proteases have been identified in mammalian genomes. To define the mechanisms that underlie pro-invasive activity, we have developed a series of ex vivo models which recapitulate each of the key steps involved in cancer progression and angiogenesis. Herein, we demonstrate that tissue-invasive activity is solely dependent on a sub-family of membrane-anchored matrix metalloproteinases (MMPs), termed the MT-MMPs, which regulate tumor cell invasion and proliferation as well as the neovascularization process. Interestingly, while MT-MMPs are synthesized as inactive zymogens, the proteinases are processed to active enzymes in the constitutive secretory pathway by an intracellular mechanism involving one or more members of the proprotein convertases, a distinct gene family of subtilisin-like serine proteinases. Therapeutic interventions directed against this enzyme couple could prove useful in the control of tissue-destructive and/or invasive disease states.

S22.3

The Role of ADAMTS-4 (Aggrecanase 1) and ADAMTS-5 (Aggrecanase 2) in the Pathophysiology of Arthritis and Cancer

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Osteoarthritis (OA) is characterized by articular cartilage erosion as a consequence of proteolytic cleavage of the major functional macromolecule aggrecan. Aggrecan degradation in OA and rheumatoid arthritis is attributed to cleavage of the core protein at the Glu373-Ala374 bond by the aggrecanases. Two aggrecanases, purified from IL-1-stimulated cartilage explants, were identified as members of the a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family, ADAMTS-4 and ADAMTS-5, and work from a number of groups has begun to provide insight into the molecular basis for the role of these proteases in aggrecan catabolism. In addition to the breakdown of aggrecan, these proteinases have been implicated in the breakdown of other aggregating proteoglycans icnluding brevican via cleavage at Glu395-Ser396. Brevican is a major brain matrix proteoglycan, and its cleavage by ADAMTS-4 may be key to the invasiveness of malignant gliomas. Therefore, development of specific inhibitors of ADAMTS-4 and -5, may provide new therapeutic strategies for the treatment of arthritis and cancer. Based on ADAMTS-4 and -5 biochemistry, one can envisage the design of three classes of inhibitors.

S22.4

Targeting the trafficking of MT-MMP for anti-cancer therapy

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Following the successful completion of the human genome and the ongoing effort in human proteome, we believe the next wave of activity will be focused on the traffickome—the localization and transportation of all cellular proteins. To this end, we have embarked on a large scale screening for intracellular mediators for the trafficking of cell surface molecules such as MT1-MMP and EGFR. The strategy involves the cloning of more than 500 likely effectors in expression vectors and the construction of their corresponding siRNA vectors. These potential regulators have been screened for their ability to perturb the trafficking patterns of MT1-MMP in MDCK and PC3 cells. The positive candidates are currently being evaluated by co-IP experiments to see if they interact directly with the previously identified adaptor proteins including dynamin, clathrin, components of the AP2 complex, and the PDZ containing Mints. The long term goal is to construct a regulatory circuit that regulate the trafficking of MT-MMPs and identify potential targets upstream of MT-MMPs for drug development.

S23.1

Na^+-Ca^{2+} Exchanger Gene Products, NCX1, NCX2 and NCX3, as Putative Targets for Neuroprotection in Brain Ischemia

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NCX is a neuronal plasmamembrane antiporter, which, by coupling Ca^{2+} and Na^+ fluxes, plays a relevant role in brain ischemia. In the CNS there are 3 different NCX gene products NCX1, NCX2, and NCX3. NCX transcript and protein expression is differently regulated after ischemia in the focal region and in the peri-infarct area. The pharmacological inhibition of NCX activity is detrimental in the development of ischemic damage. NCX knocking out through antisense strategy showed that NCX1 and NCX3 play a major role in NCX neuroprotective action. Accordingly, NCX3 transfected BHK cells are less vulnerable to chemical hypoxia. Interestingly, the expression of NCX1 and NCX3 is up-regulated by NGF in PC12 cells. This modulation occurs via two pathways activated by tyrosine kinase receptors, Ras-Raf-Mek-MAPK pathway and PI-3K/AKT pathway. In fact, the Mek inhibitor PD 98059 or the PI-3K inhibitor LY 294002 prevented this up-regulation, whereas AKT-1 PC12 cell positive mutarts showed an increase in NCX1 and NCX3 expression. These data demonstrate that NCX products display a differential expression in the development of ischemic damage and can be selectively upregulated through NGF-mediated trasductional pathways

S23.2

Na^+/Ca^{2+} Exchange Inhibitors: Therapeutic Potential in Cardiovascular Diseases

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Intracellular Ca^{2+} is the key regulator in cardiac and arterial functions during the contraction-relaxation cycle. Myocyte Ca^{2+} imbalance thus produces mechanical dysfunction, electrical instability (arrhythmia), and muscle remodeling. The Na^+/Ca^{2+} exchanger (NCX), that exchanges Na^+ and Ca^{2+} in either Ca^{2+} efflux or Ca^{2+} influx mode, is one of the major Ca^{2+}-handing proteins in myocytes. Evidence is currently accumulating to suggest that NCX1 is up-regulated in various cardiovascular diseases. Recently developed benzyloxyphenyl NCX inhibitors effectively prevent myocardial ischemia/reperfusion injury and salt-sensitive hypertension in animal models. Furthermore, several experiments with genetically engineered mice provide compelling evidence that these diseases are triggered by pathological Ca^{2+} entry through NCX1 in cardiac and arterial myocytes, respectively. Thus, NCX inhibitors may have therapeutic potential as novel cardiovascular drugs for myocardial reperfusion injury and salt-sensitive hypertension. However, the efficacy of NCX inhibitors, as well as the role of NCX1, in heart failure or arrhythmias requires more detailed study.

Key words: Na^+/Ca^{2+} exchanger, SEA0400, ischemia, hypertension

S23.3

Na^+/Ca^{2+} exchanger of neurons was modulated during acute and chronic brain ischemia in rat

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Sodium-calcium exchanger (NCX), an antiporter localized on the plasma membrane of neurons and glia, is thought to provide an important pathway for Ca^{2+} extrusion. It may also play a prominent role in inducing Ca^{2+} overload during

brain ischemia and reperfusion. In this study the alterations of NCX isoforms in neurons during acute and chronic brain ischemia of rats were investigated. The acute brain ischemia of rats was induced by MCAO. It showed that the expression in cortex in mRNA level of NCX1 was decreased by 42%, 28% respectively, after 2 and 6 h reperfusion, following 2 h ischemia. However, it was restored to control level at 12 and 24 h reperfusion. NCX2 and NCX3 were not changed significantly after ischemia and reperfusion. The chronic global cerebral ischemia was induced in rats by bilateral common carotid artery ligation (BCAL) for 1, 2 and 4 weeks. It was found that in cortex the mRNA expression of NCX1 was reduced by 35%, 54% and 27%, respectively, after BCAL 1, 2, and 4 weeks. For NCX2, its expression was decreased by 41%, 29% and 12% after BCAL 1, 2 and 4 weeks, respectively. For NCX3, it was reduced by 29%, 27% and 12% after BCAL 1, 2 and 4 weeks, respectively. However, in hippocampus, the expressions of NCX1 and NCX3 were not significantly changed after BCAL, whereas NCX2 increased by 60% after BCAL 1 week. The expressions of NCXs in protein level were also studied. Our results indicate that Na^+/Ca^{2+} exchanger may play an important role in acute and chronic brain injury from ischemia. It might also play a role as a drug target in neuronal protection.

Key words: Sodium-calcium exchanger; brain ischemia; mRNA expression

Acknowledgements: The work was supported by the National Science Foundation of China, No.30371644 and Specialized Research Fund for the Doctoral Program of Higher Education, NO.20020023030

S23.4

Na and Ca Regulation in Normal and Failing Hearts

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Ca in cardiac myocytes regulates contractility and electrophysiology. Ca and Na regulation are linked via Na/Ca exchange (NCX), and Na/K-ATPase (NKA) is the main means of Na extrusion. Heart failure (HF) exhibits contractile dysfunction and arrhythmias, and both are due to altered Ca & Na handling. Triggered arrhythmias (e.g. DADs) are prominent in HF. DADs are due to spontaneous SR Ca release and activation of transient inward NCX current. Thus NCX and Na are critical in systolic & diastolic function and arrhythmias. $[Na]_i$ is elevated in HF which may limit SR unloading and provide Ca influx during the HF action potential, thus limiting depressed systolic function. High $[Na]_i$ in HF is due to enhanced Na influx. Cellular NKA function appears unaltered, despite reduced NKA expression. We find that phospholemman (PLM) regulates NKA by an inhibition which is relieved by PLM phosphorylation. Intermolecular FRET between PLM and NKA is substantial and is reduced upon PLM phosphorylation. The lower expression level of more phosphory-lated PLM in HF may explain unaltered NKA function with lower expression. Thus, altered Ca and Na handling are important in contractile function and arrhythmogenesis in HF.

S23.5

Na^+/Ca^{2+} exchanger: Lessons from NCX1 overexpression and from NCX1 and NCX3 knockout mice

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The Na^+/Ca^{2+} exchanger is a unique mechanism allowing Ca^{2+} extrusion from the cell against its gradient without consuming any energy. The Na^+/Ca^{2+} exchanger has been recently cloned and this has offered new research possibilities to better investigate this transporter and to use it to improve cellular function. In recent work, we showed that overexpression of the Na^+/Ca^{2+} exchanger (NCX1) in an insulin secreting -cell line shaped stimulus-induced cytosolic Ca^{2+} oscillations indicating that Na^+/Ca^{2+} exchange contributes to both Ca^{2+} entry and outflow, but also generates an inward current that influences the pattern of electrical activity and Ca^{2+} oscillations. Overexpression of the exchanger also induced ER Ca^{2+} depletion, ER stress, caspase 12 activation, with subsequent increase in β cell death by apoptosis and decrease in cell proliferation. Conversely, βcells from NCX1 knockout mice ($NCX1^{+/-}$) showed a disruption in glucose-induced Ca^{2+} oscillations, an increase in glucose-induced insulin release, and resistance to apoptotic cell death. Mice lacking the Ncx3 gene showed localized skeletal muscle fibre necrosis, impaired neuromuscular transmission, muscle weakness and ease in fatigability. Conclusions: the present data underscore the interest of developing activators and inhibitors of the Na^+/Ca^{2+} exchanger(s) and open perspectives for their therapeutical interests.

Key words: Na/Ca exchange, apoptosis

S24.1

Pharmacological evaluation of TP receptor antagonists on alpha and beta receptor isoforms

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Thromboxane A2 (TXA_2) is a mediator implicated in pathologies such as myocardial infarction. The TXA_2 receptor is a GPCR of which two alternative spliced isoforms, TP_α and TP_β, have been described. In this study, we present the pharmacological evaluation on the individual TP_α and TP_β isoforms of a series of original compounds. We developed cell lines expressing TP_α or TP_β, and measured the calcium mobilization triggered by TXA_2 agonist. Several compounds displayed interesting pharmacological profiles, many exhibiting greater antagonistic functional activity for either TP_α or TP_β. For example, JH90 was characterized by a selectivity $TP_\alpha IC_{50}$/ $TP_\beta IC_{50}$ ratio of ~10 ($TP_\alpha IC_{50}$ = 1590nM ±1320nM; $TP_\beta IC_{50}$ = 151nM ±110nM). In conclusion, we have pharmacologically defined several TP receptor antagonists characterized by differential activity on the TP isoforms receptors. Moreover, from our results, we can propose several structural moieties conferring isoform specificity. These agents could lead to development of pharmacological tools useful for the study of the specific role of TP isoform receptors.

S24.2

Coupling of b2-adrenoceptor to Gs and XLas. Different conformational states of the receptor are percieved diferantially by the two variants

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XLas (Extra Large Alpha S) is an alternatively spliced variant of Gas, the G protein alpha subunit that couples various extracellular stimuli to the activation of adenylyl cyclase. XLas is identical with Gas except for its long amino terminus and appears to share its functional properties such as receptor selectivity, bg binding and adenylyl cyclase activation. In this study, coupling properties of Gas and XLas to b2 adrenergic receptor (β2AR) and adenylyl cyclase are investigated in transfected HEK-293 cells. Adenylyl cyclase activity measurements show that, compared to Gas, Xlas exhibits a high basal and agonist-induced activity, both of which are enhanced by the increased expression of β2AR. Thus, spontaneous receptor activity of the β2AR seems to contribute to the basal activities of both Gas and XLas. However, while the basal activity of Gas can be inhibited by inverse agonists that suppress the spontaneous activity of the β2AR, the basal activity of XLas is insensitive to these ligands. This suggests that the conformation induced by these ligands is sensed as an inactive state by Gas but not by XLas. This study is supported partly by the research grants A. BAP. 2002-08-09-088 and TUBITAK 104s472

S24.3

Repeated intermittent administration of MDMA affects serotonin receptor mRNA in the rat brain using qPCR Kindlundh-Hogberg

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The aim of the present study was to investigate whether the recreational drug (+/-)-3,4-methylenedioxymetamphetamine (MDMA) would affect mRNA of serotonin and dopamine receptors. Male Sprague-Dawley rats received either 3x1 or 3x5 mg/kg/day (3 hours apart) every 7:th day during 4 weeks. Real time RT-PCR was used to determine the mRNA levels of serotonin $5HT_{1A}$, $5HT_{1B}$, $5HT_{2A}$, $5HT_{2C}$, $5HT_3$, $5HT_6$ receptors and dopamine D1, D2, D3 receptors in seven brain nuclei. Results could be highlighted by profound MDMA-induced increase of the $5HT_{1B}$-receptor mRNA in the cortex, caudate putamen, nucleus accumbens, and hypothalamus at the highest dose. In addition, an inverse correlation between MDMA-induced pellet consumption and $5HT_{2C}$ mRNA levels was observed in the hypothalamus. This study is concluded to provide evidence for a unique implication of serotonin rather than dopamine receptor mRNA levels, in response to repeated intermittent MDMA administration.

KEY words: MDMA, serotonin receptor, dopamine receptor, drug abuse

S24.4

Hypoxia-induced endothelial dysfunction in human umbilical vein endothelial cells

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We have studied changes in protein expression in human umbilical vein endothelial cells (ECs) exposed to hypoxia using proteomics. Cells were exposed to 5% CO2 for 24 hours or 10 mM CoCl2 for 4 hours. Two separate sets of 2D gel experiments (n = 4 each) revealed several proteins that were upregulated by hypoxia. These included glucose regulated protein (Grp) 78, cyclophilin A, cofilin, calmodulin and tubulin which was confirmed by immunoblotting (n = 8-12). Also by immunoblotting Grp94 and caspase 12 were shown to be increased whereas actin was reduced. CoCl2, a stabilizer of HIF-1a was able to regulate many of the proteins indicating an important role for this transcription factor. The upregulation of Grp78, Grp94 and caspase 12 is indicative of ER stress, a novel finding concerning hypoxic ECs. The upregulation of cofilin and tubulin suggests migration or angiogenesis, while the increase in cyclophilin A can involve migration, caspase activation and protein folding. The fall in actin could imply a shift from G to F actin perhaps as stress fibers. We conclude that hypoxia has direct effects on ECs, which should be taken into account when treating patients.

Key words: Hypoxia, endothelium, ER stress, angiogenesis

S24.5

Novel effects of nadolol on desensitization and heterologous sensitization systems

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Nadolol is a nonselective beta-adrenergic receptor (βAR) blocker with inverse agonist activity at β2ARs. In this work, the effect of nadolol on forskolin-stimulated phosphorylation of the cAMP-dependent protein kinase (PKA) site of the human β2AR overexpressed in human embryonic kidney (HEK) 293 cells was tested with phosphoserine-specific antibodies. Western blot data showed acute nadolol treatment decreased forskolin stimulated phosphorylation at the PKA site while chronic nadolol treatment increased it. Also biotin labeling results indicated chronic nadolol treatment may prevent degradation of human β2 AR in HEK 293 cells. Furthermore, for tracheal rings from our asthmatic mouse model that were precontracted with methachcholine, acute and chronic nadolol treatment produced an enhanced responsiveness (relaxation) to the prostacyclin receptor (IP) agonist cicaprost. Experimental data suggested the effects of nadolol on phosphorylation at the PKA site, on receptor degradation and on heterologous sensitization to IPs might contribute to a beneficial effect of nadolol in asthma.

Key words: Nadolol; desensitization; heterologous sensitization
Acknowledgement: Sandler Program for Asthma Research

S24.6

The involvement of TASK-1 channels in the development of a novel spontaneous myogenic waveform in guinea pig trachea

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Studies were undertaken to determine the anatomical localization of dual-pore domain potassium (K2P) channels in guinea pig trachea and to establish their involvement in spontaneous myogenic wave formation. RT-PCR and immunohistochemical studies illustrated the presence of mRNA and protein encoding K2P α subunits in tracheal smooth muscle. Tracheal segments exposed to 6% or 12% CO2 developed myogenic-wave activity. Acidic (TASK-1 blocking) and alkaline (TASK-1 opening) conditions respectively inhibited and had no effect on myogenic-wave activity. Furthermore, treatment with the TASK-1 inhibitors bupivacaine (1-100 μM), anandamide and methanandamide (each 1-10 μM in the presence of 1 μM AM251 and 1 μM iodoresiniferatoxin) caused a concentration-dependent abolition of myogenic-waves and markedly increased myocyte tone. These results indicate that TASK-1 channels may play an important role in the production of a novel spontaneous myogenic waveform in guinea-pig trachea. Speculatively, the opening of K2P channels by drugs may perhaps represent a promising bronchodilator mechanism worthy of further exploitation.

Supported by Medical Research Council UK (MRC) and Novartis
Key words: TASK-1, K2P channels, trachea

S24.7

Peptide Inhibitors of Regulators of G-Protein Signaling 4 (RGS4): Enhancing Potency by Combinatorial Library Screening

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Objective: To identify potent peptide inhibitors of Regulator of G-Protein Signaling 4 (RGS4). The cyclic peptide YJ34 (Ac-Val-Lys-[Cys-Thr-Gly-Ile-Cys]-Glu-NH2, S-S) which inhibits RGS activity with uM potency [1] was designed to mimic the switch 1 region of Galphai in the RGS4-Galphai crystal structure [2]. The present study aims to find related constrained peptide inhibitors with nM potency. Methods: A focused One-Bead, One-Compound peptide library [3] which retains key residues of YJ34 was created. The library with 2.5 billion peptides was screened and beads with increased binding of a fluorescently labelled RGS4 were sequenced. Results: A series of bead-bound peptides that bind RGS4 more tightly than our lead compound, YJ34 were identified. Structural similarities and functional inhibitory activity of the potent peptides will be described. Conclusions: Two approaches to drug design, rational design and combinatorial screening were applied to the identification of peptide RGS inhibitors. The combination of the two can be much more effective then either alone.

S24.8

Effects of Diabetes Mellitus and High Glucose on Brain-Pancreas Relative Protein(BPRP)

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Brain-Pancreas Relative Protein (BPRP) is a novel protein identified in our Lab. It was primarily localized in brain neurons and islet cells, which implies its function in these tissues. We examined the effects of alloxan-induced diabetes in rats on the level of the BPRP in the brain. Diabetes resulted in significant increase in blood glucose, and decrease in BPRP levels in the brain at both 4 and 8 weeks of diabetes duration. To investigate whether the changes of blood glucose could regulate the alterations of BPRP, we use the PC12 cells to examine the effects of high glucose on the level of BPRP. Treatment of PC12 cells with different concentration of glucose significantly decreased BPRP level in the dose-dependent and time-dependent manners. The effect of glucose couldn't be mimicked by mannitol. In addition, high glucose-induced down-regulation of BPRP was reversed by ALLN, an inhibitor of calpain and not affected by treatment with the MG132, a specific proteasome inhibitor. These results suggest that this protein was probably destroyed by proteolytic degradation and the down-regulation of BPRP and the activiy of calpain may contribute to the complications of diabetes in Central Nervous System.

S24.9

Bradykinin (BK) potentiates the cholinergic EPSCs via B2 kinin receptor in acutely dissociated paratracheal ganglion (PTG) neurons of rat

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PTG neurons localize on the serosal surface of the dorsal tracheal wall and predominantly control lower air way function. We previously reported that BK, a potent inflammatory mediator, depolarized PTG neurons via M-current inhibition and potentiated nicotinic current in PTG neurons. In this study, we further studied its effect on excitatory postsynaptic currents (EPSCs) in dissociated PTG neurons attached with presynaptic boutons. Method: Nystatin-perforated patch clamp technique was applied to the PTG neurons acutely dissociated from 10- to 18-day-old Wistar rats. Result: EPSC frequency was increased in high K^+ external solution without changing its amplitude. Cd^{2+} and mecamylamine inhibited EPSCs. Contrary, BK at 100 nM potentiated the amplitude and its frequency to 1.39 ±0.11 and 2.62 ±0.81 times of pre-application control, respectively. BK potentiation of EPSCs was mimicked by [Hyp(3)]-bradykinin, a B2 receptor agonist but abolished by HOE 140, a B2 receptor antagonist. These results suggest that BK

potentiates the cholinergic EPSCs via B2 kinin receptor in PTG neurons. Present results might provide a new mechanism underlying the BK-induced hyper-reactivity of the vagal nerve in the airway.

S24.10

β Adrenergic Modulation of the Intermolecular Signaling between a single L-type Ca^{2+} Channel and Ryanodine Receptors in Rat Heart Cells

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Signaling of β Adrenergic receptors (βAR) upregulates cardiac contractility by enhancing Ca^{2+} release from ryanodine receptors (RyRs) in the sarcoplasmic reticulum. Using the state-of-the-art loose-patch confocal imaging technique, here we have for the first time investigated the βAR modulation of the intermolecular process that a single L-type Ca^{2+} channel (LCC) activates RyR Ca^{2+} release. In the presence of 20 mM Ca^{2+} and 10 μM FPL64176 in pipette electrodes, line-scan imaging of fluo-4 fluorescence detected that Ca^{2+} sparklets from a single LCC activated Ca^{2+} sparks from RyRs in a stochastic manner. βAR agonist, isoproterenol (1 μM), increased spark amplitude by more than 60%. This effect could not be reversed by adjusting SR Ca^{2+} loads to comparable level. Rather, the latency time constant for an LCC sparklet to activate a RyR spark was shortened from 4.2 to 2.9 ms, indicating that the RyR responsivity was enhanced by βAR stimulation. We conclude that βAR signaling upregulates RyR Ca^{2+} release at least in part by direct sensitization of RyRs. This study clarified a long-debating controversy in this field, and provided intermolecular insight into the βAR regulation of heart function.

S25.1

Gaseous transmitters : Introduction and the pharmacology of inhibitors of key enzymes, nitric oxide synthase and heme oxygenase

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This presentation will start with a review on the gaseous transmitters, and the pharmacological tools that have contributed to our understanding of their roles in mammals. For the nitric oxide (NO)/NO synthase (NOS) system, NOS inhibitors and NO donors have been the major such tools. We will then focus on the carbon monoxide/heme oxygenase (CO/HO) system, and our work on the development of inhibitors selective for HO-1. We have synthesized imidazole-containing compounds which were tested for their ability to inhibit the in vitro HO activity of rat spleen microsomes (HO-1) and rat brain microsomes (HO-2). Several of these imidazole-containing compounds were selective for HO-1 as the IC50 values for HO-2 are in the order of 600 times greater than those for HO-1. In addition, these drugs have little or no effect on NOS and soluble guanylyl cyclase, in contrast to the metalloporphyrins which have been used previously to elucidate the CO/HO system. These novel drugs will be useful tools for studies on the CO/HO system, and might have useful therapeutic applications.

Key words: selective heme oxygenase-1 inhibitor, imidazole dioxolane

Supported by the Canadian Institutes for Health Research

S25.2

Carbon monoxide-releasing molecules (CO-RMs) : bioactive properties and therapeutic potentials

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Carbon monoxide (CO) is emerging as a versatile mediator of physiological processes to the extent that treatment of animals with exogenous CO gas has beneficial effects in a range of vascular- and inflammatory-related disease models. The recent discovery that certain transition metal carbonyls function as CO-releasing molecules (CO-RMs) in biological systems highlighted the potential of exploiting this and similar classes of compounds as a stratagem to deliver CO. We have succeeded in synthesizing compounds that release CO either very rapidly ("fast releasers") or with a slow kinetic ("slow releasers") and demonstrated that CO-RMs possess vasodilatory and antiinflammatory properties as well as cytoprotective activities. Most recently, we are discovering that CO-RMs can used therapeutically to maintain the integrity of organs for transplantation as they significantly improve the function of isolated kidneys preserved in cold storage solutions. Thus, CO-RMs may help to identify new cellular targets that are responsive to CO and facilitate the therapeutic delivery of this gas in a safe, measurable and controllable fashion.

S25.3

The possible role of hydrogen sulfide on the pathogenesis of renovascular hypertension in rats

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Hydrogen sulfide (H_2S) is a newly found gasotransmitter in vascular system and was involved in both the maintenance of basal blood pressure and the development of hypertension such as in spontaneous hypertension rat (SHR). This work showed that activity of cystathionine-γ lyase (CSE), a H_2S generating enzyme, in thoracic aorta and kidney, were suppressed in two-kidney-one-clip [2K-1C] renovascular hypertension rats. The plasma level of H_2S also decreased in those rats. Exogenous administration of H_2S former-NaHS could increase the plasma level of H_2S and enhance the CSE activity of aorta and kidney. Exogenous administration of H_2S also attenuated the elevation of pressure and lessened the aorta structural remodeling during the development of hypertension. The results showed that endogenous H_2S system was involved in the development of renovascular hypertension. Exogenous H_2S could exert beneficial effect on the pathogenesis of renovascular hypertension.

Key words: renovascular Hypertension; Hydrogen sulfide; cystathionine-γ lyase (CSE)

S25.4

Molecular and cellular targets of endogenous hydrogen sulfide and prospects for the future

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Cystathionine gamma-lyase (CSE) plays an important role in catalyzing endogenous production of hydrogen sulfide (H_2S). The objective of the present study was to investigate the effects of CSE/H_2S system on apoptosis-proliferation balance of different types of cells. Human aorta smooth muscle cells (HASMCs) and INS-1E cells from an insulin-secreting cell line were transfected with a recombinant defective adenovirus containing CSE gene (Ad.CSE) or treated with exogenous H_2S at physiologically relevant concentrations. Under these conditions, increased apoptosis of HASMCs and INS-1E cells was observed, both associated with p38 MAPK activation. Inhibition of p38 MAPK in INS-1E cells not only suppressed the endoplasmic reticulum (ER) stress but also decreased apoptosis induced by H_2S, suggesting that p38 MAPK activation functions upstream of ER stress to initiate the H_2S-induced apoptosis. Our results demonstrated that the CSE/H_2S system may offer a novel therapeutic target for management of apoptosis of different types of cells under various pathophysiological situations.

Key words: Gasotransmitter, H_2S, apoptosis, p38 MAP kinase.

Acknowledgement: This study has been supported by CIHR and NSERC.

S25.5

The role of NO as a neurotransmitter in the cerebral vasculature

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Neural control of smooth muscle tone affects tissue functions. We have reported that dilating transmitter derived from nerves innervating blood vesssels, penile corpus, GI tract etc. is nitric oxide (NO). In anesthetized dogs and monkeys, electrical stimulation (ES) of a pterygopalatine ganglion (PPG) dilated cerebral arteries only in the stimulated side. NO synthase inhibitors abolished the dilation. Surgical denervation at the PPG instantly constricted the cerebral artery. In rats, ES of the nerve bundles from the PPG increased the cerebral blood flow, which was inhibited by NO synthase inhibitors.

After FITC-dextran (10 kD) was systemically infused in anesthetized dogs, ES was applied to one side of the PPG. The fluorescent intensity in certain areas of the brain was higher in the stimulated side. Similar findings were histochemically obtained. T1-weighted MRI enhanced by gadolinium-DTPA during ES in the monkey showed higher signal intensities in certain brain regions in the stimulated side. These findings suggest that nitrergic nerve derived from PPG, tonically dilates the cerebral artery to maintain the cerebral circulation. Further, the nitrergic nerve seems to regulate the BBB permeability.

S25.6

Pharmacological modulation of oxidative-nitrosative stress and downstream effectors in heart failure.

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Heart failure is the major cause of hospitalization, morbidity and mortality worldwide. Experimental and clinical studies have suggested that there is an increased production of reactive oxygen species (ROS) both in animals and in patients with acute and chronic heart failure. The possible source of increased ROS in the failing myocardium include xanthine and NAD(P)H oxidoreductases, cyclooxygenase, the mitochondrial electron transport chain and activated neutrophils among many others. Dysregulation of nitric oxide (NO) synthases has also been implicated in the pathogenesis of chronic heart failure. The combination of NO and superoxide yields peroxynitrite, a reactive oxidant, which has been shown to impair cardiac function via multiple mechanisms including activation of matrix metalloproteinases (MMPs) and nuclear enzyme poly(ADP-ribose) polymerase (PARP). Recent studies have demonstrated that pharmacological inhibition of xanthine oxidase derived superoxide formation, neutralization of peroxynitrite or inhibition of PARP provide significant benefit in various forms of myocardial injury. This talk focuses on the role of nitrosative stress and downstream mechanisms in heart failure.

S26.1

Mouse metabonomics for the analysis and prediction of drug metabolism

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Objective: To study the the metabolism of drugs and other foreign compounds in vivo and to examine pharmaco- and toxicokinetics of drugs. Methods are being developed that predict human metabolism of drugs and susceptibility to chemical toxicity.

Methods: P450-null and P450-humanized mice were developed. UPLC-QTOFMS, LC/MS/MS and GC/MS were used as analytic tools to examine the metabolism of drugs in these models.

Results: Comparing P450-null with wild-type mice yields metabolic patterns that reflect the catalytic activities of specific P450 forms. The humanized mice determine the catalytic activities and regulation of P450 genes found in humans. To study metabolism, drugs or other xenobiotic chemicals were administered to mice and serum and urine examined for production of metabolites that are not found in untreated mice. These metabolites can be derived from the drug or from organ toxicities. Specific examples will be presented with dietary alkaloids and anticancer drugs.

Conclusions: By accurate mass determination using UPLC-QTOFMS, pathways of metabolism of drugs can be rapidly assessed and data extrapolated to humans.

Key words: drug metabolism, metabonomics, P450s, UPLC-QTOFMS

S26.2

Regulation of Cytochrome P_{450} gene expression and activity

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Major cytochrome P_{450} (CYP), drug metabolism enzymes and transporters are regulated by nuclear receptors: aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR) and pregnane X receptor (PXR). These receptors establish cross-talk with other signalling pathways. For example, CAR and PXR are regulated by the glucocorticoid receptor (GR) and the hypothesis of a GR-[CAR/PXR]-CYP cascade has been proposed (Pascussi et al. Mol Pharmacol 2000; Mol Endocrinol 2003). Consistent with this hypothesis, we showed that the repressive effect of interleukin-1β on drug metabolism is linked to the inhibitory effect of NFkB on GR transcriptional activity (Assenat et al. Hepatology 2004). Another important cross-talk concerns the vitamin D receptor (VDR): VDR binds to and transactivates PXR/CAR responsive elements of CYP2B6/2C9/3A4 (Drocourt et al. J Biol Chem 2002), while PXR activates VDR-target genes including CYP24 encoding a mitochondrial P450 involved in catabolism of vitamin D (Pascussi et al. J Clin Invest 2005). Other examples will be presented. These cross-talks provide new views for understanding how physiopathological stimuli affect drug metabolism and how drugs might excert adverse effects.

S26.3

P_{450} and carcinogenesis

Alan R Boobis, Section of Experimental and Toxicology, Division of Medicine, Hammersmith Campus, Imperial College London, Ducane Road, London W12 0NN, UK

P_{450} is known to activate many chemical carcinogens into their ultimate DNA reactive metabolites. Differences in P_{450} expression and specificity can explain many species and tissue differences in carcinogen susceptibility. However, evidence that genetic differences in P_{450} enzymes in humans explain differences in cancer susceptibility is much less convincing. P_{450} may play a role in the carcinogenicity of some chemicals acting by a non-genotoxic mechanism. The mitogenic effects of compounds such as phenobarbital result in hepatocarcinogenicity in rodents. The CAR receptor is involved in this response, but the possible role of induced CYP2B enzymes remains to be determined. Certain forms of P_{450}, e.g. CYP1B1, are over-expressed in tumours. This has led to interest in their diagnostic and therapeutic potential, for example in the activation of prodrugs. Some P_{450} enzymes metabolise endogenous compounds, such as hormones and prostanoids. There is evidence that some of these pathways play a key role in certain types of cancer, for example those that are estrogen-dependent. Understanding the specificity and regulation of P_{450} therefore has implications for many different aspects of carcinogenesis.

S26.4

Therapeutic Implications of Cytochrome P_{450} Polymorphism and Expression

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Polymorphism of genes encoding drug metabolising enzymes is a major known genetic cause for the interindividual variability in drug toxicity and response. Based on the occurrence of mutations in these genes, gene deletions and gene duplications, the populations can be divided into poor (PM), intermediate (IM), efficient (EM) or ultrarapid (UM) metabolisers. A major role of this polymorphism is seen for individual susceptibility to drug toxicity, where subjects lacking a particular enzyme can achieve too high plasma levels at ordinary dosage, and with respect to non response of drug treatment where UMs are overrepresented. An increasing amount of literature indicates that the genetic variability of cytochrome P450 enzymes influences the therapeutic outcome of treatment of HIV, cancer, depression, ulcer, psychosis, cardiovascular disorders, epilepsy and pain. The number of important CYP variants identified increases and recently a common CYP2C19 gene causing increased drug metabolism was identified in our laboratory. The lecture will give a state of the art view of the field today with illustrations from the clinical perspective.

S27.1

ENDOCANNABINOIDS AND THE CONTROL OF ENERGY HOMEOSTASIS

George Kunos, Douglas Osei-Hyiaman, Lei Wang, Jie Liu, Pal Pacher, Sandor Batkai and Svetlana Radaeva; NIAAA, National Institutes of Health, Bethesda, MD 20892, USA

The endocannabinoid system has emerged as a regulator of energy homeostasis. Studies in our laboratory, using leptin-deficient obese rodents and CB1 cannabinoid receptor (CB1)-deficient mice, indicated that endocannabinoids acting via CB1 mediate the hunger-induced increase in food intake and are negatively regulated by leptin in brain areas of appetite control, including the hypothalamus, limbic forebrain and amygdala. CB1-/- mice are lean and resistant to diet-induced obesity (DIO) despite similar energy intake to wild-type mice with DIO, suggesting that CB1 regulates body weight through additional targets, such as adipose tissue and liver. Endocannabinoids and CB1 are present in the liver and are upregulated in DIO. CB1 stimulation increases de novo hepatic lipogenesis through activation of the fatty acid biosynthetic pathway, whereas CB1 blockade inhibits lipogenesis and increases fatty acid βoxidation. In the hypothalamus, the fatty acid synthetic pathway has been implicated in the regulation of appetite, and may thus represent a common molecular target for the central appetitive and peripheral metabolic effects of endocannabinoids.

Key words: cannabinoid, appetite, fat metabolism, obesity

S27.2

Endocannabinoid modulation of pain perception

Billy R. Martin, Aron H. Lichtman, and M. Imad Damaj; Department of Pharmacology and Toxicology; Virginia Commonwealth University; Richmond, VA, USA

The endocannabinoid system is comprised of two receptor subtypes, two major endocannabinoids, along with synthetic and metabolic enzymes for the endocannabinoids. One of the many actions of the endocannabinoid system is to control pain perception. It has long been recognized that cannabinoid agonists, such as tetrahydrocannabinol, produce analgesia in a wide range of pain models in laboratory animals and humans. The major analgesic effects of tetrahydrocannabinol result from its activation of CB1 cannabinoid receptors. Recently, it has been shown that activation of the CB2 cannabinoid receptor will also produce analgesia and well as reduce inflammation. Selective CB2 agonists have been developed that produce analgesia in formalin- and caraageenan-induced pain models in mice as well as in selected neuropathic pain models. In addition, administration of endocannabinoids to laboratory animals produce analgesia. Elevation of the endocannabinoid anandamide through genetic deletion of its primary catabolic enzyme, fatty acid amide hydrolase, produces a CB1-mediated analgesia.

Key words: endocannabinoids, pain, analgesia

Acknowledgment: This research was supported by NIH Grant DA 03672.

S27.3

Role of Endocannbinoids in Drug Addiction

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The presence of the endogenous cannabinoid system in reward circuits and its role in motivational and emotional homeostasis suggests that they might be involved in drug addiction. Animal models of drug reward provide evidence that the endogenous cannabinoids do have a role in the rewarding effect of several addictive drugs such as opioids, cocaine, alcohol, nicotine and psychostimulants. The pharmacological management of endocannbinoid signalling not only blocks the direct reinforcing effects of opioids, nicotine and ethanol, but also prevents relapse to the various drugs of abuse, including opioid, alcohol, cocaine and metamphetamine. Finally, as recently demonstrated, Delta-9 tetrahydrocannabinol and the non psychoactive cannabidiol at low doses potentiated the extiction of cocaine and amphetamine place preference. This could be a new strategy to accelerate extinction, thus potentially reducing the likelihood of relapse. The efficacy of cannabinoid drugs in treating drug addiction, a disorder that still lacks effective therapeutic approaches, will be discussed.

S27.4

The Endocannabinoid System in Neuroprotection

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In the 1990's we identified 2 major endogenous cannabinoids - anandamide and 2-arachidonoyl glycerol (2-AG). We shall present some of our results related to their neuroprotective properties.

Traumatic brain injury leads to secondary events that include the release of harmful mediators, as well as to the promotion of neuroprotective mechanisms. Using a mouse model of closed head injury (CHI) we have shown that the levels of 2-AG are elevated in the brain after CHI. This is apparently a neuroprotective effect, as administration of 2-AG reduced brain edema, decreased infarct volume, partly preserved the blood brain barrier and protected hippocampal cells. The mechanism of action also involved inhibition of the inflammatory response. 2-AG inhibited the acute expression of proinflammatory cytokines, TNF-α, IL-1β and IL-6. 2-AG abolished the increase of the transcription factor NF-κB transactivation. The association of these effects with the endocannabinoid system was indicated by work with CB1 knock out mice that showed minor spontaneous recovery after 24 hours, in contrast with wild type mice. These mice also failed to respond to treatment with exogenous 2-AG.

S27.5

Metabotropic glutamate receptors and endocannabinoids in post-ischemic hippocampal neuronal death

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We examined whether agents acting on the endocannabinoid receptor CB1 could play a role in the mechanisms of mGlu1-mediated neuroprotection. We used organotypic rat hippocampal slices exposed to 30 min oxygen-glucose deprivation (OGD), which promotes CA1 injury 24 h later. When present in the incubation medium, the CB1 receptor agonist WIN 55212-2 exacerbated CA1 injury induced by a 20 min, sublethal period of OGD. Conversely, incubation with the CB1 receptor antagonist AM251 significantly attenuated 30 min OGD injury. The CB1 receptor agonist WIN 55212-2, but not AM251, significantly reverted the neuroprotective effects of the mGlu1 receptor antagonist LY367385. On the other hand, AM251, but not WIN 55212-2, was able to revert the neurotoxic effects of the mGlu1 agonist DHPG. Finally, WIN 55212-2 reduced the increase in the hippocampal output of GABA evoked by LY367385 in freely moving gerbils. Our results suggest that in CA1 the release of GABA contributes to the attenuation of OGD injury induced by mGlu1 receptor antagonists and that endocannabinoid receptors are involved in mediating the GABAergic effects of mGlu1 receptors.

Key words: mGlu receptors, CB1 receptors, neuroprotection, oxygen-gluc

S27.6

The endocannabinoid (EC) system and the potential for its therapeutic use

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N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol are the best studied endocannabinoids (ECs). Together with proteins catalyzing their biosynthesis and inactivation, which have been characterized to a large extent, and the two cannabinoid CB1 and CB2 receptors, which they activate, ECs form the EC system. A picture is now emerging suggesting that the EC system is transiently activated only "when and where needed" to afford protection against cell excitotoxicity, damage and malignant transformation, or to mitigate pain, inflammation and other stressful conditions that acutely or chronically affect mammals and humans. When this happens, selective inhibitors of EC inactivation might be used with a protective function. On the other hand, during certain chronic conditions, the temporal and/or spatial selectivity of EC action is lost, thus contributing to the symptoms of the disorders. In these cases, cannabinoid receptor antagonists or selective inhibitors of EC biosynthesis might be exploited therapeutically. Examples of animal models of central and peripheral disorders where the pharmacological manipulation of EC levels and actions can be used with therapeutic purpose will be described.

S28.1

Signaling through stress-activated ASK1 JNK/p38 pathways and their therapeutic implications for human diseases

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein (MAP) kinase kinase kinase family, which activates both the MKK4/MKK7-JNK and MKK3/MKK6-p38 MAP kinase pathways. ASK1-JNK/p38 cascade constitutes an important signaling pathway in various types of stress-induced apoptosis. We have shown by deletion of Ask1 gene in mice that ASK1 plays pivotal roles in oxidative stress- and endoplasmic reticulum (ER) stress-induced apoptosis. These stresses are closely linked to various physiological phenomena in the control of cell fate, and the resultant apoptosis is implicated in the pathophysiology of a broad range of human diseases. Moreover, ASK1-p38 pathway was recently found to play important roles in the innate immune responses. In this symposium, I will review our recent findings on the pathophysiological roles of ASK family proteins in stress responses.

S28.2

Rho-Kinase Is an Important Therapeutic Target in Cardiovascular Medicine

Hiroaki Shimokawa, Department of Cardiovascular Medicine, Tohoku University Graduate Schod of Medicine, Sendai, Japan.

Rho-kinase has been identified as one of the effectors of the small GTP-binding protein Rho. Accumulating evidence has demonstrated that Rho/Rho-kinase pathway plays an important role in various cellular functions, not only in vascular smooth muscle cell (VSMC) contraction but also in actin cytoskeleton organization, cell adhesion and motility, cytokinesis, and gene expressions, all of which may be involved in the pathogenesis of cardiovascular disease. At molecular level, Rho-kinase up-regulates various molecules that accelerate inflammation/oxidative stress, thrombus formation, and fibrosis, while it down-regulates endothelial nitric oxide synthase. The expression of Rho-kinase itself is mediated by protein kinase C/NF-κB pathway with an inhibitory and stimulatory modulation by estrogen and nicotine, respectively. At cellular level, Rho-kinase mediates VSMC hypercontraction, stimulates VSMC proliferation and migration, and enhances inflammatory cell motility. In animal studies, Rho-kinase has been shown to be substantially involved in the pathogenesis of vasospasm, arteriosclerosis, ischemia/reperfusion injury, hypertension, pulmonary hypertension, stroke and heart failure, and to enhance central sympathetic nerve activity. Finally, in clinical studies, fasudil, a Rho-kinase inhibitor, is effective for the treatment of a wide range of cardiovascular disease, including cerebral and coronary vasospasm, angina, hypertension, pulmonary hypertension, and heart failure, with a reasonable safety. Thus, Rho-kinase is an important therapeutic target in cardiovascular medicine.

S28.3

Targeting protein-protein interactions in signaling pathways for therapeutic interventions

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Protein-protein interactions are critical for mediating signal transduction pathways, such as cell survival signaling, under physiological and pathological conditions. Targeting such interactions have been a challenge for small molecule discovery. 14-3-3 is a phosphoserine/threonine binding protein and has been implicated in regulating diverse cellular processes in normal cells and cancer. For example, overexpression of 14-3-3 has been correlated with poor survival of cancer patients. Thus, targeting 14-3-3 proteins may lead to the development of a novel class of anticancer agents. The 14-3-3/client protein interaction will be used as a model system to address strategies for the development of protein-protein interaction antagonists.

S28.4

Recent advances in estrogen-signaling and new sites for pharmacological interventions

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Both in vitro and in vivo, ERβ acts as an antiproliferative principle in several tissues, e g the prostate where ERβ is antiproliferative, proapoptotic and prodifferentiative. An ERβ agonist developed by Eli Lilly shows all of the anticipated biological effects on the prostate, namely reduced cellular proliferation and increased apoptosis, leading to a diminished size of both mouse and rat prostate. This opens up hitherto unthought-of pharmaceutical possibilities in treating prostate disorders in humans, including both benign prostatic hyperplasia and prostate carcinoma.

It now appears quite clear that ERβ also exerts an antiproliferative effect on human breast cancer cells both in vitro and in vivo; there seems to be sufficient indications for a pharmaceutical potential of ERβ agonists in treatment of breast cancer. A similar case can be made for use of ERβ agonists in treatment of colon cancer. Furthermore, ERβ is of major importance in estrogenic regulation of the immune system. Wyeth-Ayerst has reported extremely encouraging results of ERβ agonists in treatment of rodent models of inflammatory bowel disease (IBD), rheumatoid arthritis and endometriosis.

S28.5

A novel antiarrhythmic target: M3R/IKM3

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This study was designed to explore the possible role of M3 subtype of acetylcholine muscarinic receptors (M3-mAChR) in cytoprotection of myocardial infarction. Studies were performed in a rat model of myocardial infarction and in isolated myocytes. We found that choline diminished ventricular arrhythmias during ischemia, which was achieved by correcting hemodynamic impairment, and protecting cardiomyocytes from apoptotic death. The beneficial effects of choline were reversed by the M3-selective antagonists but not by the M2-selective antagonist. Choline/M3-mAChR activated several survival signaling molecules (anti-apoptotic proteins Bcl-2 and ERKs), increased endogenous antioxidant reserve (SOD), and reduced apoptotic mediators (proapoptotic proteins Fas and p38 MAPK) and intracellular Ca^{2+} overload. In addition, we also found that administration of choline attenuated the ischemia-induced suppression of the association between connexin 43 and M3-mAChR. We concluded that choline reduced ischemic arrhythmias via stimulating the cardiac M3-mAChRs which in turn result in alterations of multiple signaling pathways.

Key words: acetylcholine muscarinic receptors; arrhythmia; choline; signaling pathways.

Lecture.3

The IUPHAR Lecture in Analytical Pharmacology: From Systems to Target to Systems: Can we Keep Caprice out of Pharmacological Numbers

Terry P. Kenakin GlaxoSmithKline Reseach and Development; Research Traingle Park, NC USA

From its 'inception, receptor theory has been used in attempts to quantify drug activity in a system independent manner in attempts to predict therapeutic activity from data obtained in test systems. This lecture will highlight key findings from various authors that have progressed these ideas to the present day state of the art. Broadly speaking, agonism can be described by the Operational model while antagonism can be discussed either through orthosteric or allosteric models. This presentation will focus on the 'prescience' of Operational theory in predicting agonist activity in systems, the impact of kinetics on observed orthosteric antagonism in different experimental assay formats, and the ability of allosteric theory to put numbers to ligand interactions with shapeshifting proteins. This latter idea will be discussed in relation to newly discovered CCR5 HIV entry inhibitors and the concept of GPCR target-salvage. The influence of receptor/G-protein pleiotropism, ligand-selective receptor active states and collateral efficacy also will be considered. Finally, a look to the future of receptor theory in drug discovery will be made.

L10

Targeting adenosine receptors

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There are four G-protein-coupled adenosine receptors denoted A1, A2A, A2B and A3. They are blocked by caffeine, the most widely used of all psychoactive drugs. Targeted deletions of the A1 and A2A receptors have revealed physiological, and especially pathophysiological, roles of these adenosine receptors. Deletion of A1 receptors slightly increases blood pressure and heart rate, as well as urinary excretion, but the tubuloglomerlular feedback is eliminated. Importantly, ischemic preconditioning in heart and kidney is defective or absent. Regulation of insulin secretion is compromised. These mice are more sensitive to painful stimuli. Neurodegeneration after epileptic seizures is aggravated in A1 KO mice, whereas tolerance to cerebral ischemia is essentially unaltered. Mice with a targeted deletion of A2A receptors have an altered immune response and have an increased platelet aggregation. A2A KO mice are not aroused by caffeine, but show an increased tolerance to neurodegeneration, including loss of dopamine neurons characteristic of Parkinson's disease. These findings suggest that one could develop novel drugs that target adenosine receptors.

Key words: Adenosine, caffeine, neurodegeneration, diabetes.

L11

Regulation of hypoxia-inducible factor, cell respiration and NO

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Physiological concentrations of nitric oxide (NO) inhibit cytochrome c oxidase reversibly and in competition with oxygen. Thus NO causes a type of "metabolic hypoxia" in which oxygen is present but cannot be used for mitochondrial respiration. We have investigated the effects of hypoxia and exposure to NO on the transcription factor hypoxia-inducible factor-1 (HIF) , whose alpha subunit becomes stabilised as the oxygen concentration decreases, resulting in the expression of HIF-dependent target genes involved in glycolysis and angiogenesis. We have found that low concentrations of NO (< 400 nM) cause a rapid decrease in HIF-1alpha stabilised by exposure of the cells to 3 % O_2. This prevention of HIF-1alpha stabilisation, which is shared by other inhibitors of mitochondrial respiration, is due to increased prolyl hydroxylase-dependent degradation of HIF-1alpha. Furthermore, inhibition of mitochondrial respiration in hypoxia results in redistribution of oxygen towards non-respiratory oxygen-dependent targets, such as prolyl hydroxylases, so that they do not register hypoxia. Thus, the signalling consequences of hypoxia are profoundly modified by NO.

L12

Prostanoid Receptors : From Physiology, Molecular Biology to Translational Research

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Prostanoids including various prostaglandins (PGs) and thromboxanes (TXs) act on cell surface receptors to maintain local homeostasis. We cloned cDNAs for all of the eight types and subtypes of prostanoid receptors, including PGD receptor、four subtypes of PGE receptor (EP1、EP2、EP3 and EP4) , PGF receptor, PGI receptor and TXA receptor. We then generated KO mice deficient in each of these receptors individually, and provided a set of receptor cDNAs to a pharmaceutical company for development of agonists and antagonists selective to each receptor. Using these KO mice and selective compounds developed, we examined roles of individual receptor in various physiological and pathophysiological conditions. Our analysis has not only identified the types of prostanoid receptors working in processes known to be inhibited by NSAIDs such as fever generation, but also revealed new functions of prostanoids that had not been expected from the NSAID effects. These findings have been explcited for clinical application of the agonists and antagonists. One examples ia a phase II study for application of an EP4 agonist in ulcerative colitis patients now being carried out as a translational research.

L13

Endothelial Dysfunction and Vascular Disease

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The endothelium mediates relaxations or contractions of the underlying smooth muscle. The best characterized endothelium-derived relaxing factor (EDRF) is nitric oxide (NO) . NO is formed by the constitutive NO synthase of the endothelial cells. The relaxations evoked by NO are due to the stimulation of soluble guanylate cyclase and the resulting accumulation of cyclic GMP. The endothelial cells endothelium-derived hyperpolarizing factor (EDHF) that causes hyperpolarization of the smooth muscle. The release of EDRF from the endothelium can be mediated by both pertussis toxin-sensitive (alpha2-adrenergic activation, serotonin, aggregating platelets) and insensitive (adenosine diphosphate, bradykinin) G-proteins. In blood vessels with regenerated endothelium, and/or atherosclerosis, there is a selective loss of the pertussis-toxin sensitive mechanisms of EDRF-release which favors the occurence of vasospasm, thrombosis and cellular growth. The endothelial cells also produce endothelium-derived contracting factors (EDCFs) which include superoxide anions, endoperoxides, thromboxane A2 and endothelin-1. The release of EDCFs is maintained or even augmented in hypertension and diabetes.

L14

Retinoid Pharmacology : Cell Growth, Differentiation and Cancer

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Retincids, retinol (vitamin A) , and related metabolites such as retinoic acid (RA) serve as cancer chemopreventive and chemotherapeutic agents by regulating cell growth and differentiation. The actions of retinoids are primarily mediated by two different families of nuclear RA receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) . RARs and RXRs act as transcription factors. Pharmacologic doses of RA are used to treat several types of cancer. Conversely, vitamin A deficiency increases the incidence of carcinogenesis. The esterification of retinol in the intestine, liver, and lung is catalyzed by the enzyme lecithin: retinol acyltransferase (LRAT) . We have generated an LRAT knock-out mouse strain in which less than 0.2 % of the retinyl esters in wild type mice are detected. These LRAT-/- mice rapidly become vitamin A-deficient and have many advantages over WT mice in studes of vitamin A deficiency. Additionally, we have shown that loss of LRAT expression is associated with invasive bladder cancer. Retinoids induce the differentiation of several types of stem cells, including embryonic stem cells. Rex-1 (Zfp-42) , a zinc finger family transcription factor which is highly expressed in em

L15

Aquaporin water channels : from atomic structure to clinical medicine

Peter Agre, Duke University, Durham, North Carolina USA

Aquaporin (AQP) water channel proteins enable high water permeability of certain biological membranes. Discovered in human red cells but expressed in multiple tissues, AQP1 has been thoroughly characterized and its atomic structure is known. Expression patterns of the thirteen known human homologs predict phenotype. Individuals lacking Colton blood group antigens have mutations in AQP1. In people with no AQP1, lack of water causes defective urine concentration and reduced fluid exchange between capillary and interstitium in lung. Mutations in AQP0, expressed in lens fiber cells, result in familial cataracts. Mutations in AQP2, expressed in renal collecting duct principal cells, result in nephrogenic diabetes insipidus. AQP2 underexpression is found in disorders with reduced urinary concentration, AQP2 overexpression in those with fluid retention. Mistargeting of AQP5, normally expressed in the apical membranes of salivary and lacrimal gland acini, can occur in Sjogren's syndrome. Aquaporins also are implicated in brain edema and muscular dystrophy (AQP4) , anhidrosis (AQP5) , renal tubular acidosis (AQP6) , conversion of glycerol to glucose during starvation (AQP7 and AQP9) and cystic fibrosis (several) .

L23

The nootropic effect of (-) clausenamide and ginsenoside Rg1 is characterized by increasing neural plasticity

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Clausenamide was isolated from the leaves of Clausena lansium Lour. Skells. It possesses four chiral center containing 16 enantiomers which have been synthesized by chemists of our institutes. After pharmacological screening, a pair of anantiomer (-) and (+) clausenamide were selected for further study. In more than 10 models of memory impairment including APP transgenic mice, aged (24-27 months) rats, diabetic mice, (-) clausenamide but not (+) clausenamide was shown to improve cognitive impairment significantly. For elucidating its mechanism, we found that firstly, (-) clausenamide increased intracellular calcium concentration about two times which show beneficial actions on CNS. Secondly, (-) clausenamide increased acetylcholine content and Ach release from synaptosome. Thirdly, (-) clausenamide increased synapses density and mossy fiber sprouting in hippocampus of adult rats and weaning mice. Fourthly, (-) clausenamide enhanced Zif/268 mRNA and protein expression. According our studies, the nootropic signal transduction pathway of (-) clausenamide is as follows:

(-) clausenamide increase intracellular Ca^{2+} → Adenylate cyclase activation → cAMP ↑ → PKC ↑ → CREB phosphorylation → Zif/268 expression and protein synthesis ↑ → facilitation of memory and LTP.

Ginsenoside Rg1 is the main active principle of ginseng which shears many pharmacological activities of ginseng. With behavioral and electrophysiological tests, results showed that Rg1 has anti-amnestic effect and could improve all stages (registration, consolidation and retrieval of memory) of memory. Its mechanism is similar to that of (-) clausenamide, importantly, Rg1 has also stimulating effect on neural stem cells, i.e. Rg1 increased hippocampal neurogenesis in adult rodent brain whether in vitro or in vivo, under physiological condition or pathological condition. This new finding suggests that Rg1 is a promising agent for treatment of stroke, Alzermer's disease and various memory impairments.

S29.1

Permeability transition pore, AQP8 and mitochondrial water transport

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Although movement of water into and out of the mitochondrion is central for its shape and activity the molecular pathways of mitochondrial water transport remain mostly elusive. By stopped flow light scattering we found striking high water permeability of isolated rat liver mitochondria and low activation energy characterizing the related osmotic transport. Experiments with mitochondria using cyclosporin A (CsA), an inhibitor of the opening of the permeability transition pore (PTP) acting as a mitochondrial coordinator of pro-apoptosis, and Hg++, an ion blocking AQP8, the aquaporin water channel located in the inner mitochondrial membrane, indicated major roles for PTP and AQP8 in mediating the mitochondrial water transport. Targeting of these two water conductive pathways may be instrumental to act on the mitochondrial volume, a function that could be used to modulate cell death in an innovative therapeutic perspective.

Key words: Mitochondria, apoptosis, PTP, aquaporin.

Acknowledgements: funding from Italian PRIN and CEGBA is gratefully acknowledged.

S29.2

Epithelial fluid transport and aquaporins: an evolving relationship. Evidence for the paracellular route in corneal endothelium

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Objective: How epithelia transport fluid remains unsolved and controverted. We investigate this issue. Methods: We use electrophysiology, optical microscopy, and determinations of fluid movement with a nanoinjector device and of cell volume by light scattering. We work with rabbit corneal endothelium in vitro, and with cultured endothelial cells from wild-type and AQP1 null mice. Results: Transtissue electrical currents generate fluid movements. The direction of the fluid movement is reversed by current reversal or by changing tight junctional electrical charges by the polycation polylysine. These effects require junctional integrity. AQP1 null mice cells display diminished osmotic permeability and regulatory volume decrease (60% of control) but normal fluid transport. A mathematical model of corneal endothelium predicts observed experimental results only when based on paracellular electro-osmosis. A separate mathematical model of the junction accounts for electro-osmosis. Conclusions: We propose a novel paradigm in which fluid is transported via the paracellular route by electro-osmotic coupling at the junctions. AQP1 has a role in regulation but not as a significant water pathway.

Support: NIH.

S29.3

AQP2 binding proteins regulate intracellular trafficking of AQP2

Sei Sasaki; Department of Nephrology, Graduate School, Tokyo Medical and Dental University

Aquaporin-2 (AQP2) is the kidney collecting duct water channel and its gene mutations cause nephrogenic diabetes insipidus manifested by polydipsia and polyuria. Regulation of water reabsorption in the collecting duct is critically important in body water homeostasis and this is handled by trafficking of AQP2 to and from the apical membrane. Although trafficking of AQP2 is known to be dependent on cAMP-mediated and other signaling cascades, further molecular mechanisms remain largely unknown. We decided to isolate proteins that directly bind to AQP2 and regulate its trafficking. We isolated 2 proteins using different methods; SPA-1, a GTPase-activating protein (GAP) for Rap1, and cytoskeletal protein actin. A large scale proteomic analysis of rat renal medulla extract identified further 11 binding proteins, and most of them have ability to interact with actin. We speculate that these proteins make a multiprotein complex and spatial and temporal analysis of the complex will be important to understand the trafficking of AQP2.

Key words; aquaporin, AQP, kidney, urine concentration

S29.4

AQP1 as a new target for anti-cancer drug discovery

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Our present study has proved that 2 carbonic anhydrase inhibitors (CAI) could significantly inhibit the expression of AQP1 in vivo and in vitro and inhibit the function of water transportation across the cell membrane in the RBC and HEK293 cells which transfected AQP1 cDNA. We also observed that the CAIs could depress the tumor metastasis and diminish the angiogenesis in the tumor tissues. In addition, we reconfirmed our previous result by using SPR recently. The result indicated that CAI could directly interact with AQP1 immobilized on the CM5 chip. Further more, we fund that the interaction of AQP1 with MHC maybe involve in the effects of CAI via the change of AQP1 conformation or/and location seemly liking AQP2 regulation by actin. Collaborated with chemists, we designed and synthesized about 100 new compounds that modified from the chemical structure of CAI and could dock with AQP1 protein. The primary pharmacological study indicated that there were 3 chemicals could significantly inhibit tumor metastasis, angiogenesis and water transportation mediated by AQP1, among them the XJ-6-A is the most potential compound. XJ-6-A might be as a specific inhibitor of AQP1 for the future development.

S30.1

CELLULAR COFACTORS IN THE REPLICATION OF HIV-1.

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Primate lentiviruses such as HIV-1 have evolved the ability to persist within the infected individual in the face of immune surveillance and potent antiretroviral pressure. As a virus with a limited genetic repertoire, it is not surprising that the replication of HIV-1 depends upon the ability of the virus to usurp cellular functions in order to complete certain aspects of its replication cycle. It also apparent that some cellular factors can inhibit viral replication thereby requiring that the virus evolve sophisticated defense mechanisms to counteract them. Our laboratory has been characterizing cellular factors that facilitate viral replication and we have identified components of the inner nuclear envelope that play an important role in the ability of the virus to engage chromatin and to undergo productive infection of a cell. Understanding how these cellular cofactors operate is important in order to devise novel strategies to counteract HIV-1 infection and AIDS.

S30.2

Macrophage Nanoparticle Delivery System for Anti-retroviral Medicines: Treatment of HIV-1 Infection in the Nervous System & Other Viral Reservoirs

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Cell-based delivery of nanoformulated drugs can improve drug bioavailability, pharmacokinetics and diminish secondary side effects. As mononuclear phagocytes (MP) serve as HIV-1 reservoirs and traffic to sites of infection we tested a nanoformulation (NP) of Indinavir (IDV) for therapeutic efficacy. NP-IDV were loaded in mouse bone marrow macrophage (BMM). Electron microscopy and RP-HPLC confirmed NP tissue uptake in a humanized rodent model of HIV-1 infection. After adoptive transfer to naive mice MP distribution and HIV-1 p24 antigen were assessed by SPECT, T2 MRI, and immunohistology. Seventy five percent BMM contained NP-IDV in vesicles. A single dose of NP-IDV loaded BMM provided plasma IDV levels of 156 and 221 μg/ml on days 7 and 10. Tissue distribution of IDV paralleled that of labeled MP. Survival of CD4+ T cells and anti-retroviral responses in NP-IDV treated mice exceeded 50% that of untreated controls. A single dose of NP-IDV BMM provides sustained drug levels and anti-retroviral activities without toxicity.

S30.3

Inhibitors of Human Immunodeficiency Virus Type I Integration: Predinical Discovery to Clinical Validation

Daria J. Hazuda, Virus and Cell Biology, Merck Research Labs; West Point PA 19486

The virally encoded enzyme integrase plays a critical role in HIV-1 replication and has long been considered a promising target for the development agents to treat HIV-1 infection. However, it is only recently that the efficacy of integrase inhibitors has been demonstrated in experimental animal model systems of retroviral infection and in HIV-1 infected subjects. MK-0518 is the most advancedof the clinical candidates in this new class. MK-0518 has demonstrated robust efficacy in short term monotherapy studies and in phase 2 combinations studies in patients with multi-class resistance. Although the first inhibitors in this new class of antiretroviral agents are in the earliest stages of clinical development, the study of integrase function and inhibitor mechanism as well as recent insights on resistance derived from in vitro analyses have played an important role in the drug discovery and development process for these inhibitors. This presentation will review the role of integrase in HIV-1 infection, the mechanism of integrase inhibitors and the results of resistance studies on preclinical compounds with an emphasis on the discovery path that led to the identificaiton of MK-0518.

S30.4

HIV drug resistance : viral strategies for treatment escape

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In order to ensure escape from the intense pharmacological pressure exerted by HAART, HIV must fulfill three main requirements:

1. Ensure HIV resistance per se, through mutations that promote structural and functional changes in the viral proteins that are targeted by antiretroviral drugs. Resistance mutations generally accumulate gradually along with viral escape, starting with low levels of resistance and evolving progressively toward levels that are relevant to the concentrations of drugs found in the most drug-permeable infected tissues in vivo. These mutations often vary from one drug to another and promote resistance through a variety of mechanisms.

2. Preserve viral "fitness" or replicative capacity, the frequently observed replicative cost of resistance mutations. Because they modfy the properties of viral proteins and most notably the catalytic efficiency of viral enzymes, most resistance mutations have a negative impact on HIV replication. HIV, however, has developed a number of compensatory mechanisms to limit resistance-associated loss of viral fitness, some of which are still poorly understood and can involve regions of the HIV genome that are distinct from those directly targeted by the drugs. Although it is well established that resistant viruses are clearly less fit in vivo than their wild-type parental counterparts, the consequences of reduced viral fitness on HIV pathogenicity still need to be fully evaluated.

3. Preserve viral diversity, an essential property of viral populations observed in treated patients. Resistance evolves through the constant coevolution of multiple viral species bearing different genotypes and phenotypes, which can act as a reservoir from where new genotypes can be recuited depending on the pharmacological pressure. Furthermore, genetic recombination, a remarkable property of retroviruses, ensures that following the strong bottlenecks that often accompany pharmacological selection of resistant viral species, HIV can rapidly reconstitute a full and vital diversity in regions of its genome that are not directly subjected to this pressure.

This work was supported by grants from ANRS and European Union FP5.

S30.5

Inhibitors of virus-cell membrane fusion

Ralf Altmeyer; HKU-Pasteur Research Centre

In 2003 the first virus entry inhibitor, the anti-HIV peptide T20 (Fuzeon enfuvirtide entered the arena of approved drugs for treatment of Human Immunodeficiency Virus type 1 infection. T20 is an unconventional antiviral drug as it does not target a viral replicase or protease but a conformational transition within the HIV1 fusion protein gp41 required for virus-cell membrane fusion. Major advances have been made over the past decade in the understanding of the molecular mechanism of HIV entry into target cells, from the identification of co-receptors to conformational transitions of envelope proteins gp120/41. The understanding of these molecular and cellular mechanisms have paved the way to the design of novel molecules that target gp120 attachment to the CD4 or CCR/CXCR4 (co)-receptors, conformational changes in the envelope or downregulation of the receptors. Recent developments in the identification of novel targets and drugs during the HIV entry process will be presented.

S32.1

Medicinal plants of India

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Traditional systems of medicine all over the world have been using plants and plants products for therapeutic purposes. India has a rich flora of medicinal plants that are potential sources of biologically active substances. Work carried out to establish the scientific basis of use of plants in various disorders including diabetes mellitus where it has been shown that Momordica charantia, Eugenia jambolana, Gymena sylvestre and Terminalia bellarica fruit are effective in controlling glucose levels and complications. Hibiscus flowers, Piper longum, Embelia ribes have been found to be effective oral contraceptives in females. Nutmeg, Ocimum sanctum are in use as oral contraceptives in males. O. sanctum has been found to be an adaptogen and Benincasa hispida juice is traditionally used to treat mercury poisoning, though present day work has shown it to be effective as a deaddicting agent. A wide variety of plants are currently under research.

Key words: Medicinal, plants, India.

Acknowledgements: I would like to thank my postgraduates who have helped me carry out this research.

S32.2

Modern pharmacological study of traditional Chinese medicinal prescription

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Luiwei Dihuang decoction (LW) is a classical "Kidney Yin-nourishing" traditional Chinese medicinal prescription. In this research, the effect of LW was studied from the angle of neuroendocrine immunomodulation (NIM) network. Learning behavioral tests, radio-immune assay and various immune experiments were used in the study. The results showed that LW significantly improved the learning behaviors in senescence-accelerated mice (SAM) and the cognitive-enhancing effect of LW was related to modulating brain monoamine transmitters, restoring the balance of hypothalamus-pituitary-adrenal axis and facilitating the induction of long-term potentiation (LTP). LW significantly promoted the secretion of testosterone by primary cultured testes cells in SAM and modulated the balance of hypothalamus-pituitary-ovary axis. Oral administration of LW significantly improved the immune functions in immunodeficient model animals. It also restored the disordered immune balances in autoimmune mice. Theses results suggested that modulation of NIM network is the main effect of LW, which may account for its effect of "Kidney-nourishing effect" in traditional Chinese medicine.

S32.3

Pharmacological study of traditional Brazilian medicine

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Brazil possesses about 20-22% of the world's biodiversity. Despite the great interest of the Brazilian population in traditional medicine, until recently, few medicinal plants have been studied scientifically. In spite of governmental initiatives, most medicinal species with their traditional knowledge, especially those derived from indigenous populations, are disappearing. A search on the Web of Science data base reveals that few areas of basic research in Brazil have progressed as rapidly as plant articles published in international journals over the last 25 years. Brazil has published 42% of all Latin American articles in this field. A great effort towards training of specialized personnel has been carried out in several areas related to phytomedicine in Brazil, notably in organic chemistry, preclinical and clinical pharmacology, and pharmaceutical sciences. In 1995, the MInistry of Health established general guiddelines for the registration of phytomedicine with scientific proof of safety, efficacy and quality. Interaction between the pharmaceutical companies and universities emerged and in 2005 the first phytomedicine - Acheflan ®, an ATP mimetic a from Cordia verbenacea, fully developed in Brazil- was approved.

S32.4

Arnica: New insights in the molecular mode of action of this traditional medicinal plant

Irmgard Merfort; Department of Pharmaceutical Biology and Biotechnology, University of Freiburg, Germany

Preparations from Arnica montana flowers have a long lasting tradition for the external use to treat haematomas, contusions, sprains, rheumatic diseases and superficial inflammations of the skin. Recent studies have considerably enhanced our knowledge on the pharmacological activity and efficacy of this traditional medical plant. The most effective compounds, the sesquiterpene lactones (SLs), such as helenalin and dihydrohelenalin esters, inhibit the transcription factors NF-kappaB and NF-AT at micromolar concentrations thus targeting inflammatory processes at a very central point. Both transcription factors regulate the transcription of genes of many inflammatory mediators. Pharmacokinetic studies have shown that SLs being part of the extract penetrate from the respective preparations into the stratum corneum of the skin and permeate in deeper skin layers. First clinical pilot studies proved the efficacy in inflammatory diseases after external application. In all cases Arnica preparations were well tolerated. Accordingly, very recent results only suggest weak sensitizing properties. Therefore, the opinion in literature that SLs are strong contact allergens has to be revised.

S32.5

PHARMACOGENETICS AND HERB-DRUG INTERACTIONS

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The purposes of this presentation are: (1) to demonstrate the usefulness of a probe-drug cocktail for assessing herb-drug interaction involving CYP inhibition/induction mechanism; (2) to illustrate the importance of applying pharmacogenetics in the investigation of such interactions.

The concept of using a cocktail approach for assessing herb-drug interaction was initially investigated using "Pittsburg cocktail" with G. biloba. Subsequently an improved cocktail for phenotyping of CYP1A2, 2C9, 2C19, 2D6 and 3A was developed and validated. Based on CYP activity before and after G. biloba, this herb was predicted to stimulate CYP2C19 activity and such an effect appeared to follow a genotype-dependent manner. A pharmacokinetic interaction study involving G. biloba and omeprazole was then carried out, and the results confirmed the initial prediction of an inductive effect of G. biloba which manifested in a CYP2C19 genotype-dependent manner.

Our new cocktail can offer a useful and convenient approach for screening herb-drug interactions. In carrying out screening studies, recruitment of subjects with different genotypes should be considered to predict the potential genotype-dependent interaction.

S33.1

Eicosanoids receptors involved in the regulation of vascular tone and reactivity

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Eicosanoids are metabolites derived from arachidonic acid (AA). Different enzymatic pathways transform AA into either prostanoids (prostaglandins (PG) and thromboxane), leukotrienes (LT), epoxyeicosatrienoic acids (EETs), hydroxyeicosatetraenoic acids (HETEs) and lipoxins. In addition, free-radical oxidation products of AA (F2 isoprostanes) may also be formed. However, for these numerous metabolites only some fifteen eicosanoid receptors present on the cell membranes have been described. The prostanoids activate 8 receptors (IP, EP1-4, DP, FP, TP), isoprostanes act also on the TP receptor and the LTs activate 4 receptors (CysLT1, 2; BLT1, 2). In addition, lipoxin, 5-oxo-ETE and PGD2 stimulate the ALX, OXE and CRTH2 receptors, respectively. Finally, at the nuclear level, the eicosanoids are also potent activators of gene transcription via the PPAR receptors. Activation of the eicosanoid receptors may result in vasoconstriction, vasodilatation, angiogenesis, migration and proliferation of the vascular or blood cells. A characterization of the eicosanoid receptors present in the vascular wall from human tissues will be presented in order to highlight and appreciate their role during vascular pathologies.

S33.2

CRTH2/DP receptors and PPAR interaction

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Prostaglandin (PG) D2 is a major prostanoid secreted from activated mast cells and has long been implicated in allergic diseases. DP and CRTH2 are receptors for PGD2, which are associated with Gs- and Gi-type of G protein, respectively, leading to different signaling pathways. Ligand selectivity on two receptors also differs each other; CRTH2, but not DP, is agonized by a PGD2 metabolite 15-deoxy-delta12,14-PGJ2, which is known as an endogenous ligand for a nuclear receptor, PPAR amma. Interestingly PPAR amma exerts anti-inflammatory effects, while CRTH2 is thought to play roles in the formation of allergic inflammations though induction of migration and/or activation of Th2 cells, basophils, and eosinophils. Thus a ligand may deliver opposite signals depending on situation and environment. We indicated implication of CRTH2 in pro-inflammation based on our results of in vitro studies. Recently we have generated CRTH2-deficient mice and demonstrated that chronic allergic inflammation of the skin is alleviated in mutant mice. In this symposium, we would like to discuss implication of the complex PGD2 system in inflammation and possible therapy of allergic diseases.

Key words: prostaglandin D2, allergy

S33.3

Roles of leukotriene B4 receptors in immunological reactions

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Leukotriene B4 (LTB4) has been known as a potent lipid mediator that activates phagocytes. We cloned two G-protein coupled receptors for LTB4, BLT1 and BLT2, a high- and low-affinity receptors, respectively. BLT1 and BLT2 couple to Gi- and Gq-classes of G-protein and activate various intracellular signals leading to calcium increase, degranulation, and chemotaxis.

To reveal the roles of BLT1 in vivo, we and others generated BLT1-deficient mice, and showed its roles in trafficking and adhesion of various subsets of leukocytes, leading to inflammatory and immunological reactions. BLT1 was found to be expressed in eosinophils, Th1- or Th2-polarized T cells and dendritic cells in addition to granulocytes. Ovalbumin-induced airway hyperresponsiveness was attenuated in BLT1-deifcient mice accompanied with reduced airway eosinophilia and goblet cells. BLT1-deficient dendritic cells induced attenuated Th1 responses in allogeneic mixed lymphocyte reaction. Thus, BLT1 is an important immunoregulator as well as inflammatory mediator.

Key words; GPCR, eicosanoid, arachidonic acid, lipoxygenase

S33.4

Therapeutic Opportunities in the Leukotriene Pathway

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Leukotrienes are potent inflammatory and constrictive molecules involved in respiratory and cardiovascular diseases. Successful leukotriene inhibitor (5-lipoxygenase) and antagonist (CysLT1 receptor) therapies have been marketed for asthma and allergic rhinitis. Recent human genetic linkage of haplotypes in leukotriene pathway genes to myocardial infarction and stroke have reignited pharmaceutical interest in the development of novel leukotriene inhibitors and antagonists. The chemistry and biology of the development of therapies targeting the leukotriene pathway proteins will be outlined. In addition, a new paradigm for patient selection by genotype and phenotype will be discussed.

S33.5

Resolution of Vascular Inflammation: Lipoxin Receptors

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Lipoxins (LX) are trihydroxytetraene-containing eicosanoids generated via transcellular biosynthesis during cell-cell interactions in vivo. Lipoxin A4 (LXA4) regulates leukocyte trafficking by activating its specific receptor, ALX, the first identified G protein-coupled receptor (GPCR) for lipoxygenase-derived eicosanoids, with cell type-specific signaling pathways. Aspirin impinges on this endogenous system initiating the biosynthesis of aspirin-triggered LX (ATL; the carbon-15 epimers of LX) within the vasculature. ATL mimics the protective actions of native LXA4 via interacting with the same GPCR (i.e. ALX) and thus can contribute in part to the clinical benefits of aspirin. Both the LXA4 and ATL systems have emerged as founding members of the protective lipid/chemical mediators, which stop neutrophils and recruit monocytes in a non-phlogistic manner leading to resolution of inflammation. This talk will provide an update of the novel endogenous anti-inflammatory circuits, highlighting this ligand-receptor pair (i.e. LXA4/ATL-ALX axis) that offers "agonist-driven" molecular mechanism(s) and "resolution-targeted" therapeutic approaches with high degree of precision in controlling inflammation.

S34.1

Regulatory Agencies Role in Educational Programs Related to Drug Development and Regulatory Science.

Lawrence J. Lesko, Clinical Pharmacology, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland, USA.

Regulatory agencies have three potential roles: (1) to render a thorough evaluation of a regulatory submission (e.g., a New Drug Application to FDA) as a step towards possible approval of a drug product for marketing, (2) to facilitate successful drug development by partnering with industry sponsors to share intellectual expertise that will lead to approval of a drug product with optimal characteristics of benefit and toxicity, and (3) to establish databases from which signicant learning can take place that could lead to best practices or guidances for industry. At FDA, we have embarked on a new initiative under the Agency's critical path initiative that is called "model-based drug development" or MBDD. The component parts of MBDD include a disease model, a drug model and clinical trial information that, when combined with historical data on placebo effects and drop-out rates, allows for simulating clinical trials and making more informed decisions about dose, entry criteria, biomarkers and clinical endpoints. FDA uses these disease-drug models in communicating with industry, and vice-versa, early in drug development at the end-of-phase 2A meetings. These meetings allow for learning and education of clinical pharmacologists, biostatisticians and clinical physicians about how to improve decision quality in drug development and regulatory review. The FDA has development, or has under development, 9-10 different disease state models which rely on interdisciplinary collaboration that in and of itself, constitutes significant learning about the pathophysiology of disease and associated disease and drug biomarkers. Another area of education for FDA is the Voluntary Genomic Data Submission Program (VGDS). Industry-FDA meetings associated with VGDS are ideal opportunities for educating all disciplines in the newer, cutting-edge technologies such as pharmacogenomics. FDA has had nearly 30 VGDS meetings dealing with a range of topics from analytical validation of microarrays, biomarker validation for predictive clinical response and clinical trial designs that involve enrichment of patient populations. Overall, FDA can serve as a training ground for quantitative scientists because of the short cycle times associated with review of New Drug Applications. Plans are underway to offer a formal program of numerous fellowships and graduate student internships to provide on the job training in MBDD and pharmacogenomics.

S34.2

Training Methods and Outcomes in Regulatory Science

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Regulatory Science is an emerging discipline driven by the increasing complexity of the drug and device development path. Effective operational skills require a combination of science, business and legal training that is best provided using a team teaching approach. The University of Southern California has now had five years of experience with a site-based program and eighteen months of experience with a parallel distance program. Students are highly heterogeneous in backgrounds and age range (age range: 24-61, mean: 35, n = 135). Most students prefer weekend condensed courses that allow concurrent full-time employment. Students are highly mobile and use the webstreamed and archived lectures extensively. Students who already have industry experience typically enroll to increase their regulatory breadth and to validate their knowledge. Most of these students aim to either move laterally from jobs in quality assurance and research into regulatory affairs or clinical research, or to move to a higher job level within regulatory affairs. Students without previous industry experience usually gain employment before program completion.

S34.3

"Gene Therapy Products in China: Regulation and Quality Research"

Sang Guowei

Gene therapy is one of the most popular bio-tech advances in the world in the last 2 decades, yet in China it is still a new field for new drug discovery and development, with inexperienced regulatory governance and premature technical guideline. In this presentation, the general China NDA application process and timeline are briefly introduced first, followed with the regulation and guidelines for gene therapy specifically, on both clinical trial and quality control research. Those key consideration points on manufacturing process and quality control for gene therapy in the latest guideline have been elaborated. The majority part of the presentation is about the quality standard research results discussion which has been done in NCPBP, with the examples of Adv-p53, Adv-hIL-2, rAVV-2/hFIX etc on assay of physicochemical characters, specification, bio-assay, impurities and safety test. In the last section of the presentation, the current gene therapy in China has been summarized with the available 18 application status and related information, in which most of the therapeutic area is oncology. It is expected to have the overall understanding of gene therapy submissions in China and the related clinical and quality control considerations.

S34.4

CIOMS: Building International Consensus between Health Authorities and Pharmaceutical Industry in Research Ethics and Safety of Medicines

Juhana E. Idänpään-Heikkilä, Council for International Organizations of Medical Sciences (CIOMS); c/o World Health Organization (WHO); Geneva, Switzerland

In collaboration with WHO, the scientific community, investigators and sponsors of research CIOMS published in 1982 Proposed International Ethical Guidelines for Biomedical Research Involving Human Subjects. The Guidelines incated how ethical principles of the WMA Declaration of Helsinki could be effectively implemented. A revised version of the Guiddelines was published in 1993 and a further revision in 2002. The 1991 CIOMS International Guidelines for Ethical Review of Epidemiological Studies are currently under revision. Both Guiddelines can be downloaded from www.cioms.ch

In 2000-2005, 52 senior scientists from drug regulatory authorities and 55 from pharmaceutical industry have prepared recommendations for solution of contentious issues in drug safety.

CIOMS WG I created world-wide recognised reporting form for drug adverse reactions. CIOMS WGs II-V prepared guidance on assessing and periodic reporting of safety during post-authorization period. CIOMS WG VI provided guidance on "Management of Safety Information from Clinical Trials" in 2005. The current CIOMS WG VII is preparing guidance on Development Safety Update Report (DSUR). The CIOMS WG on Pharmacogenetics published its report in 2005.

S34.5

Putting the "science" into drug development and regulatory science programs

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The core sciences of clinical drug development and regulatory science include biopharmaceutics, clinical pharmacology, biostatistics, pharmacometrics, and medicine. These disciplines also fuel the scientifice basis for regulatory science.

Most students in drug development and regulatory science training programs typically have advanced scientific degrees, positioning them to understand and extend their knowledge in the context of case studies and study of regulatory rules and guidelines. In this presentation, the science content and methods of learning in contemporary training programs in drug development and regulatory science will be presented.

S35.1

Chiral inversion of NG-nitro-D-arginine by D-amino acid oxidase accounts for its in vivo blockade of nitric oxide synthesis

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NG-nitro-L-arginine (L-NNA) inhibits nitric oxide synthase in a sterospecific manner. However, administration of both L-NNA and D-NNA into rats produced pressor responses that were blocked by L-arginine. It was speculated D-NNA underwent a chiral inversion and L-NNA was produced. The current study was to examine the possible role of renal D-amino acid oxidase (DAAO) in the chiral inversion of D-NNA. L-NNA and D-NNA were separately I.V injected into rats, plasma L-NNA and D-NNA were detected by capillary electrochromatography (CEC) and blood pressure was recorded. L-NNA was detected in the blood sample immediately after D-NNA injection while no D-NNA was detected after L-NNA injection. Renal ligation nearly completely blocked the pressor response and the conversion of D-NNA. Injection of benzoate, a selective inhibitor of DAAO into rats completely blocked the pressor response to D-NNA but not to D-NNA pre-incubated with kidney homogenates. Sodium benzoate also completely abolished D-NNA inversion. Our results reveal a novel pathway of unidirectional chiral inversion of D-amino acids where the renal DAAO plays an indispensable role accounting for the biological activity of D-NNA.

S35.2

TARGETING NO AND PEROXYNITRITE IN ACUTE AND CHRONIC HEART FAILURE

Richard Schulz, Cardiovascular Research Group, Departments of Pediatrics and Pharmacology, University of Alberta, Edmonton, Alberta, Canada

Oxidative stress injury to the heart is central to both acute ischemia-reperfusion injury and chronic pathologies involving proinflammatory cytokines. Peroxynitrite, the product of NO and superoxide, is enhanced in both of these injuries. Several biomolecules are targets for peroxynitrite. We found that a key early response to peroxynitrite-stress in the heart is activation of matrix metalloproteinase-2 (MMP-2), an ubiquitous MMP, via direct activation of the proenzyme. Although MMPs are commonly thought to only proteolyze extracellular matrix proteins, we demonstrated that MMP-2 is also localized in the cardiac myocyte within the sarcomere. It co-localizes with the sarcomeric proteins troponin I (TnI) and myosin light chain-1. In both acute and chronic heart injury models the loss of contractile function can be prevented by inhibitors of MMP activity, which also prevent the proteolytic degradation of these novel intracellular targets. Thus a new paradigm has emerged whereby inhibition of MMP activity can protect the heart, preventing the early response to oxidative stress by peroxynitrite by blocking the degradation of intracellular proteins which are susceptible to MMP-2.

S35.3

Effects of inhibitors of inducible nitric oxide synthase (iNOS) on cardiovascular function in experimental diabetes mellitus

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Hyperglycemia and diabetes mellitus (DM) are known to induce the expression of iNOS in the heart and blood vessels. We determined if iNOS depresses cardiovascular function in rats with streptozotocin (STZ, 60 mg/kg iv)-induced DM (type 1) and in Zucker diabetic fatty rats (ZDF, type 2 DM). Catheters were inserted into the iliac artery and left ventricle (LV) for measurement of pressures. Rats with STZ-induced DM for 3 wk, relative to control rats, had impaired pressor, venous and LV contractile responses to noradrenaline. These responses were improved by acute i.v. injection of 1400 W (iNOS inhibitor) or AMD6221 (nitric oxide scavenger). The ZDF rats (20 wk old), relative to the Zucker lean rats, had impaired LV contraction to dobutamine, and the response was also improved by 1400 W. The inhibitors did not affect responses of the control rats. Immunostaining of iNOS was higher in the hearts of both groups of diabetic rats than those of controls. These results show that iNOS contributes to vascular and cardiac contractile dysfunction in type 1 and type 2 DM.

Key words: diabetes mellitus, iNOS

Supported by the Heart & Stroke Fdtn of Canada and the Canadian Diabetes Assn

S35.4

Development of Mice Lacking All Nitric Oxide Synthase Isoforms

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Nitric oxide (NO) is produced in almost all tissues and organs, exerting a variety of biological actions under both physiological and pathological conditions. NO is synthesized by three different isoforms of NO synthase (NOS): neuronal (nNOS), inducible (iNOS), and endothelial NOS (eNOS). Since there are substantial compensatory interactions among the NOS isoforms, the ultimate roles of endogenous NO in our body still remain to be fully elucidated. Here, we have successfully developed mice in which all three NOS genes are completely deleted, by crossbreeding singly NOS-/- mice. NOS expression and activities were totally absent in the triply n/i/eNOS-/- mice before and after treatment with lipopolysaccharide. While the triply n/i/eNOS-/- mice were viable, their survival rate was markedly reduced as compared with wild-type mice. Furthermore, the triply n/i/eNOS-/- mice exhibited spontaneous development of systemic arteriosclerosis and a clustering of cardiovascular risk factors. These results provide the first evidence that the NOS system plays a critical role in maintaining body homeostasis, especially in the cardiovascular system (PNAS 2005).

Key words: mice, nitric oxide, nitric oxide synthase

S35.5

Functional Roles and Mechanisms of Nitric Oxide in Human Diseases

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Lecture 4

Molecular Insights Into the Mechanisms of Electron Transfer by Nitric Oxide Synthases (NOSs)

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NOSs catalyze the step-wise oxidation of L-arginine to NO and L-citrulline - a reaction dependent on the availability of NADPH, O2, and the cofactor tetrahydrobiopeterin (H4B). The three isoforms of NOS are comprised of an N-terminal heme-containing oxygenase domain (OXD) fused to a di-flavin-containing reductase domain (RD) via a calmodulin binding linker. The RD accepts electrons from NADPH and transfers them to the heme iron of the OXD. This process is triggered by calmodulin binding. All these steps must be orchestrated in a timely fashion for NOS to generate NO. For example, a decrease in the bioavailability of H4B leads to superoxide generation. A structural mechanism for this, involving the key interaction of the pyrimadone group of the pterin with the heme propionates groups, has been provided [Raman et al.,(1998) Cell]. Using a battery of techniques, e.g. EPR, electron flow through eNOS and nNOS recombinant proteins under normal and uncoupled conditions was studied. A comprehensive

overview, based on these results, of the factors that regulate electron transfer and thus couple it to L-Arg oxidation is given.

Key words: Nitric oxide, NO, NOS, electron transfer
Acknowledgement: Supported by grants MSMT 0021620806 and GACR 303/05/0336.

Lecture 5

Role of tetrahydrobiopterin in nitric oxide synthase catalysis

Bernd Mayer and Antonius Gorren, Department of Pharmacology and Toxicology, Karl-Franzens University Graz Univ.-Platz 2, 8010 Graz, Austria Nitric oxide synthases (NOS) are cytochrome P450 heme proteins that require the pterin cofactor tetrahydrobiopterin (BH4) to catalyze two-step conversion of L-arg to L-cit and NO. Unlike other pterin-dependent enzymes, all three NOS isoforms contain BH4 as tightly bound prosthetic group. BH4 binding confers the unusually high stability of NOS homodimers and shifts the heme to the active high-spin state. In addition to these allosteric effects, BH4 provides the second electron that is required for P450-mediated substrate oxidation. Although the concept of BH4 undergoing two consecutive one-electron redox-cycles in the course of L-arg oxidation might explain how NOS generates the free radical NO, recent observations with the 4-amino analog of BH4 revealed an additional function of the pterin as proton donor to support protonation of the ferrous-superoxy complex, which is an essential step in P450 catalysis. Thus, the NOS reaction involves a unique redox function of BH4 that is without precedence in biology. The consequences of this complex redox function of BH4 for NOS uncouling in conditions of oxidative cellular stress will be discussed.

Key words: nitric oxide synthase, cytochrome P450, tetrahydrobiopterin, superoxide
Acknowledgements: This work was supported by the Austrian Science Foundation (FWF).

Lecture 6

Contribution of uncoupled endothelial NO synthase (eNOS) to vascular disease

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Nitric oxide (NO) produced by eNOS is an important protective molecule in the vasculature. A functional eNOS oxidizes L-arginine to L-citrulline and NO. This normal function of eNOS requires dimerization of the enzyme, the presence of the substrate L-arginine, and the essential cofactor (6R)-5,6,7,8-tetrahydro-L-biopterin (BH4). Cardiovascular risk factors stimulate the production of reactive oxygen species (ROS) in the vasculature. NADPH oxidases represent major sources of ROS and have been found upregulated and activated in cardiovascular disease. Superoxide avidly reacts with vascular NO to form peroxynitrite. The cofactor BH4 is highly sensitive to oxidation by peroxynitrite. Diminished levels of BH4 promote eNOS uncoupling (i.e. superoxide production by eNOS). Uncoupling of eNOS has been observed in several in vitro models, in animal models of cardiovascular diseases, and in patients with cardiovascular risk factors. BH4 has been shown to correct eNOS dysfunction in animal models and patients. In addition, folic acid and infusions of vitamin C are able to restore eNOS functionality, most probably by enhancing BH4 levels as well.

Key words: endothelial NO synthase, tetrahydro-L-biopterin, oxidative stress, peroxynitrite

Lecture 7

Uncoupling of endothelial nitric oxide synthase in response to plasma factors

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Plasma accumulation of homocysteine or LDLs is associated with nitric oxide (NO)-dependent endothelial dysfunction and increased oxidative stress. Our goal is to examine the participation of endothelial NO synthase (NOS3) in this oxidative stress. Release of NO at surface of human endothelial cells is detected by electrochemistry, that of free radical using fluorescent indicators. The oxidized LDLs and their lipid constituents decreased the agonist-activated NOS3 phosphorylation and NO release. The effects of LDLs and lysophosphatitylcholine were dependent on extracellular superoxide anions, but not those of oxysterols. The latter increased NOS3 translocation from plasma membrane to cytosol. Homocysteine inhibited NO release and citrulline formation without affecting NOS3 phosphorylation. The homocysteine-induced oxidative stress was independent of external superoxide anions, but depended on NOS3 activity. Intracellular synthesis of superoxide anions was associated with reduced levels of tetrahydrobiopterin and inhibition of sepiapterin effects. Thus, hyperhomocysteinemia results in uncoupling of NOS3 activity while LDL accumulation leads to activation of the membrane NAD(P)H oxidase.

Lecture 8

Approaches to prevent endothelial nitric oxide synthase (eNOS) uncoupling as potential therapeutic concepts

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Tetrahydrobiopterin (BH4) has emerged as a critical determinant of eNOS activity and a potential therapeutic target in the vasculature. When BH4 availability is limiting, eNOS no longer produces NO but instead generates superoxide. Deficiency of BH4 may be caused by oxidative stress. Upon reaction with oxidants and in particular with peroxynitrite, BH4 forms a neutral trihydrobiopterin radical which disproportionates to the quinonoid 6,7-[8H]-dihydrobiopterin. These compounds can be regenerated or further oxidized to biopterin. Strategies to maintain BH4 availability include reduction of oxidative degradation and improvement of regeneration. Generally, prevention of peroxnitrite formation by targeting superoxide generating enzymes such as NADPH oxidases or scavenging of peroxynitrite may be important in maintaining BH4 levels. Regeneration of BH4 seems to be a major function of ascorbic acid which was found to be highly reactive towards the trihydrobiopterin radical as well as towards the quinonoid 6,7-[8H]-dihydrobiopterin. Other antioxidants such as glutathione, flavanols or alpha-tocopherol did not stabilize BH4 levels suggesting that ascorbate may specifically adjust BH4-dependent eNOS function.

L16

Understanding Drug Glucuronidation - New Insights Into An Old Enzyme

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Glucuronidation reactions, catalysed by the enzyme UDP-glucuronosyltransferase (UGT), represents both a clearance and detoxification pathway for a myriad of substrates including drugs, environmental pollutants, and endogenous compounds. Although the existence of glucuronide conjugates has been known for 150 years and the pharmacological, toxicological and physiological significance of this metabolic pathway is well recognised, only recently has knowledge of UGT structure-function relationships reached a level that permits rationalisation of drug and chemical glucuronidation in humans. Like cytochrome P450, UGT exists as a superfamily of enzymes. Expression of the individual UGTs in cell culture has allowed definition of substrate selectivity and the development of computational models for the reaction phenotyping of any glucuronidated compound. Moreover, chimeragenesis and site-directed mutagenesis studies have provided important insights into the domains and individual amino acids that contribute to substrate selectivity and binding. Accumulating evidence also indicates that UGTs form homo- and hetero-dimers and complexes with other cellular proteins.

Key words: drug metabolism, glucuronidation

L17

ANALGESICS: STIMULATORS OF THE NO-cGMP-PKG-K^+ ATP CHANNEL

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Prevention of the development of sensitization of a special group of nociceptive neurons (hyperalgesia/hypernociception) constitutes the mechanism of analgesic action of COX inhibitors and Steroidal drugs. Steroids block the release of hyperalgesic cytokines as well as activation of COX. Aspirin-like drugs inhibit the synthesis of prostaglandins. Those agents have no direct effect upon ongoing nociceptors sensitization. This contrasts with the direct antihypernociceptive effect of drugs like dipyrone, diclofenac, flurbiprofen and peripheral acting opiates. Hypernociception is associated with closure of K^+ channels and priming of the TTX

resistant Na channels. Thus, change of K^+ channels conductance promoting the out flow current of the ion, may contribute to restore nociceptors resting potential. In this presentation we shall discuss the experimental behavioural and biochemical molecular evidences that supports that this group of analgesics/antinociceptive drugs block ongoing hyperalgesia. This direct blockade results from the opening of ATP-sensitive K^+ channels via the stimulation of the Arginine/NO/cGMP/PKG biochemical pathway.

Key words: analgesics, hyperalgesia direct blockers.

L18

Adrenoceptor Trafficking in a Living Cell

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Great knowledge has been accumulated on G protein couple receptors (GRCP) by conventional approaches. But seldom evidence has ever been established on the analysis of dynamic behaviors of GPCRs in a living cell. We observed single alpha1 adrenergic receptor (AR) dynamic trafficking by fluorescence imaging with high temporal and spatial resolution in living HEK293 cells., Heterogeneity of the motion was found by the delineation of the trajectories of alpha1B-AR. Two apparent patterns of movements were extracted from the trajectory analysis: immobile motion and directed motion. The internalization of Membrane alpha1A-AR labeled with fluorescence antibody has been seen after an agonist stimulation. The endosomes of alpha1A-AR were transported along actin filaments in a step-by-step manner. The average step-size was found to be 33 nanometers. Our current work provides several new insights into the mechanism and dynamic properties of adrenogic receptor transport.

Key words: adrenergic receptor; single molecule imaging; traficking; living cell.
Acknowledgement: This work was supported by grants from the National Science Foundation of China (30490172, 30200342).

L19

Myoblast-mediated gene transfer for therapeutic angiogenesis

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Therapeutic angiogenesis requires growing functional and stable vessels. Genetically engineered myoblasts are uniquely suited to systematically investigate the effects of dosing, timing and combination of angiogenic factors. Following intramuscular injection, myoblasts fuse to the host fibers and provide essentially lifelong gene expression. Single cells can be isolated from a polyclonal parent population of VEGF-expressing myoblasts and expanded into monoclonal populations that provide uniform VEGF expression levels in vivo. Surprisingly, microenvironmental VEGF concentrations, and not the total dose, were found to determine whether normal capillaries or hemangiomas are induced, and whether functional improvements are achieved in ischemia. Myoblasts can be engineered to express any combination of factors, e.g. VEGF together with the vascular "maturation factor" PDGF. This combination avoids hemangioma growth and improves regional blood flow in ischemia. The studies performed with myoblasts indicate that safety and efficacy are not mutually exclusive in therapeutic angiogenesis. In addition to their experimental value, myoblasts hold therapeutic promise for patients with heart failure.

L20

Ethnicity, genetics and tailored pharmacotherapy

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EEthnic differences in both pharmacodynamics and pharmacokinetics usually reflect differences in the distribution of polymorphic traits, which occur at different frequencies in different population. Asians metabolize CYP2D6-mediated drugs more slowly than Caucasians, due predominantly to high frequencies of variants of 2D6-10, a reduced function allele. Since in most cases the genotype of drug metabolizing enzymes, transporters and receptor determine the drug toxicity and efficacy, the determination of genotype of such proteins plays an important role in optimization of therapy for the individual patient. To translate pharmacogenetics knowledge to the treatment of patients, a Tailored Therapy Center was founded at the Central South University. The Center is pioneering the use of patient tailored therapy. The goal of this tailored approach is to deliver the most effective therapy, while minimizing possible side effects related to drug dosing. Over 4000 hypertensive patients were treated through the Central. We have demonstrated that patient tailored therapy improves quality of life and is a superior treatment model.

S36.1

ENDOGENOUS MEDIATORS OF MUCOSAL PROTECTION: OPPORTUNITIES FOR DRUG DEVELOPMENT

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The endogenous mediators that coordinate mucosal defence have become more clearly understood in recent years. Prostaglandins (PGs) play a key role in modulating mucosal defence. The ulcerogenic effects of NSAIDs are related to inhibition of PG synthesis. More recently, important roles for two gaseous mediators have become clear. Nitric oxide (NO) exerts many of the same effects on mucosal defence as PGs. Suppression NO renders the mucosa more susceptible to injury, while administration of NO donors can protect the stomach. NO-releasing NSAIDs have greatly reduced gastrointestinal toxicity as compared to NSAIDs themselves. Hydrogen sulfide (H_2S), like NO, is an endogenous gas with a wide range of actions. H_2S as an important mediator of mucosal defence: it is a vasodilator and potent inhibitor of leukocyte adhesion. NSAIDs reduce endogenous H_2S synthesis. H_2S-releasing derivatives of a number of drugs exhibit increased potency and GI safety. H_2S-releasing drugs may have utility for treatment of disorders of the gastrointestinal tract characterized by inflammation and pain.

Key words: Nitric oxide; Hydrogen sulfide; Ulcer; Inflammation

S36.2

Effects of lipoxin A4 and lipoxygenase inhibitors on gastric mucosal defense

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Aspirin leads to formation of protective 15(R)-epi-lipoxin (LX) A4 via acetylated cyclooxygenase (COX)-2 and further metabolism by 5-lipoxygenase (LO) (Fiorucci et al., 2002). Serhan et al. (2000) have described that in the presence of indomethacin and acetaminophen arrays of anti-inflammatory lipid mediators are produced from mucosal eicosapentaenoic acid via COX-2-dependent oxygenations and 5-LO. Whereas in rats ischemia-reperfusion alone induced minor gastric damage pretreatment with the COX-2-inhibitor celecoxib markedly increased injury. Low doses of indomethacin, acetaminophen, S- or R-flurbiprofen, before or after celecoxib protected against the damage-aggravating effect of celecoxib. The protective effects of the drugs were reversed by pretreatment with inhibitors of 5-LO(A63162), 12-LO(baicalein) or 15-LO(PD146176) or the LXA4/annexin 1-receptor antagonist BOC1. The findings show that the protection by these non-steroidal anti-inflammatory drugs is not mediated by COX-2 as it operates when COX-2 is inhibited, but is modulated by LO activities.

Key words: Lipoxygenases, cyclooxygenase-2, non-steroidal anti-inflammatory drugs, gastric injury
Acknowledgement: This study was supported by the DFG

S36.3

Mucosal Protective ("Cytoprotective") Agents - Novel Molecular Mechanisms of Action

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Following Robert's discovery that prostaglandins (PGs) E and I type protect GI mucosa against necrotizing agents (cytoprotection) and accelerate ulcer healing, other drugs have been shown to exert cytoprotective action. In early 1980's we demonstrated that antacids and sucralfate protect gastric mucosa against injury, while H2 RA are not effective (Am. J. Med 1985 &1987). Next we showed that antacid hydrotalcite (Talcid) protects gastric mucosa, stimulates angiogenesis, accelerates ulcer healing and improves quality of scar; the latter was recently confirmed in human ulcers. We found that novel molecular mechanisms of Talcid's action in gastric mucosa are: activation of Cox2, HSP-70, EGF, its receptor and bFGF genes, which are important for protection and healing. Talcid also absorbs and neutralizes all H. pylori toxins and reduces H. pylori adherence to human

gastric cells. Sucralfate and rebamipide (Mucosta) also exert mucosal protective and ulcer healing actions through induction of prostaglandins and growth factors. In regard to PGs, our studies demonstrated that PGE transactivates EGF receptor (Nature Med 2002), activates CREB and stimulates VEGF expression, angiogenesis and ulcer healing.

S36.4

Unique Profile of Lafutidine, A Novel Histamine H2 Antagonist: Mucosal Protection Throughout GI Tract Mediated by Capsaicin-Sensitive Afferen

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Lafutidine is a histamine H2 receptor antagonist with a mucosal protective action. This agent prevents gastric lesions as induced by a variety of noxious agents, the effect being attenuated by pretreatment with the antagonist of calcitonin gene-related peptide (CGRP) and a blocker of nitric oxide (NO) production as well as chemical ablation of capsaicin-sensitive sensory neurons, but not a cyclooxygenase inhibitor indomethacin. Lafutidine also exhibits a protective activity against the experimentally-induced mucosal lesions in the gastrointestinal tissues other than the stomach; including acid reflux esophagitis; indomethacin-induced small intestinal lesions; colonic inflammation induced by dextran sulfate sodium. Furthermore, lafutidine also promotes the healing of gastric ulcers and reduces the ulcer relapse after discontinuation of the treatment. Given the above findings, we conclude that lafutidine has a protective action throughout the gastrointestinal tract from the esophagus to large intestine, and these effects are mainly mediated by capsaicin-sensitive afferent neurons and dependent on CGRP and NO but no prostaglandins.

S36.5

The use of Proton Pump Inhibitors in Acute Peptic Ulcer Bleeding

Joseph JY Sung, The Chinese University of Hong Kong

Bleeding from peptic ulcer disease is still one of the most common medical emergency with an average mortality of 10% worldwide. The use of endoscopic hemostasis has revolutionized the treatment of ulcer bleeding. Suppression of gastric acid secretion is important for platelet aggregation and hence control of bleeding from peptic ulcer diseases. We have conducted a few clinical trials to demonstrate that

1. The combination of intravenous proton pump inhibitors at high dose (80 mg bolus followed by 8 mg/hour for 72 hours) with endoscopic therapy is superior to endoscopic therapy alone in active ulcer bleeding.
2. Intravenous proton pump inhibitors alone does not obviate the need of endosocpic hemostasis, especially in patients with an ulcer with clot or protuberant vessel.
3. The effect of high dose omeprazole infusion on intragastric acid suppression among those with CYP2C19 extensive metabolizers is superior to others receiving intravenous repeated bolus injection
4. In the presence of intravenous and oral pantoprazole, immediate reintroduction of aspirin has not substantially increased the risk of recurrent bleeding from peptic ulcers. Discontinuation of aspirin is associated with increased mortality

S37.1

Optimizing energy metabolism as a pharmacological approach to treating ischemic heart disease

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During ischemia cardiac glycolytic rates increase, while mitochondrial oxidation of glucose decreases. This leads to myocardial acidosis due to the accumulation of lactate and protons. Inhibition of fatty acid oxidation is a novel approach to treating ischemia, because it results in a stimulation of glucose oxidation and a decrease in proton production. This improves cardiac efficiency (work/CO_2 consumed), as less energy is required for non-contractile purposes. The anti-anginal agent trimetazidine inhibits fatty acid oxidation, secondary to inhibition of the fatty acid βoxidation enzyme 3-ketoacyl CoA thiolase (3-KAT). The resultant stimulation of glucose oxidation decreases acidosis, thereby increasing cardiac efficiency. Clinically, trimetazidine is the most widely used anti-anginal agent with a mechanism of action that can be attributed to metabolic modulation and improvement of cardiac efficiency. This therapeutic approach not only lessens the severity and symptoms of an angina attack in patients with coronary artery disease, but also decreases the incidence of angina attacks. Using trimetazidine to optimize energy substrate preference is a novel approach to treating ischemic heart disease.

S37.2

Reactive oxygen species involvment in diabetic cardiomyopathy: Effects of treatment with N-acetylcysteine

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Hyperglycemia increases the production of reactive oxygen species (ROS) and the subsequent activation of PKCβ2 isoform in the myocardium that is attributable to the development of cardiomyopathy through mechanisms that involve increased expression of connective tissue growth factor (CTGF) and the resultant increase of cardiac fibrosis and cardiomyocyte hypertrophy, features of cardiomyopathy. We hypothesized that the antioxidant N-acetylcysteine (NAC) would normalize hyperglycemia-induced overexpression of myocardial PKCβ2 and CTGF and prevent the development of diabetic cardiomyopathy. Control and streptozotocin-induced diabetic rats were treated with NAC for 8 weeks before measurements were performed. Myocardial hypertrophy, characterized by an increased ratio of ventricle weight to body weight and cardiomyocyte cross-sectional area was found to be higher in untreated diabetic rats, accompanied by increased fibrosis. Further, increased myocardial levels of NADPH oxidase, a source of ROS formation, were accompanied by an increased expression of PKCβ2 and CTGF. NAC attenuated or prevented these changes. The results suggest that ROS plays a role in the development of diabetic cardiomyopathy.

S37.3

Metabolic Modulation as an Approach to Protect the Failing Heart

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Inspite of the advances in treatment heart failure continues to have a poor prognosis. With an aging population the prevalence of cardiac failure is increasing. A metabolic approach to therapy addresses the negative effects of increased free fatty acid (FFA) metabolism and reduced glucose oxidation and compliments established therapy with beta-blockade, angiotensin-converting enzyme inhibitors etc. Trimetazidine increases glucose oxidation by shifting the energy substrate from FFA's. Patients with ischemic cardiomyopathy treated with trimetazidine for 6 months increased their ejection fraction by 9% compared to placebo.1 Using dobutamine stress echocardiography an increase in contractility was demonstrated compared to placebo identifying protection at a cellular level as the two groups had a similar haemodynamic stress.2 The benefits have also been shown in the elderly.3 The metabolic approach to the failing heart reduces ischaemia and improves left ventricular function and therefore may have prognostic importance.

S37.4

Inhibition of fatty acid oxidation as a pharmacological approach to treating heart failure

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Alterations in myocardial energy substrate metabolism in heart failure patients can contribute to contractile dysfunction and to the progressive left ventricular remodeling. In general, the metabolic changes that occur in chronic heart failure are difficult to study due to the diverse etiology of clinical heart failure and the limitation of animal models. Recent evidence suggests that myocardial energy metabolism is relatively normal during the early stages of heart failure, however in the advanced stages there is a reduced mitochondrial oxidative metabolism due to defects in the electron transport chain, an increase in glycolysis, and a down-regulation of the capacity for carbohydrate and fatty acid oxidation. The consequences of these metabolic changes on cardiac function are not well understood. We have observed in animal models that long-term treatment with partial inhibitors of myocardial fatty acid oxidation with either trimetazidine, ranolazine, or oxfenicine can prevent the development of some of the molecular and functional abnormalities of heart failure. This pharmacological approach is particular attractive because it is independent of current drugs aimed at the hemodynamic and neuro-e

S37.5

Pharmacological Modulation of Energy Metabolism in the Treatment of Diabetic Cardiomyopathy

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Diabetes is associated with a specific cardiomyopathy which occurs in the absence of ischemic or valvular heart disease. The prevalence of diabetic cardiomyopathy is alarmingly high, affecting as many as 40-60% of diabetic patients. Our laboratory was among the first to initiate studies on experimental cardiomyopathy in streptozotocin (STZ) and alloxan-induced diabetic rats and rabbits. The cardiomyopathy first appears at six weeks in this model. Heart structure is not significantly disrupted at six weeks, but there are widespread disturbances in calcium handling. We have attempted to find drugs which can ameliorate the cardiac dysfunction induced by the diabetic state. Insulin itself, as well as insulin enhancing agents such as metformin or trace metal insulin enhancers (vanadium, selenium, tungsten) improve cardiac function. Other treatments which improve cardiac function include fish oil, certain amino acids, antioxidants such as vitamin C and CPT-1 inhibitors. Most recently, we have studied the effects of beta-blockers in diabetic cardiomyopathy, and found that they act partly by inhibiting fatty acid oxidation and promoting glucose oxidation. The mechanisms of these effects will be discussed.

S38.1

Bilirubin (BR) activates the Aryl hydrocarbon Receptor (AhR) by modifying the cellular redox state

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Excretion of BR, a neurotoxic end-product of heme catabolism, is normally mediated by hepatic UDP-glucuronosyltransferase (BR-UGT). In the absence of BR-UGT, BR stimulates the AhR-mediated transcription of the CYP1A1 gene. This study was designed to identify the mechanism of this stimulation.

AhR function was studied by reporter gene expression in HeLa cells transiently transfected with w.t. and mutant AhR constructs. Intracellular AhR localization was monitored by fluorescence microscopy using GFP-tagged AhR constructs. The intracellular content of reactive oxygen species (ROS) was assayed by fluorescence microscopy using carboxy-H_2DCFDA staining.

Results: BR increased nuclear AhR localization and reporter gene expression in cells transfected with either w.t. or mutant receptor lacking the minimal ligand-binding domain (LBD).

The BR dose-response relationship obtained for AhR activation exhibited a bell-shaped pattern, similar to that obtained for the reducing agent N-acetyl-L-cysteine (NAC).

Oxidative stress, induced by H_2O_2, abolished the stimulatory effects of BR and TCDD on AhR activation. BR, as well as NAC, restored the effects of TCDD on w.t. AhR nuclear accumulation, when added to cells after 2 hours of exposure to H_2O_2. Nuclear accumulation of the mutant receptor, lacking the LBD, was also increased by BR and NAC in the presence of H_2O_2.

Both BR, in a low concentration (50 μM), and NAC markedly reduced the generation of ROS formed during oxidative stress.

Conclusions: BR exerts its stimulatory effects on AhR signal transduction by virtue of its anti-oxidant, redox-modifying properties, without requiring binding of the inducer to the receptor.

Key words: bilirubin, cytochrome P450, AhR, redox.

S38.2

Regulation of Gene Expression and Oncogenesis by the Aryl Hydrocarbon Receptor

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The aryl hydrocarbon (dioxin) receptor is a nuclear gene regulatory protein that functions as an intracellular receptor for environmental pollutants, notably dioxin (2,3,7,8,-tetrachlorodibenzo-p-dioxin). A number of loss-of-function studies in mice have implicated the receptor in dioxin-induced chemical carcinogenesis. We have performed gain-of-function studies in mice to assess the biological function of the receptor in the absence of exposure to environmental pollutants. These studies indicate that a constitutively active form of the receptor induces development of gastric tumors due to dysregulation of gastric epithelium cell homeostasis. The possible mechanisms, gene regulatory potential of the receptor will be discussed.

S38.3

Diverse roles of Biliverdin-Reductase in regulation of the oxidative response gene: heme oxygenase-1

Mahin D. Maines, University of Rochester, School of Medicine

Oxidative stress signals dynamic changes in the expression of stress responsive genes that are induced through activation of AP-1/ATF-2 elements. HO-1 and HO-2 are members of the HSP32 family that controls cellular levels of heme and hemoproteins. HO-1 is the oxidative stress responsive form while HO-2 is GC inducible, O2/NO/CO sensor of the cell (1,2). The enzymes are key components of cellular defense mechanisms and produce biliverdin and CO. Biliverdin is reduced to bilirubin by biliverdin reductase (BVR). Recent studies reveal that HO-1 activity is regulated by the serine/threonine/tyrosine kinase, BVR. BVR is a b-Zip transcription factor for AP-1/ATF-2 regulated genes. BVR relays information from activated insulin/growth factor receptor to down stream signal cascades of MAPK and PKC's to elicit oxidative response gene expression (3).

Supported by NIH Grants ESRO104066 and RO1-ES12187

S38.4

Cross-talk between xenobiotics signalling and oxidative stress

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The Ah Receptor mediates the induction of a large number of genes by dioxin and Polyaromatic Hydrocarbons. The functions of these genes products contribute to the toxic consequences of exposure to dioxin and PAHs. Induction of cytochrome P4501A1 has been thoroughly characterized. CYP1A1 mediates both adaptive and toxic pathways through the generation of reactive intermediates. Reactive Oxygen Species which are released by CYP1A1 could contribute to toxicity, but we have shown that they also play a regulatory role in the fine tuning of the balance between phase1 and phase 2 enzymes. We have recently shown that the IGFBP-1 (Insulin-like Growth Factor Binding Protein-1) is a target of dioxin in human hepatocytes and hepatoma cells. A cross talk between dioxin induction and hormonal or stress regulation of this gene is mediated by its promoter. Paraoxonase 1 which hydrolyses oxidized lipids and organophosphates is also induced by the Ah Receptor. However dioxin is a poor inducer while polyphenols are potent inducers of this gene. Thus xenobiotics signalling displays several cross talks with physiological or stress signalling pathways.

Key words: dioxin, oxidative stress, CYP1A1, IGFBP1, paraoxonase1

S39.1

Mechanisms underlying neuroprotection by PARP inhibitors

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Accumulating evidence indicates that poly(ADP-ribose) polymerase-1 (PARP-1) activity plays active roles in neurodegeneration, and that pharmacological inhibitors of PARP-1 are efficacious neuroprotective drugs. Yet, the molecular mechanisms underlying such pharmacodynamic properties remain controversial. We aim at understanding such mechanisms by developing selective and powerful inhibitors of PARP isoforms, as well as using transgenic mice carrying alterations in genes involved in poly(ADP-ribose) metabolism. In this presentation, we will provide data showing that poly(ADP-ribosyl)ation promotes neurodegeneration through mechanisms encompassing dysfunction of energy dynamics, facilitation of the inflammatory response as well as activation of the apoptotic machinery. Experiments are in progress to understand which molecules [i.e. poly(ADP-ribose) itself and/or poly(ADP-ribosyl)ated factors] underpin the neurotoxic effect of poly(ADP-ribosyl)ation. Answering this question could identify new target(s) of relevance to pharmacological intervention.

S39.2

Poly(ADP-ribose) Polymerases (PARPs) as Potential Drug Targets

Valérie Schreiber, Francoise Dantzer, Jean-Christophe Amé, Josiane Ménissier-de Murcia and Gilbert de Murcia; Département Intégrité du génome, UMR 7175 CNRS-ULP; ESBS; Boulevard Sébastien Brant, BP 10413; 67412 ILLKIRCH, France

Poly(ADP-ribosyl)ation is an immediate DNA damage-dependent post-translational modification of histones and other nuclear proteins that contributes to the survival of injured proliferating cells. Poly(ADP-ribose) polymerases (PARPs) now constitute a large family of 17 proteins, encoded by different genes and displaying a conserved catalytic domain. PARP-1 (113 kDa), the founding member, and PARP-2 (62 kDa) are so far the sole enzymes whose catalytic activity is immediately stimulated by DNA strand-breaks. A large repertoire of sequences encoding novel PARPs now extend considerably the field of poly(ADP-ribosyl)ation reactions to various aspects of the cell biology including cell proliferation and cell death. Some of these new members interact with each other, share common partners and common subcellular localizations suggesting possible fine tuning in the regulation of this posttranslational modification of proteins. This review summarizes our present knowledge of this emerging superfamily that might ultimately improve pharmacological strategies to enhance both antitumor efficacy as well as the treatment of a number of inflammatory and neurodegenerative disorders.

S39.3

Poly (ADP-ribose) polymerase (PARP) Inhibitors for the Treatment of Ischemia-Reperfusion Injury

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There is now good evidence that inhibitors of the activity of poly (ADP-ribose) polymerase (PARP) reduce the tissue injury caused by ischemia and reperfusion (I/R) of the heart, skeletal muscle, brain, kidney & liver. For instance, inhibitors of PARP activity reduce the infarct size and cardiac dysfunction caused by I/R injury of the heart (and other organs) of i.e. the rat, rabbit and pig. The tissue injury caused by coronary artery occlusion and reperfusion in PARP-1 knock out mice is substantially reduced (when compared to their wild-type litter mates). The beneficial results obtained in the last years with relatively weak PARP-inhibitors (3-AB) have recently been confirmed with more potent, water-soluble PARP-inhibitors (5-AIQ, PJ-34, INO-1001, KU-0058684, KU-0059434). Taken together, these findings support the view that the excessive activation of PARP contributes to I/R injury of the heart and other organs. Phase II clinical trials evaluating the effects and side-effects of the PARP-inhibitor INO-1001 in patients with acute myocardial infarction, coronary angioplasty and bypass heart surgery are currently ongoing.

S39.4

Biochemistry and pharmacology of enzymes involved in NAD metabolism

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The pyridine nucleotides are the major redox carriers in all organisms. However, recent research has also established a wide array of signalling pathways that involve NAD. The dinucleotide serves as substrate for protein modifications including protein deacetylation and ADP-ribosylation. It also is a precursor of intracellular calcium-mobilising molecules. Thus, NAD-mediated signal transduction does not merely regulate metabolic pathways, but holds key positions in the control of fundamental cellular processes.

Our recent research has revealed an unexpected subcellular distribution of NAD biosynthesis. The three human isoforms of NMN adenylyltransferases (NMNAT), which catalyse the final biosynthetic step, were localised to the nucleus, mitochondria and the Golgi complex. Moreover, we found that the nuclear NMNAT is recruited to sites of poly-ADP-ribosylation and stimulates the activity of poly-ADP-ribose polymerase-1 (PARP-1). Therefore, NAD biosynthesis is directly linked to processes such as DNA repair and apoptosis. Possibly, isozyme-specific pharmacological modulation of NMNATs could not only influence individual NAD pools, but also become a tool to selectively regulate signalling pathways.

S40.1

Vanilloid Receptors and Airway Hyperresponsiveness

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The transient receptor potential vanilloid 1 (TRPV1) is rather selectively expressed in a subpopulation of nociceptive primary sensory neurons that promote neurogenic inflammation. TRPV1 is activated by noxious temperature, low extracellular pH and diverse lipid derivatives, and is uniquely sensitive to vanilloid molecules, including capsaicin. Various G-protein-coupled and tyrosine kinase receptors, including the NGF, bradykinin and prostaglandin receptors upregulate TRPV1 expression and sensitivity. Other exogenous or endogenosus chemical agents, relevant for airway pathophysiology, and including reactive oxygen species, ethanol and hydrogen sulphide sensitise/activate TRPV1. In the airways, TRPV1 agonists cause cough, bronchoconstriction, microvascular leakage, hyperreactivity and hypersecretion. A higher density of TRPV1-positive nerve fibres has been found in patients with chronic cough and patients with asthma and chronic obstructive pulmonary disease are more sensitive to TRPV1-induced cough. In asthma exacerbations or in other inflammatory conditions of the airways TRPV1 activation may contribute to respiratory symptoms and TRPV1 antagonists may be useful in the treatment of these conditions.

S40.2

Reactive oxygen / nitrogen species in airways inflammation

YAMAGATA TOSHIYUKI; Third Department of Internal Medicine, Wakayama Medical University; ICHINOSE MASAKAZU; Third Department of Internal Medicine, Wakayama Medical University

Although the participant cells and mediators are different, bronchial asthma (BA) and chronic obstructive pulmonary disease (COPD) are both characterized by chronic airway inflammation. Oxidative / nitrosative stress play an important role in the pathophysiology in both diseases. In BA, the production of nitric oxide (NO) is increased probably via the upregulation of inducible NO synthase. It has been reported that the level of exhaled NO is correlated with the severity of airflow limitation, airway hyperresponsiveness or eosinophils infiltration. The anti-inflammatory agent, corticosteroid, which is a key drug for BA, can reduce the NO production as well as airway inflammation and hyperresponsiveness. On the contrary, in COPD airways, the formation of 3-nitrotyrosine rather than NO is much more increased than bronchial asthma. We have found that the several agents including theophylline, corticosteroid and allopurinol can inhibit the oxidative / nitrosative stress. These agents improve the airway inflammation and may prevent the progression of COPD. In this symposium, the importance of oxidative / nitrosative in the airway inflammation and its pharmacotherapeutic modification will be reviewed.

S40.3

Pharmacological approaches for the treatment of COPD

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Chronic obstructive pulmonary disease (COPD) is a disease of the airways with an underlying inflammatory component. The prevalence and healthcare burden of COPD is still rising and is predicted to continue to rise in the foreseeable future. The current mainstays of therapy for COPD are bronchodilators and corticosteroids, despite the first only treating symptoms and the second lacking good evidence for their use, except during exacerbations. The use of inhaled corticosteroids in COPD is widespread but controversial and their early introduction to asymptomatic patients with stable disease does not appear to alter the rate of decline in lung function. The inflammatory processes underlying the pathology of COPD have begun to be elucidated. This has resulted in the identification of new targets (eg. matrix metalloproteinases, p38 kinase, phosphodiesterase 4, IκB kinase-2) which will allow the development of novel approaches in order to provide

new and improved therapies to treat this debilitating disease.

Key words: COPD, inflammation, elastase
Acknowledgements: GlaxoSmithKline for financial support in the form of research project grants

S40.4

Histamine and anti-histamine drugs: what's new

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Until 2000, histamine was thought to act via three receptors (H1, H2 and H3). After the successful clinical development of H1- and H2-antagonists, H3-receptor agonists or antagonists are now developped in different therapeutic areas such as obesity and cognition. Dual H1- and H3-antagonists may be also of clinical benefit in allergic rhinitis to improve efficacy on nasal congestion. At the end of 2000, cloning of the human H4 receptor was reported. This subtype is related most closely to the human H3-receptor. The H4-receptor is mainly expressed on eosinophils, mast cells, $CD8^+$ T cells and dendritic cells. This receptor is involved in histamine-induced eosinophil and mast cell chemotaxis as well as IL-16 secretion from $CD8^+$ T cells and suppression of IL-12 secretion from dendritic cells. However, the H4-receptor is not involved in histamine-mediated increase in vascular permeability. These functions of the H4-receptor implies that it has a role in inflammatory and immune responses. Expression of H1- and H2-receptors on antigen-presenting cells enhances the potential of histamine in immune responses and inflammation. H4-antagonists have already demonstrated anti-inflammatory activities in animal models.

S41.1

The Unique Renal Myogenic Response: A Mechanism Protecting Against Hypertensive Injury

Rodger Loutzenhiser; Smooth Muscle Research Group; University of Calgary Faculty of Medicine; Calgary, Alberta, Canada

Hypertension is a leading factor in the onset and progression of chronic kidney disease (CKD). Elevations in systolic blood pressure (SBP) are most closely linked to CKD as is impaired renal autoregulation. Indeed, animal studies show an invariant relationship between impaired autoregulation and hypertensive glomerular injury. The myogenic response of the afferent arteriole (AA) contributes to autoregulation and is uniquely suited to a role in renal protection. We have found that, in the rat, pressure increases initiate a rapid AA vasoconstriction within 200-300 ms. When pressure is reduced, vasodilation is intitiated after a much longer delay (~1 s) and high-speed video studies show that vasoconstrictor responses initiated by short pressure pulses (< 300 ms) continue during this delay. Experimental and modeling approaches demonstrate that these features allow the AA to sense and adjust steady-state myogenic tone in response to the rapidly oscillating SBP signal, thereby attenuating the transmission of pressure transients to the glomerulus. Studies of the mechanisms underlying the unusual kinetics implicate intracellular Ca release and the AA expression of the cardiac ryanodine receptor isoform (RyR2).

S41.2

Gap junctions and communication in the renal afferent arterioles

Niels-Henrik Holstein-Rathlou, Max Salomonsson, Thomas Braunstein and Charlotte Mehlin Sørensen; Department of Medical Physiology, The University of Copenhagen, Denmark..

Nephrons arising from the same cortical radial artery (interlobular artery) interacts, so that. activation of the tubuloglomerular feedback mechanism (TGF) in one nephron results in the lowering of stop flow pressure both in the perfused nephron and in the neighboring non-perfused nephrons arising from the same cortical radial artery. This cross-talk is due to a conducted vascular response (CVR) where vasomotor stimuli elicited at one site travels through the vascular wall to cause constrictions and/or dilatations at remote sites. In the preglomerular vasculature the endothelial cells contain different connexins that electrically and chemically couples neighboring cells. In contrast, except for the juxtaglomerular, renin containing cells, it has been difficult to detect the presence of connexins in the media. Although the nature of the conducted signal remains unknown, it appears likely that it involves an electronic spread of a local change in membrane potential along the vessel. CVR's and the nephron-nephron interaction is increased in SHR. In the talk we will discuss possible mechanisms for the nephron-nephron interaction, and the changes in afferent arteriolar expression of Cx's in SHR.

S41.3

Renal Blood Flow in Pathological States: Hypertension and Diabetes

Sadayoshi Ito; Division of Nephrology, Endocrinology, and Vascular medicine, Department of Medicine; Tohoku University School of Medicine

Renal hemodynamics plays an important role for the pathogenesis and progression of diabetic and hypertensive renal diseases. Alteration of glomerular hemodynamics is one of the determinarts of glomerular injuries, proteinuria and subsequent tubulointerstitial damages. Recent studies indicate that the renin angiotensin system (RAS) plays an important role in the renal damages, and the mechanism for this seems to involve inflammation and oxidative stress. In addition to glomerular hemodynamics, medullary circulation is also involved in the pathogenesis hypertension. Decreases medullary blood flow causes sodium retention and hypertension. Studies indicate that oxidative stress is produced by tubules by various stimuli and alters endothelial function of nearby vascular beds, a phenomenon called "tubulo-vascular crosstalk". In this presentation, I will discuss recent in vivo and in vitro evidences for the role of oxidative stress and inflammation in the pathophysiology of hypertension and diabetic renal diseases.

S42.1

Overview of the history and therapeutic potential of purinergic signalling

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ATP is an extracellular signalling molecule and was proposed as a neurotransmitter of non-adrenergic, non-cholinergic nerves supplying the gut and bladder in the early 1970's and later as a cotransmitter in most nerve types in both peripheral and central nervous systems. Subdivision into P1 and P2 receptors responsive to adenosine and ATP respectively was proposed in 1978. Four subtypes of P1 receptors were cloned and subdivision of P2 receptors into P2X ionotropic and P2Y metabotropic families followed. Currently, 7 subtypes of P2X receptors and 8 subtypes of P2Y receptors have been cloned and characterised. The P2X form heteromultimers and some P2Y receptor subtypes are responsive to pyrimidines. Short-term purinergic signalling occurs in neurotransmission and secretion. Long-term (trophic) purinergic signalling occurs in cell proliferation, differentiation and death during development and regeneration. There is strong current interest in the therapeutic potential of purinergic agents in diseases such as thrombosis, stroke, pain, cystic fibrosis, dry eye, osteoporosis, kidney failure, diabetes and cancer.

Key words: adenosine, ATP, purinergic, purinoceptors

S42.2

Purinergic mechanisms involved in neuropathic pain

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There is abundant evidence that extracellular ATP and other nucleotides have an important role in pain signaling at both the periphery and in the CNS. Recent findings suggest that endogenous ATP and its receptor system might be involved in neuropathic pain. Neuropathic pain is often a consequence of nerve injury through surgery, bone compression, diabetes or infection. This type of pain can be so severe that even light touching can be intensely painful; unfortunately, this state is generally resistant to currently available treatments. We recently reported that the expression of P2X4 receptors in the spinal cord is enhanced in spinal microglia after peripheral nerve injury, and blocking pharmacologically and suppressing molecularly P2X4 receptors produce a reduction of the neuropathic pain behaviour (Nature 424, 778-783, 2003), and that brain-derived neurotrophic factor (BDNF) released from microglia by the stimulation of P2X4 causes the depolarizing shift in reversal potential of anion in LI neurons of rats with nerve injury (Nature, 438, 1017-1021, 2005), resulting in causing neuropathic pain. Understanding the key roles of these ATP receptors may lead to new strategies for controling the pain.

S42.3

Medicinal Chemistry of Purine Receptor Antagonists

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Purine receptors are classified to P1 (adenosine) and P2 (nucleotide) receptors, which have been known to mediate diverse physiological functions in various cells and organs. Among 4 different subtypes of adenosine receptors, A2B and A3 receptors are closely related with several diseases including asthma and ischemia. In the case of P2 receptors, which are further classified to P2X and P2Y receptors, P2X3 and P2X7 receptors are mostly interesting subtypes, because of their actions of pain signaling involved in chronic inflammatory nociception and neuropathic pain by nerve injury and joint inflammation including rheumatoid arthritis or osteoarthritis. In the efforts of modulating disease related purine receptors, we have developed selective and potent A2B, A3, P2X3 and P2X7 receptor antagonists. The representative example with a strategy of medicinal chemistry of each purine receptor antagonist will be presented in the area of design or screening, synthesis, biological assays using ligand binding, electrophysiological, and cell based assay systems and functional evaluation of the antagonists.

Key words: Purine Receptors, Antagonists, Adenosine, Nuclectides

S42.4

P2 receptors in glial cells

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Astrocytes and microglia express many ionotropic P2X and G-protein-coupled P2Y receptors, which are differentially recruited under specific conditions. Astrocytes release and respond to ATP with a propagating wave of intracellular calcium increases, allowing a homotypic and heterotypic signaling which also involves microglia, neurons and oligodendroglia. This form of short-term signaling primarily involves astrocytic P2Y1,2,4, and, maybe, P2X7 receptors. Multiple P2 receptors (i.e., P2Y1,2,6,12,13 and P2X7) seem instead to cooperate to long-term astrocytic changes during inflammatory gliosis. In microglia, inflammatory stimuli produce differential changes of distinct P2 receptors, suggesting highly specific roles in acquisition of the activated microglia phenotype. It is believed that nucleotide-induced gliosis may start as an acute, defense mechanism and that its dysregulation in chronic inflammation may contribute to neuronal death. Thus, the elucidation of the roles of P2 receptors may help exploiting the beneficial features of activated glia while attenuating their harmful properties, thus providing the basis for novel neuroprotective strategies specifically targeting the purinergic system.

S42.5

Hydrolysis of extracellular nucleotides by CD39/ENTPD family members: prominent effects on thrombosis, vascular inflammation and immune reactions.

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Ecto-nucleotidases of the CD39/E-NTPDase family are expressed in the vasculature and immune systems. These ecto-enzymes hydrolyze extracellular nucleotides, ultimately to the respective nucleosides, to regulate P2-receptor signaling. Spatial and temporal expression of CD39/NTPDase1 by vascular and immune cells could regulate thrombotic and immune reactions in vivo. CD39 has the potential to modulate thrombotic reactions viz. platelet activation after ischemia reperfusion in vivo. Increases of NTPDase1 biochemical activity within microparticles associated with evolving arteriolar thrombi also seems to impede further ADP-mediated platelet activation. CD39 is also a surface marker of T regulatory cells (Treg). Co-ordinated expression of CD39 on Treg and the adenosine A2A receptor on activated effector T cells (Teff) generates an immunosuppressive loop. Adoptive transfer of Cd39 null Treg fails to inhibit allograft rejection in vivo and null mice also develop autoimmune manifestations and exhibit vascular thrombophilia. Pharmacologic modalities to modulate or boost NTPDase1 expression may suppress deleterious vascular or immune reactions, as seen in autoimmune disease and transplant graft rejection.

S43.1

Use of Gene Targeting Technology to Understand the Roles of CNS Muscarinic Receptors

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The precise roles of the individual muscarinic acetylcholine (ACh) receptor subtypes (M1-M5 mAChRs) in mediating the diverse central actions of ACh are not well understood at present. To address this issue, we used gene targeting technology to generate M1-M5 mAChR-deficient mice (KO mice). During the past few years, we, together with many collaborators, have subjected the M1-M5 mAChR KO mice to a series of physiological, pharmacological, behavioral, biochemical, and neurochemical tests. More recently, we started to employ Cre/loxP technology to generate mutant mouse lines that lack specific mAChRs only in neurons or in certain regions of the brain. Phenotypic analysis of these mutant mouse strains revealed that distinct mAChR subtypes play key roles in the regulation of body weight and growth, cognition, drug-seeking behavior, analgesia, and various other important functions of the CNS. These studies should provide a rational basis for the development of novel muscarinic drugs for the treatment of several important diseases of the CNS.

Key words: acetylcholine, receptor knockout mice, muscarinic receptors

This research was supported by the Intramural Research Program of the NIH, NIDDK

S43.2

CHANGES IN MUSCARINIC RECEPTORS IN SCHIZOPHRENIA: REGIONAL SPECIFICITY AND POSSIBLE OUTCOMES

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Recent studies using the selective muscarinic receptor radioligand, [^{3}H] pirenzepine, have consistently shown decreases in binding in the CNS from subjects with schizophrenia. These finding are consistent with a recent neuroimaging study which showed decreased in muscarinic receptors in a number of CNS regions in "drug-free" schizophrenic subjects. Limitations in the selectivity of radioligands do not allow binding studies to identify which of the 5 muscarinic receptors is altered in schizophrenia. By contrast, our more recent studies have used receptor specific antibodies to measure the levels of M1 and M4 receptors in post mortem CNS from subjects with schizophrenia, the two receptors targeted by [^{3}H] pirenzepine. These studies have shown that the M1 receptor is decreased in Brodmann's area (BA) 9, but not BA 40 or the thalamus from subjects with schizophrenia and that the levels of the M4 receptor was not altered in any of these regions. These data suggest that decreases in the M1 receptor may be of particular import in the dorsolateral prefrontal cortex from subjects with schizophrenia. Moreover, it is possible that both clozapine and olanzapine may act as antagonists at all muscarinic receptors, including the presynaptic M2 receptor. This means these drugs could produce some of their therapeutic effects by causing an increase efflux of acetylcholine from the innervating cholinergic neuron. The potential outcomes from such complex pharmacology will be discussed.

S43.3

Imaging of the muscarinic cholinergic receptors in vivo in schizophrenia

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Preclinical and clinical pharmacology, neuroimaging, post-mortem studies and treatment studies suggest an alteration of the muscarinic system in schizophrenia. Likewise, muscarinic mechanisms may mediate some of the effects of different antipsychotics.

123I-IQNB is a SPECT-ligand that binds very selectively and with high affinity to all five subtypes of the muscarinic cholinergic receptors. IQNB-SPECT-imaging offers a tool to image muscarinic receptors in vivo. Comparing unmedicated schizophrenic subjects with age-and sex-matched healthy controls, we found a significant decrease of muscarinic receptor availability in different cortical and subcortical brain-regions. Treatment with the atypical antipsychotics olanzapine and clozapine significantly reduced the availability of the muscarinic receptors. In direct comparison, treatment with clozapine leads to a significantly stronger reduction of muscarinic receptor availability than olanzapine. Thus imaging of muscarinic receptors can be used to assess the effects of medications on this receptor in vivo.

Key words: Acetylcholine, PET/SPECT, schizophrenia

S43.4

Comparative muscarinic receptor pathology in developmental and degenerative disorders of the human brain

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Muscarinic receptors mediate the effect of the cholinergic system, central to cognition and consciousness, whose dysfunction is linked to abnormal developmental and degenerative disorders and psychotic states. In autism a 30% reduction in cortical M1 receptors is similar to the reduction in schizophrenia but is in contrast to raised M1 in dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD). In Alzheimer's disease (AD) M1 cortical activation is compromised by defective coupling to G-proteins. In progressive supranuclear palsy preserved striatal M1 may explain increased Parkinsonism with AChE inhibitor, in contrast to DLB where low striatal M1 may be the corollary of no additional movement impairment with AChEI. Reduced cortical cholinergic innervation correlates with cognitive impairment and visual hallucinations in DLB/PDD (indeed the presence of hallucinations indicates likelihood of benefit from AChEI therapy). In autism and schizophrenia no presynaptic cholinergic loss is reported. Separate M2 and M4 density was measured by AFDX384 selective blockade. M4 were higher in DLB/PDD cortex but unchanged in AD. Determination of M2 and M4 receptors in chronic schizophrenia is in progress

S43.5

Desensitization of Nicotinic Receptor in the Brain

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S44.1

Bone metabolism and pathophysiology of oestrogen-related osteoporosis: cellular and molecular mechanisms of control

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The association between oestrogen deficiency and osteoporosis is well documented and oestrogen replacement during and after the menopause prevents bone loss and reduces fracture risk. The mechanisms by which oestrogen deficiency induces bone loss are not fully defined, but increased bone turnover and osteoclastic activity play a major role, whilst osteoblastic activity is decreased. The former changes are most prominent early in the menopause and result both in reduction of bone mass but and microarchitectural deterioration of cancellous and cortical bone.

The effects of oestrogen deficiency on bone are mediated by a number of mechanisms, including release of pro-resorptive cytokines from bone cells and other cells in the bone microenvironment. In particular, there is an increase in production of RANKL (receptor activator of NFkB ligand) and reduced production of osteoprotegerin (OPG). Collectively, these changes result in an increase in development and activity of osteoclasts, the latter resulting at least in part from reduced apoptosis. Prevention of these changes can be achieved not only by oestrogen but also by pharmacological agents such as the bisphosphonates, strontium ranelate and raloxifene

S44.2

In vitro and in vivo models used to study bone metabolism

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Several in vitro and in vivo models proved to be useful to study bone cell metabolism. In vitro, bone formation and bone resorption activity can be analysed using organ cultures of calvaria or long bones. Bone cell proliferation, differentiation, apoptosis, and the osteogenic and resorptive capacity can be tested using osteoblastic or osteoclastic cell cultures. Ex-vivo cultures of bone cells allow to compare in vivo and in vitro cell phenotypes. Useful in vivo models of bone loss include ovariectomy, immobilization, glucocorticoid treatment and protein/mineral deficiency. Multiple models of transgenic (TG) and knock-out (KO) mice were developped. Most relevant models are LRP5, Wnt, leptin, RANKL and OPG TG/KO which show altered bone mass, microarchitecture and resistance (analysed by BMD, micro-CT, biomechanics), and altered cell activity (measured by histomorphometry, ex-vivo cultures, gene expression). In humans, analysis of patients with genetic mutations causing increased bone formation or bone mass were also shown to be useful. These models may allow to identify novel mechanisms that control bone metabolism and to develop new treatments for osteoporosis.

Key words: Bone metabolism, osteoblast, osteoclast

S44.3

Bone biochemical markers and techniques for bone mass evaluation to be used in pharmacology

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Osteoporosis is defined as a bone mineral density (BMD) of 2.5 standard deviation or more below the young normal mean. BMD. Dual X-Ray densitometry is the state of the art technique in assessing BMD. Ultrasound assessment is correlated with BMD measurements by DEXA, but is less repeatable and less applicable clinically.

QCT and MRI are techniques which are mainly used to assess BMD in the research setting.

Biochemical markers of bone formation include Bone specific alkaline phosphatase, Procollagen type I propeptides (PINP) and Osteocalcin

Biochemical markers of bone resorption include Deoxypyridinoline cross-link (in urine), C and N-telopeptides of type I collagen cross-link (in serum and urine)

In clinical trials, the percentage decrease in bone turnover markers correlates with the change in BMD at 2 years. In women aged 75 years or older, urine C-telopeptide and free deoxypyridinoline cross-link of type I collagen have been shown to be independent predictors of an increased risk of hip fracture, and their combination with low BMD is an even stronger predictor.

Biochemical markers can hence be used to complement BMD testing for assessment of fracture risk and to monitor response to drugs. However, it cannot replace DEXA in the assessment of fracture risk and response to treatment.

S44.4

Biomechanical Exploration of Bone Tissue

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Bone mechanical properties represent the most important end point in preclinical study of osteoporosis since bone fragility is a key element of fracture risk. Bone strength can be measured at different skeletal sites taking into account the relative contribution of cancellous and cortical tissue. Axial compression of vertebrae or metaphysis and bending test of long bones provide information on maximal load, stiffness and energy. Bone strength is determined by bone geometry (diameter, thickness), trabecular microarchitecture and intrinsic properties of bony tissue (material quality). Recently, nanoindentation has been proved to be a reliable method to assess the intrinsic mechanical properties of single bone structural units like hardness, modulus and working energy. Investigation of these determinants is of major importance since new antiosteoporotic drugs potentially influence all these determinants, including intrinsic bone tissue quality. Similar investigations are also feasible using human bone biopsy. Careful investigation of biomechanics and all its determinants should be considered to fully understand the mechanisms of action of antiosteoporotic drugs.

Key words: Biomechanics, bone, osteoporosis, safety

S44.5

Molecular mechanisms underlying the effects of anabolic agents

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Agents able to increase bone mass in adults by stimulating bone formation are an important therapeutic advancement. Recent discovery of signal transduction pathways and transcription factors critical for osteoblast differentiation and function have opened new approaches. The transcription factors Runx2 and osterix are critical for osteoblast differentiation. Recent identification of the Wnt signalling pathway is of particular interest as LRP5 a co-receptor for Wnt has been shown to play an important role in determining bone mass. BMP2, a growth factor inducing osteoblast differentiation can interact with the Wnt pathway. Inactivation of sclerostin, an inhibitor of both Wnt and BMP2, induces high bone mass. All of these proteins or transcription factors could be target for anabolic treatment. However, the precise targets of existing anabolic agents for bone are is still elusive. Parathyroid hormone administrated intermittently at low dose has been shown to prevent osteoblast apoptosis. Stontium ranelate increases osteoblast proliferation but its molecular target is unknown. Mechanical loading is anabolic for bone and Wnt signalling is important for its action.

S44.6

Optimization of bone formation and bone resorption: mode of action and clinical benefits of strontium ranelate

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Current anti-osteoporotic therapies are based on anticatabolic or anabolic effect on bone remodeling. Strontium ranelate decreases bone resorption and increases bone formation in vitro and in vivo in animal models. Efficacy of strontium ranelate (2 g orally per day) was assessed two double-blind placebo-controlled trials for the prevention of vertebral and peripheral fractures. The SOTI study included 1649 osteoporotic postmenopausal women with at least one prevalent vertebral fracture. A risk reduction of vertebral fracture was obtained after 3 years with strontium ranelate (RR 0.59). An early and significant increased in bone-specific alkaline phosphatase (8.1%) and a reduction in serum CTX (12.2%) levels was observed and sustained for 3 years. The TROPOS study, designed to assess the prevention of nonvertebral fractures, .included 5091 postmenopausal women with osteoporosis. Fracture risk was reduced by 19% for major fragility fractures and in a subgroup of 1977 women at high risk of hip fractures. Strontium ranelate was well tolerated without difference in serious adverse events between groups. This demonstrates that strontium ranelate is effective for the treatment of postmenopausal osteoporosis.

S45.1

The Microvasculature as a Therapeutic Target in Diabetes: Possible Influence of Vascular Heterogeneity.

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Vascular disease remains a major cause of morbidity and mortality in diabetes mellitus, in spite of recent improvements in outcome. Therapeutic strategies aimed directly at preventing, or minimizing the extent of, these sequelae are required as an adjunct to treatments directed at normalizing the metabolic state. The microvasculature, and the endothelium in particular, are early contributors to vascular dysfunction, thus raising the question as to how best to specifically target this aspect of the vasculature. However, the expansive nature of the microvasculature, the varying demands that tissues have in terms of blood flow and the heterogeneity that exists between endothelial cells in different sites raises potential problems as to the practicality of such an approach. Similarly, heterogeneity exists at the level of microvascular smooth muscle. For example, variation exists with respect to mechanisms regulating basal myogenic tone and modes of cellular communication between the muscle and endothelial layers. Further, temporal and genetic factors in the genesis of diabetic microvascular dysfunction may impact on therapeutic strategies. It is suggested that a systematic approach is required to understand the heterogeneity of the microvasculature, with particular emphasis on relating differences in gene and protein expression with functional properties. Such an approach may then provide the necessary information to allow exploitation of microvascular heterogeneity for unique targeted interventions as well as providing the necessary rationale for pharmacological interventions (both prophylactic and corrective) aimed at the microvasculature as a whole.

S45.2

Oxidant mechanisms in diabetic complications

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Hyperglycemia and hyperlipidemia decrease formation of the vasodilators, nitric oxide and prostacyclin, and increase formation of vasoconstrictor eicosanoids which exacerbate diabetic vascular disease. A key alteration in endothelial cell phenotype is increased formation of reactive oxygen species. This is in part due to the functional uncoupling of endothelial nitric oxide synthase, such that it generates superoxide anion in addition to NO. This is responsible for nitric oxide synthase to produce peroxynitrite, a damaging molecule. Peroxynitrite inactivates prostacyclin synthase leading to the accumulation of inflammatory and prothrombotic eicosanoids. This not only helps to explain the impairment of endothelial vasodilator mechanisms, but also increased progression of vascular disease. Many of the cellular abnormalities can be prevented by adequate scavenging of oxygen-derived free radicals or by blocking the actions of the eicosanoids at TP receptors. This pathophysiological mechanism is highly relevant to diabetic complications involving not only the aorta, but also the microvasculature, including that of the kidney and heart.

S45.3

OXIDATIVE STRESS AND PROINFLAMMATORY MECHANISMS IN THE VASCULAR COMPLICATIONS OF DIABETES

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Diabetic vessels present both pro-oxidant and pro-inflammatory status, as diabetes is a low grade inflammatory disease. In human vascular smooth muscle (HASMC) and endothelial cells (HUVEC) the expression of pro-inflammatory molecules, like inducible nitric oxide synthase (iNOS), adhesion molecules (VCAM-1 and ICAM-1), the transcription factors activator protein-1 (AP-1) and nuclear factor k-B (NF-kB) was analyzed in response to high glucose or non-enzymatic glycosylated hemoglobin. In HASMC, high glucose by itself had no significant effects, although it concentration-dependent enhanced the stimulatory effect of the cytokine interleukin-1beta on iNOS, ICAM-1 and NF-kB expression. Furthermore, glycosylated hemoglobin also stimulated the expression of the inflammation-related transcription factors AP-1 and NF-kB. When HUVEC were analyzed, high glucose levels leaded increased expression of adhesion molecules (VCAM-1 and ICAM-1), which indeed markers of vascular inflammation. In conclusion, non-enzymatic glycosylation adducts can directly promote inflammation of vascular cells, while high glucose levels dramatically increase the effects of pro-inflammatory cytokines on the vascular wall.

S45.4

Cardiovascular complications of diabetic rats respond to a novel endothelin receptor antagonist CPU0213.

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Cardiovascular complications of diabetes are likely the consequence to insults to vascular endothelium. It is to test if an upregulation of the endothelin system is involved and responds to a novel low selective endothelin receptor antagonist CPU0213. METHODS: The diabetic rats were developed by single injection of streptozotocin 65 mg/kg ip. The expression of the calcium regulating system, the endothelin system, the iNOS and cNOS in tissue (heart, kidney and aortic wall) were evaluated in the control, untreated and treated with CPU0213. RESULTS: An impairment in vasorelaxation and compromised NO bioavailability were found in the untreated against control. A down-regulation of the RyR2, FKBP12.6,

SERCA2a and PLB was found in diabetic cardiomyopathy, together with an up-regulation of the iNOS and cNOS. An alteration in the redox system showed a state of oxidative stress and an up-regiulated ET system was found in the three tissues. These were attenuated significantly by CPU0213. CONCLUSION: Cardiovascular complications of disbetes are mediated by an activated ET system in the myocardium, renal tissue and vasculature.

Key words: diabetes; endothelin; heart; kidney
Supported by NSFC No: 30231703

S46.1

Gastroesophageal Reflux Disease in Asian Pacific, A New Disease

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Gastroesophageal reflux disease (GERD) occurs commonly in the west: 10-30% of the western population have GERD, and affects 20-44% of Americans monthly or weekly. GERD, historically considered absent in Asia, has emerged as a new and common condition in this region. Using a validated questionnaire, we estimated the annual, monthly and weekly prevalence of GERD of 29.8%, 8.9% and 2.5% respectively in Chinese. The symptoms were associated with non-cardiac chest pain (OR 2.3, 95%CI 1.7-3.1), dyspepsia (OR 1.9, 95%CI 1.4-2.5), globus (OR 1.8, 95%CI 1.2-2.7), acid feeling in stomach (OR 5.8, 95%CI 4.5-7.5) and NSAID use (OR 2.3, 95%CI 1.5-3.6). After one-year, the prevalence in this cohort increased to 34.1%, 10.1% and 2.7% respectively. A long-term prospective study in Chinese found that the endoscopic prevalence of esophagitis, hiatus hernia, benign esophageal stricture and Barrett's esophagus was 3.8%, 1.7%, 0.08% and 0.06% respectively, and most esophagitis cases (94%) were mild (LA grade A/B). Similar data have been reported in other Asian populations. Chinese patients with GERD had a lower rate of transient lower esophageal sphincter relaxations compared to the western population.

S46.2

Acid Supressant Agents: Do Differences in Pharmacokinetics Translate into Differences in Clinical Outcome

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PPIs are more effective than H2RA in treating GERD, healing DU & GU, and preventing NSAID induced ulcers. In peptic ulcer bleeding, PPI significantly reduced rebleeding rate.

Current PPIs are delayed-release enteric-coated preparations. A new immediate-release omeprazole (IR-OME) has been introduced. The time to maximum concentration was shorter and the reduction of gastric acid concentration was faster than delayed release omeprazole. Repeated bedtime dosing with IR-OME was significantly better in preventing nocturnal acid breakthrough (NAB) than other PPIs. Tenatoprazole is a new PPI that has a 7-fold longer plasma half-life than other PPIs. It prevented NAB more effectively than other PPIs. There is genetic polymorphism in PPI metabolism via CYP2C19. In H.pylori eradication, a significantly lower eradication rate was seen in extensive metabolizers (EM) for omeprazole and lansoprazole but not rabeprazole. Esophagitis healing rate was lower for EM with lansoprazole but not rabeprazole.

PPIs are superior to H2RA in the management of acid-related disorders. Among PPIs, differences in pharmacokinetics such as bioavailability, half life and metabolism may translate into differences in clinical outcome.

S46.3

The Putative Mechanisms for Proton Pump Inhibitor's failure in Patients with Gastroesophageal Reflux Disease

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The most common manifestation of proton pump inhibitor failure is continuation of classic GERD symptoms (heartburn, acid regurgitation) despite PPI therapy. Other less common manifestations include antacid consumption, the presence of erosive esophagitis and abnormal acid exposure while on PPI. PPI failure is generally defined as patients who have failed to obtain satisfactory symptomatic response to a course of standard dose PPI (once a day). It has been estimated that up to 30% of the patients that consume PPI once daily continue to report typical GERD symptoms despite treatment.

Putative mechanisms for PPI failure include compliance, Helicobacter pylori infection status, bioavailability, nocturnal acid breakthrough, rapid metabolism, PPI resistance, duodenogastroesophageal reflux, non-acidic gastroesophageal reflux, delayed gastric emptying, visceral hypersensitivity, psychological co-morbidity and emotional stress. Of those, compliance, delayed gastric emptying and visceral hypersensitivity have the highest clinical relevance. The role of duodenogastroesophageal reflux and non-acidic gastroesophageal reflux in PPI failure remains to be elucidated.

Key words: GERD, PPI, Heartburn and Compliance

S46.4

Current situation of GERD in China

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In recent years GERD has been gradually recognized as a new disease in China. The risk factors of GERD are lifestyle, changes of the structure of diet, and obesity, etc. In 1999 the epidemiological study in Beijing and Shanghai showed that the incidence of GER symptoms were 8.97%, GERD 5.77% and reflux esophagitis 1.92%. In Guangdong, the prevalence of GERD was 2.5%, but in Xi'an, the GER symptoms were as high as 16.98%. Non-erosive reflux disease (NERD) is more common than erosive esophagitis (EE) in China. The endoscopic findings of EE in Chinese are usually mild or moderate. The macroscopic observation of NERD may be normal at endoscopy, but there are mini changes at the distal part of esophagus by chromoendoscopy. Currently PPI Test (standard dose of PPI b.i.d. for 7 days) is a most simple and convenient method for diagnosis of GERD with the sensitivity 88.1%, and specificity 44%. The treatment of GERD includes the change of lifestyle and suppression of acid secretion (PPI and H2-RA) with step-down manner; long-term or on-demand treatment with PPI is necessary. Surgical and endoscopic treatment of GERD are rarely performed in China.

S47.1

The Renin-Expressing Cell and Development of the Kidney Vasculature.

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The renin-angiotensin system (RAS) is known for its regulation of blood pressure and electrolyte homeostasis through renin release from juxtaglomerular (JG) cells. It is now clear that the RAS is, also, required for normal renal development and that renin expression occurs throughout the developing renal vasculature. To characterize the transcriptome of the renin-expressing cell at different stages of development, transgenic mice expressing green fluorescent protein (GFP) under control of the renin promoter were used as a source for renin-expressing cells which were collected by fluorescence-activated cell sorting (FACS). Expression profiles were determined for both the GFP-positive and the total presorted cell populations using Affymetrix microarrays. Transcripts exhibiting enrichment or de-enrichment in the GFP-positive cell fraction were identified and results for selected transcripts of interest were validated by real time RT-PCR. The results support the hypothesis that the renin-expressing cell found in association with the renal vasculature during kidney organogenesis is an activated vascular pericyte.

Key words: renin-expressing cell, gene expression
Supported by HL48459 and CA16056.

S47.2

Electrophysiology of the Renin-Producing Juxtaglomerular Cells

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The rate of renin secretion from renal juxtaglomerular (JG) cells is the major determinant of the activity of the renin-angiotensin system. The whole-cell patch clamp method allows the study of exocytosis in JG-cells. β-Adrenoceptors, IP, EP2 and EP4 receptors are all associated with JG-cells and their activation leads to rapid cAMP/PKA-mediated exocytotic fusion and release of renin granules.

Degradation of cAMP by PDE3 and PDE4 contributes to regulation of renin release. Thus, stimulation of renin release by cGMP involves inhibition of PDE3 resulting in enhanced cAMP formation. Electrophysiological studies of JG cells demonstrate the presence of large voltage-sensitive, calcium-activated potassium channels (BKCa) of the ZERO splice variant, which is also activated by cAMP. These channels explain the hyperpolarisation, which has been observed after stimulation of renin release with cAMP. In addition, JG cells express functional L-type voltage-dependent calcium channels (Cav 1.2), which in situations with strong depolarization lead to calcium influx and inhibition of renin release. In most in vivo situations the membrane potential is probably protected against depolarisation by the BKCa channels.

S47.3

Differentiation of Renin Cells and Homeostasis

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When the integrity of our extracellular volume is compromised or there is a threat to tissue perfusion (hypotension, dehydration, hemorrhage), there is an increase in circulating renin, achieved by increasing the number of cells that synthesize renin. This increase occurs by de-differentiation of pre-existing cells (smooth muscle, mesangial, adrenal cells) that re-acquire the ability to synthesize renin until blood pressure and fluid/electrolyte balance are back to normal. Once normality is re-established, the cells differentiate again but maintain their capacity for de-differentiation, ready to be activated again when the physiological circumstances require them to maintain homeostasis. This ability is determined and constrained by the developmental history of our cells and constitutes a fundamental mechanism to preserve well being. The molecules controlling the identity of renin cells are beginning to be identified. Recent experiments implicate the Ets-1 gene as an important regulator of renin cell identity and therefore homeostasis

S47.4

Posttranscriptional Regulation of Renin Synthesis, Function and Potential Targets

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New potential targets for the renin angiotensin system are localized at the site of transcription and posttranscriptional control. Conservation of nucleotide sequences throughout different species suggests that there are functionally important binding regions. Quantitatively, a very important regulatory step of renin (REN) synthesis occurs after transcription. Although posttranscriptional REN mRNA stabilization contributes to developmental or cAMP-based upregulation of renin synthesis, very little is known about the mediators of mRNA stability. Moreover, it remains to be unraveled how REN mRNA interacts with intracellular structures to target REN mRNA in such a way that renin can be efficiently deposited in storing vesicles. Determinants involved in control of functional properties of mRNAs such as translational efficiency, metabolic stability, or intracellular localization reside predominantly in 5'- or 3'-untranslated regions (UTRs) of the mRNA. Here we report of proteins that interact with REN mRNA 3'-UTR. Functionally, we can show that the cAMP-based increase of REN mRNA stability is accompanied by an upregulation of REN mRNA binding proteins that are known for their mRNA stabilizing potentia

S48.1

Pacemaker channels in the heart: physiology, pathology and pharmacology

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"Funny" (f) channels underlie the cardiac "pacemaker" If current, an inward current activated on hyperpolarization to voltages that are in the diastolic range of sino-atrial node cells. The degree of current activation determines the slope of diastolic depolarization, and hence heart rate; If being directly modulated by cAMP, underlies the regulation of cardiac rate by - adrenergic and muscarinic stimulation. If is also present in non automatic cardiac tissue. Electrophysiological and molecular data demonstrated that f-channels are present in ventricular cardiomyocytes. In cardiac hypertrophy and failure, If current density and/or mRNA levels of f-channels are increased compared with controls. Over-expression of f-channels in non-pacemaker cells may represent an arrhythmogenic mechanism in heart failure. Inhibition of the pacemaker If current to induce a direct and selective decrease in heart rate represents an attractive therapeutic approach for coronary artery disease. Substances acting as selective f-channel inhibitors, such as ivabradine, will be useful in treating diseases such as chronic angina and heart failure and will help to assess the potential arrhythmogenic role of If in heart disease.

S48.2

Molecular analysis of HCN pacemaker channels: from genes to drug targets

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Hyperpolarization-activated cyclic nucleotide-gated channels (HCN1-4) play a crucial role in the control of rhythmic activity in heart and brain. Impaired HCN channel function has been linked to a variety of diseases including cardiac arrhythmia, epilepsy and neuropathic pain. Hence, HCN channels represent promising targets for the development of novel drugs. We have analyzed cellular factors that control the activity of HCN channels in a physiological setting. We show that HCN1-4 are regulated to different extent by a number of low molecular factors (e.g. Cl-, cAMP and protons) as well as by tyrosine phosphorylation. We provide evidence that HCN channels are efficiently blocked by some members of the class of imidazolines. In particular, clonidine, an established agonist of alpha-2-adrenoceptors, reversibly binds to and inhibits the sinoatrial HCN channel. As a consequence, clonidine profoundly reduces the frequency of pacemaker potentials in sinoatrial cells and induces bradycardia in mice deficient for all three isoforms of alpha-2 adrenoceptors. Our results suggest that clonidine may serve as a template for the design of novel HCN channel blockers.

Key words: pacemaker, HCN channel, clonidine.

S48.3

Analysis of pacemaker channel function by gene deletion

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The hyperpolarization-activated current Ih has been implicated in diverse physiological functions including pacemaking and motor learning. Ih is perhaps best known as cardiac pacemaker current because it is thought that this current constitutes the major component of the spontaneous diastolic depolarization. The increase in heart rate following β-adrenergic stimulation has been attributed to cAMP-mediated enhancement of the current. Ih is generated by four HCN channel genes. As HCN4 constitutes the predominant HCN isoform in the sinoatrial node, we tried to determine the function of this channel by generating HCN4-deficient mice. Global knockout of HCN4 results in embryonic lethality. Hence, we used a ligand-inducible Cre recombinase to delete HCN4 in a temporally controlled manner. We demonstrate that HCN4 generates the main part of Ih in sinoatrial node cells. Adult mice lacking HCN4 in the cardiac conduction system display repetitive asystolic phases. However, the mutants can increase their heart rate during sympathetic stimulation. These results indicate that HCN4 is necessary for maintaining a regular cardiac rhythm especially in the transition phase from an increased to basal heart rate.

S48.4

Anti-ischemic efficacy of the cardiac pacemaker channel inhibitor ivabradine

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Ivabradine induces a selective heart rate reduction by inhibiting the cardiac pacemaker current If. Ivabradine and propranolol were compared in an experimental model of exercise-induced regional myocardial ischemia in Yucatan micropigs. Both compounds, administered orally at 5 mg/kg, induced a similar heart rate reduction at rest and during exercise. Ivabradine, unlike propranolol, did not de-

crease myocardial contractility at rest and during exercise. Ivabradine reduced by 80% the exercise-induced ST segment shift of ECG in the ischemic area as propranolol, but better improved the myocardial contractile dysfunction.

The anti-ischemic efficacy of ivabradine was tested in approximately 5000 patients with stable angina. In a double-blinded trial, including 939 patients with stable angina, ivabradine (5 mg bid for 4 weeks followed by either 7.5 or 10 mg bid for 12 weeks) was at least as effective as atenolol (50 mg od for 4 weeks and 100 mg od for 12 weeks) in improving all criteria of exercise tolerance tests and in decreasing the number of angina attacks.

Ivabradine is at least as potent as a β-blocker in limiting exercise-induced myocardial ischemia

Key words: Heart rate, ivabradine, myocardial ischemia, exercise

S48.5

Cardioprotective Effects Induced by Heart Rate Reduction

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Heart failure is a major health problem, and is one of the few cardiovascular diseases that increased its prevalence over the last decade. Increased heart rate, generally observed in patients with heart failure, is involved in the deterioration of cardiac pump function. However, the effects of 'pure' heart rate reduction on the progression of heart failure are unknown. In a rat model of heart failure, ivabradine, a blocker of If channels. reduced dose-dependently heart rate without modification of blood pressure. This heart rate reduction was associated with an improvement in cardiac function. After chronic administration, this improvement of cardiac function persisted after ivabradine withdrawal, revealing an improvement in intrinsic myocardial function. This beneficial effect could be explained by direct effects of heart rate reduction induced by ivabradine, i.e. improved myocardial oxygen supply to demand ratio, and/or myocardial tissular effects induced by chronic decrease in heart rate such, i.e. decreased extracellular collagen accumulation, increased myocardial microcirculation. In conclusion, we show for the first time that a chronic decrease in heart rate can be beneficial in heart.

L21

ANGIOGENESIS: FROM PLANTS TO BLOOD VESSELS

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Since the first reports on tumour angiogenesis in the 1970s, major advances have been made in the understanding of the cells and agents that are involved in this process. Of all the angiogenesis factors, vascular endothelial growth factor (VEGF) has been recognised as the most important target. To date, stimulation or inhibition of angiogenesis by VEGF-based approaches has produced encouraging clinical results in treating angiogenic diseases such as coronary heart disease and malignancy. Recent studies have shown that plant-derived anticancer drugs such as taxol and camptothecin are also antiangiogenic. In our quest of angiogenesis modulators from traditional Chinese medicine (TCM), we revealed distinct "sterol ginsenoside" fingerprints of Chinese, Korean, Sanqi and American ginseng by mass spectrometric compositional analysis. Parallel functional studies demonstrated that the angiogenic or antiangiogenic property of a ginseng preparation is determined by its triol/diol ratio. We also identified several angiogenesis modulators from Sinomenium acutum and Salvia miltiorrhiza. The future prospects of TCM and other medicinal plants in the development of multi-targeted angiotherapy will be discussed.

L22

Modular Assembly of G Protein-Coupled Receptor Signalosomes

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G protein-coupled receptors (GPCRs) form functional homo- and heterodimers that assemble into modular signalling complexes. In addition to their cognate G proteins, various scaffolding and signalling partners can be recruited to the receptors thus determining the selectivity and efficacy of the "signalosomes". Although the occurrence of these complexes has been well investigated in vitro, their ontogeny and dynamic regulation in cells are still poorly understood. To directly assess the real-time assembly of GPCR oligomers and signalling complexes in living cells, we used a combination of biochemical and biophysical approaches. In particular, multiplexing Bioluminescence and Fluorescence Resonance Energy Transfer (BRET and FRET) techniques allowed to monitoring the assembly of multiple partners simultaneously. In addition to play an important role in the ontogeny and trafficking of the signalling complexes, the occurrence of receptor oligomerization offers combinatorial possibilities to increase the pharmacological and functional potential of GPCRs. Modulation of the oligomeric assemblies offers new strategies to pharmacologically regulate signalling eficacy through these important drug targets.

L24

COX-2: A Pivotal Enzyme in Mucosal Defence and Resolution of Inflammation

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The discovery of a second isoform of cyclooxygenase, called COX-2, resulted in enormous efforts to develop selective inhibitors of this enzyme as anti-inflammatory and analgesic agents that would not induced damage in the gastrointestinal tract. While this promise has only been partially fulfilled, and the cardiovascular toxicity of some NSAIDs has become better recognized, the advent of these compounds did open the door to investigations of the roles of COX-2 in various physiological and pathological conditions. It is now clear that COX-2 plays very important roles in mucosal defence in the gastrointestinal tract and lung, as well as in the repair of injury in these and other tissues. COX-2 also contributes to the process of resolution of inflammation. Moreover, prolonged elevation of COX-2 expression and activity after an inflammatory event may contribute significantly to persistent symptom generation and to predisposition to dysplastic changes. COX-2, and its downstream products, therefore remain of great interest as potential therapeutic targets for a wide range of disorders.

S49.1

Genetic manipulations of hormonal signaling in the hippocampus.

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Glucocorticoids (GCs), the adrenal steroids released during stress, compromise the ability of neurons to survive neurological injury. In contrast, estrogen protects neurons against such injuries. We designed three genetic interventions to manipulate GCs actions, which reduced their deleterious effects in rat both in vitro and in vivo. The most effective was a chimeric receptor combining the ligand-binding domain of the glucocorticoid receptor (GR) and DNA-binding domain of the estrogen receptor. Expression of this receptor reduced hippocampal lesion size after neurological damage by 63%, and reversed the outcome of the stress response by rendering GCs protective rather than destructive. Our findings elucidate three principal steps in the neuronal stress response pathway, all of which are amenable to therapeutic intervention.

GCs are also implicated in reducing adult hippocampal neurogenesis. There has been little evidence for the presence of type 1 GR or type 2 (mineralcorticoid) receptors in neuronal precursor cells (NPC), and therefore suggested that GCs must indirectly inhibit NPC proliferation, though the mechanism has remained obscure. We demonstrate that GR mRNA is transcribed and yields a cytoplasmalocalized receptor in isolated NPC from the adult hippocampus. Treatment of NPC grown in vitro with GCs induces decreased proliferation index, and a down-regulation of Nestin, a protein marker that is down regulated as NPC stop dividing and differentiate. This response is blocked using the GR-specific antagonist indicating that the GCs response is mediated by the glucocorticoid receptor. The apparent responsiveness of NPC to GCs suggests that neurogenesis may be directly modulated via GR signalling pathways.

S49.2

The neurovascular unit: new targets in the prevention and treatment of neurological disorders

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Common brain insults such as trauma, ischemia, infectiouns and neurodegenerative diseases are associated with vascular pertubations and opening of the blood-brain barrier (BBB). To explore the role of BBB lesion in pathogenesis of brain

insults we induced a prolonged, focal BBB disruption in the rat neocortex using bile salts. We show that penetration of serum albumin into the brain's extracellular space is associated with uptake of albumin into astrocytes. This triggers a transcriptional change and consequent alteration in the structure and function of astrocytes. Transcriptional changes include the down-regulation of the inward rectifying potassium channels and glutamine synthase, leading to the accummulation of potassium and glutamate in the extracellular space. The resulted activity-dependent enhanced neuronal excitability lead to non-specific synaptic plasticity followed by neuronal toxicity. We further show a critical role for transforming growth factor beta receptors in the uptake of albumin. We propose that interactions between endothelial cells, astrocyte and neurons leads to astrocytic dysfunction, hypersynchronous neuronal epileptiform activity and brain dysfunction.

S49.3

Specific inactivation of the Glucocorticoid Receptor gene in the Dopaminergic system: New insights on drug addiction.

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Stress release of glucocorticoid hormones modulates behaviors including addiction and emotional behavior. We previously showed using the Cre/loxP system that the selective inactivation of the GR gene in mice brains (GRNesCre) profoundly reduces motivation for cocaine. More recently, we showed that these behavioral effects are associated with a change in the impulse activity of midbrain DA. To determine in which cell type the function of GR is required to modulate motivation for cocaine, we generated animal models in which GR is selectively inactivated in either pre-synaptic DA neurons (GRDATCre) or post-synaptic cells (GRD1Cre). For this, we generated a mouse transgenic line that expresses the Cre recombinase under the control of the DA Transporter gene (BAC-DATCre) and used a transgenic mouse line that expresses the Cre under the control of the DA receptor 1A gene (YAC-D1Cre, T. Lemberger). Characterization of these models will be presented. To address the question of the interaction of GCs and serotonergic pathway, we generated a mouse transgenic line that allow Cre recombination in all 5-HT1A neurones and obtained conditional GR inactivation. Analysis of this animal model will be presented.

S49.4

Consolidation of fear memories

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Fear is an adaptive response that initiates defensive behavior to protect animals and humans from danger. During fear conditioning, animals receive an aversive electric shock in a precise relationship with environmental cues. This paradigm has been demonstrated to be a valuable model of learning and memory. Previous exposure to stressful events can either facilitate or inhibit fear conditioning. Inappropriately regulated fear is at the root of a variety of mental disorders such as phobias, generalized anxiety and posttraumatic stress disorder. Corticotropin-releasing factor (CRF) plays an important role in mediating neuroendocrine, autonomic, and behavioral responses to stress. CRF stimulates the HPA axis by increasing the secretion of ACTH and glucocorticoid hormones and also acts centrally as neuromodulator. Luciferase reporter assays deciphered molecular and electrophysiological mechanisms of CRF and of potential glucocorticoid downstream effectors underlying stress-related modulation of fear memory consolidation in mice.

Key words: stress, fear conditioning, hippocampus, LTP

Acknowledgments: This work was supported by the Max-Planck Society and NIH grant 2U54NSO39406-06.

S49.5

Anxiety Reactions as a Neuroprotection Strategy: Acetylcholinesterase modulations under stress and neurodegenerative diseases as a case study

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Anxiety reactions involve complex interactions of genomic, environmental and experience-derived factors, and anxiety disorders present a major health problem. Anxiety induced changes in cholinergic neurotransmission modulate the motor control over movement, the consolidation of traumatic memories, and brain-to-body communication through the neuron-immune interface. Specifically, anxiety-associated changes in acetylcholinesterase (ACHE) gene expressin modify the composition of protein variants all hydrolyzing acetylcholine but possessing distinct N- and C-termini due to alternate promoter usage and 3'-alternative splicing, and showing distinct non-hydrolytic properties, protein partner interactions and signaling properties. Changes in their composition protect both blood and nerve cells from acute insults, but also entail long-term damages. Variant-specific involvement of distinct AChE variants in Alzheimer's and Parkinson's disease and in neuromuscular syndromes like myastheria gravis anticipate therapeutic needs for drugs targeting the corresponding RNA transcripts.

Key words: Acetylcholinesterase; Anxiety; Neurodegeneration; Neurogenetics.

Supported by the Israel Science Fund.

S49.6

Chromatin in embryonic stem cell neuronal differentiation

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Embryonic stem (ES) cells, derived from the inner cell mass of the blastocyst, are unique in their ability to both self-renew and to differentiate into the three germ layers. Here we show global changes in nuclear architecture and chromatin structure during ES cell neuronal differentiation. A hyperdynamic pool of chromatin proteins is loosely bound in ES cells and become tightly associated with chromatin in neuronal progenitor cells (NPCs). The levels of activity-associated histone marks, such as acetylated histones H3 and H4, are reduced during neuronal differentiation, while H3-tri MeK9 is increased. Moreover, genomic tiling arrays, uridine incorporation assays and RT-PCR experiments revealed higher global transcriptional activity in ES cells compared with ES-derived NPCs and neurons. Taken together, these results suggest that chromatin is a fundamental regulator of neuronal commitment and that gene silencing by chromatin condensation and heterochromatin formation promotes neuronal differentiation.

S50.1

Contrd of cardiovascular and renal function through COX1 and COX2 derived prostanoids

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Prostaglandins (PGs) are are derived from cyclooxygenases (COX1 and 2) and critically regulate cardio-renal function. We performed studies to determine whether COX1 versus COX2 derived (PGs) exert different effects on blood pressure and renal function in mice.

Mice were treated with COX1 or COX2 selective inhibitors followed by an infusion of Angiotensin-II (AngII). COX2 inhibition augmented the pressor effects of AngII, whereas COX1 inhibition reduced the pressor action of AngII. PGE2 wa the major AngII stimulated product in renal cortex and medulla followed by PGI2 (6-keto PGF1), and PGF2. Intravenous infusion of Ang II significantly increased renal medullary PGE2 and PGI2 via COX2. Thus COX2 derived PGE2 may counteract the pressor effects of AngII. EP2 knockout mice lose the normal depressor effect of PGE2 and exhibit increased AngII pressor activity consistent with EP2 receptors buffering AngII hypertension. Conversely the pressor effect of AngII was reduced in EP1 knockout mice consistent with a pressor effect of EP1 receptors. These studies further suggest COX1 and COX2 derived PGE2 may have differential access to specific vasoconstrictor and vasodilator PGE2 receptors respectively.

S50.2

Cydooxygenases and prostaglandin receptors in human kidneys

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Selective inhibitors of cyclooxygenase-2 (COX-2) cause adverse renal effects. We hypothesized that COX-2 is expressed constitutively in human kidneys. Analysis of nephrectomy samples showed that both COX-isozymes were expressed in all zones of normal kidneys at the mRNA and protein levels. COX-1 was associated with collecting ducts and mesangial cells. In contrast to rodents, COX-2 immunoreactivity appeared in pre- and postglomerular vessels and was particularly

prominent in vasa recta bundles and medullary capillaries. COX-2 was observed in macula densa in fetal but not adult kidneys. COX-2 co-localized with the PGE2-EP4 receptor. Chronic renal artery stenosis was associated with a significantly increased renal vascular COX-2 expression. Serum exposure stimulated COX-2 mRNA accumulation and prostacyclin production in cultured human arterial smooth muscle. A calcineurin inhibitor, cyclosporine, and glucocorticoid inhibited serum-induced COX-2 mRNA and prostacyclin accumulation. We conclude that COX-2 expression in human kidneys is markedly different from rodent kidneys and that inhibition of COX-2 may have different consequences in humans compared to rodents related to renal vascular function

S50.3

Function of renal prostaglandins in clinical syndromes

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Prostanoids are important fatty acid metabolites involved in different physiological and pathophysiological processes of different organs. Prostanoids signal via specific G-protein coupled receptors linked to intracellular signaling systems, such as cAMP and Ca^{2+}. Within the kidney, prostanoids participate in the regulation of renal blood flow, electrolyte reabsorption, renin activation and probably also in nephron development. The underlying molecular mechanisms are not delighted in detail. Clues to such mechanisms may be given by renal pathologies. An outstanding role of PGE2 is attributed to salt losing tubulopathies, such as the antenatal Bartter Syndrome which is characterized by increased PGE2 excretion, high renin activity and normotension. Another example represents cyclooxygenase-2 (COX-2) dependent nephrogenesis. Lack of COX-2 gene or inhibition of COX-2 activity leads to renal dysgenesis. A role of prostanoids for normal renal maturation is suggested. Elucidation of the pathogenesis of such renal diseases will not only help us to develop therapeutical approaches, but also to expand our knowledge of prostanoid dependent regulation of kidney functions.

S50.4

Protection from Lipopolysaccharide (LPS) induced organ failure by COX-2 inhibitors

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LPS mimicking bacterial endotoxemia commonly induces expression of cyclooxygenase-2 and NO synthase-II and suppresses the expression of vasoconstrictor receptors. In the kidney LPS induces COX-2 expression predominantly in the renal medulla, mainly in interstitial and collecting duct cells. We determined the relevance of COX-2 for the adverse effects of LPS on cardiovascular function including the kidney. Therefore SD-rats received a single i.v. dose of LPS and were treated with different cyclooxygenase inhibitors. LPS markedly lowered systolic arterial pressure and increased heart rate from. Both cardiovascular changes induced by LPS were almost prevented by the COX-2 blocker rofecoxib. The characteristic LPS-induced increases of NO synthase II and COX-2 gene expression, as well as the downregulation of vasoconstrictor receptors were not affected by rofecoxib. Rofecoxib markedly improved LPS-induced liver damage and the overall well-being of the animals was markedly improved, an observation that was recently also confirmed in COX-2 deficient mice. Together, our data suggest that COX-2-derived prostanoids are major mediators for the detrimental effects of LPS on cardiovascular and organ function.

S50.5

Renal medullary COX-2 in blood pressure regulation

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Our previous study demonstrates that COX-2 expression in renal medulla is stimulated by salt loading. The present study explored cellular localization and function of high salt-induced renal medullary COX-2 expression. Expression of COX-2 in renal medulla, determined by immunohistochemistry, was remarkably induced by the chronic salt loading and the COX-2 induction was found predominantly in inner medullary interstitial cells. NS-398 was chronically infused at 10 mg/kg/day to renal medulla of Sprague-Dawley rats for 5 days and blood pressure was monitored by telemetry. Intravenous infusion of NS-398 at the same infusion rate was performed to control the spillover. All animals were fed a high salt diet containing 8% NaCl during the entire experimental period. Mean blood pressure (MAP) gradually and significantly increased following intramedullary infusion of NS-398 (159.8 + 6.6 in intramedullary NS-398 group vs. 127.6 + 2.4 in intramedullary vehicle group or 133.0 + 6.2 mmHg in intravenous NS-398 group). Therefore, we conclude that COX-2 expression in renal medullary interstitial cells increases in response to chronic salt loadng and this response is essential for stabilizing blood pressure during ch

S51.1

Pharmacogenetics: theoretical background and practical problems

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Pharmacogenetics holds great promise for improving drug therapy by increasing response rates and reducing rates of drug toxicity. However, in the 50 years since the term was coined, clinical implementation has been achieved in few areas. The completion of the human genome project has re-invigorated interest in the area. We now have available an enormous amount of information on the structure of the human genome. This has been accompanied by rapid advances in genotyping technologies. This provides us with strong foundations by which to undertake studies that will enable clinical implementation. Ideally, studies need to be adequately powered, prospective in nature (except for rare adverse events), ensure that the phenotype is accurate, utilise the most modern genotyping strategies, undertake genomic control and incorporate social science elements in the design. Currently, most studies are based on candidate genes-these should look at all genes in the pharmacological pathway and evaluate haplotypes in each gene. As technologies and statistical techniques advance, and costs come down, it may be possible to undertake whole genome unbiased screens to predict drug efficacy and toxicity.

S51.2

Molecularly Targeted Cancer Chemotherapy-A Shifting Paradigm

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An improved scientific understanding of the pathogenesis of neoplasia at a molecular level has identified novel targets and strategies to treat cancer. Imatinib mesylate (Gleevec®), an ATP mimetic and competitive inhibitor of the Bcr-Abl tyrosine kinase, proved effective in treating refractory CML and GIST. The Erb2 (Her-2/Neu) receptor is overexpressed in several solid tumors and receptor blockade/inhibition of down stream signaling has clinical benefit especially in tumors with gain of function EGFR mutations. Targeting the proteosome, inhibiting intracellular protein degradation with bortezomib, has proven effective therapy in refractory myeloma. Cell cycle checkpoint (Chk1 and Chk2) inhibitors are also under intensive pre-clinical and early clinical investigation. Such cell cycle regulation modifiers have already led to novel combination strategies such as combining DNA damaging agents (e.g. alkylators) with Chk1 inhibitors to abrogate cell cycle arrest. Modulating apoptosis via suppression-inhibition of pro-apoptotic proteins is being investigated in man e.g. Bcl-2 anti-sense and YM155. Novel targeted anticancer drugs pose therapeutic challenges but offer considerable therapeutic potential.

S51.3

Analysis of Genetic Variations in the Androgen Receptor and Enzymes that Regulate Androgens

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Polymorphisms within key androgen regulatory genes may play a role in individual susceptibility to the development of prostate cancer. In order to develop individual molecular profiles for the assessment of prostate cancer, genes involved in the biosynthesis, activation, metabolism, and degradation of androgens are all potentially important. We hypothesize that men with polymorphisms within genes that positively impact androgen levels will be at higher risk for developing prostate cancer and more aggressive forms of the disease than men with the wild-type alleles. Polymorphic variations have been found in a number of potentially important genes, but most have only been studied in small, defined populations, and with-

out good control groups. To date, few if any studies have been able to assess the importance of the combination of mechanistically relevant polymorphisms on prostate cancer, or the role these variations play in the development of high-grade disease. By investigating polymorphic androgen regulatory genes, we hope to gain a better understanding of important markers of prostate cancer risk and susceptibility.

Key words: Prostate Cancer, polymorphism, androgen

S51.4

Pharmacogenetics in cancer therapy

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The association between DNA variants, treatment outcome and toxicity of selected anticancer agents is established. The application of pharmacogenetics to tumor treatment allowed the discovery of the relationship between DPD gene mutations and severe 5-FU toxicity (Ezzeldin H, et al. Clin Cancer Res 2003;9:3021-8), activating mutations of EGFR and response of NSCLC to gefitinib (Lynch TJ, et al. N Engl J Med 2004;350:2129-39), hENT expression and overall survival in pancreas cancer treated with gemcitabine (Giovannetti E, et al. Cancer Res. 2006;66:3928-35). Genetic variants of UGT1A1 gene promoter are related to severe neutropenia by irinotecan (Innocenti F, et al. J Clin Oncol 2004;22:1382-8), while MTHFR and TPMT genotypes predict for treatment response to MTX and 6-MP in childhood acute lymphoblastic leukemia (Aplenc R, et al. Cancer Res 2005;65:2482-7; Stanulla M, et al. J Am Med Assoc 2005;293:1485-9). These findings should be incorporated in current clinical practice to allow treatment selection based on individual characteristics of cancer patients instead of empiric choice of chemotherapy, to reduce the toxicity burden and personalize the treatment.

Key words: pharmacogenetics, cancer, therapy

S52.1

EDHF in Human Blood Vessels: an Overview

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Endothelium-derived hyperpolarizing factor (EDHF) is believed to hyperpolarize smooth muscle cells as transferable chemicals or electrical signals through the myoendothelial gap junctions and to relax the vessel. In the last decade, we have demonstrated that 1) in the human systemic and coronary circulations EDHF plays an important role in large and micro vessels and in arteries and veins through K+ channels; 2) EDHF-mediated hyperpolarization and relaxation are more significant in the large and small coronary arteries than in the cardiac veins; 3) in the human systemic circulation EDHF-responses are more prominent in the conduit arteries than in the large veins and are different among arteries; 4) EDHF-mediated responses are impaired under pathological conditions such as hypoxia-reoxygenation, open heart surgery, or donor heart preservation; and 5) the impaired EDHF-function may be recovered by using various EDHF analogues. These studies emphasize the physiological role of EDHF and propose the way to recover EDHF-function under pathological conditions.

Key words: EDHF; Endothelium; Human vessels
Supported by RGC of Hong Kong (CUHK4127/01M & CUHK4383/03M) and St. Vincent Medical Foundation, Portland, OR, U.S.A.

S52.2

EDHF and the vascular calciumsensing receptor

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In small arteries, endothelium-dependent myocyte hyperpolarisation was previously ascribed to a 'factor', EDHF. It is now recognised that agonist activation of endothelial cells produces the EDHF effect (myocyte hyperpolarisation and vascular relaxation) by two mechanisms, both dependent on the opening of endothelial cell intermediate-conductance and small-conductance Ca2+-sensitive K-channels (IKCa and SKCa, respectively).

A calcium-sensing receptor (CaR) (which is partially activated under resting conditions) has recently been identified in vascular endothelial cells. Activation of this receptor by calindol, a positive allosteric modulator of CaR, leads to the selective opening of endothelial IKCa channels and thus, by an EDHF-like mechanism, produces hyperpolarisation and relaxation of the vascular myocytes. Modulation of the activity of endothelial cell CaRs may provide an additional mechanism by which smooth muscle tone is regulated in arteries. Stimulation of CaR by diet-derived amino acids or polyamines may produce an EDHF-like endothelium-dependent vasorelaxation which contributes to post-prandial hyperaemia.

Key words: CaR, EDHF, K-channel
Supported by the British Heart Foundation

S52.3

Calcium events in communication between novel cells with processes and vascular smooth muscle cells

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The walls of both arteries and veins have been recently discovered to possess cells which have many long and fine processes. These cells are poorly or non-contractile, unlike the surrounding smooth muscle cells. We have called these cells with processes "Interstitial Cells" (ICs) as in the rabbit portal vein and elsewhere they stain positive for c-Kit (CD117) a tyrosine kinase often used to identify the Interstitial Cells of Cajal in the gut and other tissues. ICs isolated by enzyme treatment expressed smooth muscle markers and when studied by tight-seal technique showed many of the electrophysiological characteristics of the surrounding smooth muscle cells. However, spontaneous electrical changes and associated increases in ionised calcium concentration were more long lasting. In rabbit portal vein it was found that the processes of ICs contacted smooth muscle cells and electrical stimulation of an IC could elicit a response in an adjacent smooth muscle cell. Increases in intracellular calcium concentration in an IC could be evoked by high-K solution, noradrenaline or by caffeine. These increases in calcium concentration extended into the fine processes of the IC.

S52.4

TRP channels in vascular endothelium

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The TRP cation channel family consists of 6 mammalian subfamilies which comprise ~30 members. Endothelial cells express TRPV4, TRPM4, TRPP2 and several canonical TRPCs. We focus on the functional role of TRPV4 in mouse aorta endothelial cells, MAEC, from wild type TRPV4+/+ mice and from TRPV4 knockout mice TRPV4-/-. TRPV4 integrates a large variety of stimuli ranging from hypotonic cell swelling (HTS), shear stress, temperature, and etphorbol ligands, to endogenous agonists such as arachidonic acid (AA) and epoxyeicosatrienoic acids. TRPV4 is involved endothelium-dependent vasorelaxation, which can be modulated via the cytochrome P450 (CYP) pathway. The loss of TRPV4 in MAEC mice attenuated responses to all TRPV4 activating stimuli. TRPV4-dependent responses can be modulated via CYP enzymes, which metabolize AA to EETs. Upregulation of CYP2C expression by nifedpine in MAEC from TRPV4+/+ mice causes a potentiated response to AA and cell swelling. Sulfaphenazole, an inhibitor of CYP2C9, decreased responses induced by AA and HTS. 1-Adamantyl-3-cyclo-hexylurea (ACU), an inhibitor of the soluble epoxide hydrolase, which converts EETs to dihydroxyeicosat

Key words: TRP channels, endothelium, vasorelaxation

S52.5

PROSTACYCLIN: EDRF, EDHF and EDCF

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Prostacyclin (PGI2), the principal metabolite of arachidonic acid produced by cyclooxygenase in endothelial cells, was the first identified endothelium-derived relaxing factor (EDRF). It activates IP receptors on vascular smooth muscle cells

and, in most arteries, produces relaxation. In some of those, PGI2 hyperpolarizes the smooth muscle cells by opening various populations of potassium channel and the release of PGI2 by the endothelial cells can contribute to the endothelium-dependent hyperpolarization (EDHF). Additionally, PGI2 can stimulate TP receptors and evoke smooth muscle depolarization or/and spontaneous electrical activity. In the aorta of spontaneously hypertensive rats and aging Wistar Kyoto normotensive rats, the endothelium-dependent contractions elicited by acetylcholine involve the generation of reactive oxygen species, the activation of endothelial cyclooxygenase-1 and PGI-synthase, the release and diffusion of PGI2 and subsequently the contraction of smooth muscle cells by the activation of TP receptors (EDCF). Therefore, PGI2 is a Janus face prostaglandin, in the rule it protects the vascular wall, but in some instances it can contribute to endothelial dysfunction.

S52.6

Endothelial function and dysfunction in normotensive and hypertensive patients

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Endothelium-derived NO is not only a potent vasodilator but also inhibits platelet aggregation, vascular smooth muscle cell migration and proliferation, monocyte adhesion and adhesion molecule expression, thus protecting the vessel wall against the development of atherosclerosis and thrombosis. Essential hypertension is associated with endothelial dysfunction, which involves enhanced production of oxygen free radicals, that can destroy NO and reduce its availability. In presence of impaired NO availability, endothelium-dependent relaxations are sustained by hyperpolarizing factors, including a cytochrome P450 2C9 derivative. Finally, the biological activity of contracting factors, such as ET-1, is increased. Endothelial dysfunction in essential hypertension is at least in part genetically determined, shows no correlation with blood pressure load, but it is a promoter of atherosclerotic and thrombotic damage, which are typical complications of hypertension. Finally, in prospective studies impaired endothelium-dependent vasodilation is associated with increased incidence of cardiovascular events.

Key words: endothelium, nitric oxide, oxidative stress, atherosclerosis

S53.1

Concepts and Principles of Chronobiology, Chronopharmacology and Chronotherapeutics.

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Chronobiology is the study of biological rhythms. Biological processes exhibit < 24 hour (ultradian), ~24 hour (circadian), ~28-30 day (menstrual) and ~365 day (circannual), among other, rhythms. Mammalian cell, tissue and organ circadian rhythms are driven by peripheral biological clocks that are coordinated by a master brain clock (suprachiasmatic nuclei). Environmental time cues, the major ones being the onset, offset and duration of the daily photoperiod, fine tune the period and phasing of body clocks and rhythms. The body's biological time structure results in day-night, menstrual and annual patterns in the occurrence and severity of many common medical conditions. Chronopharmacology is the study of how rhythms [e.g., in gastric pH, motility and emptying; blood flow to vital organs; liver enzyme activity; kidney and biliary function; free-to-bound drug fraction and rate-limiting steps in metabolic pathways] result in dosing-time differences in PK and PD. Chronotherapeutics is a means of optimizing the desired effects and safety of medications by proportioning their level to rhythms in disease pathophysiology and host tolerance. The first chronotherapy in the 1960s was the morning oral dosing schedule of conventional methylprednisolone tablets to minimize adrenal suppression. Thereafter, nighttime dosing schedules of conventional H2-receptor and HMG-CoA reductase inhibitor therapies were used to better control ulcer disease and hypercholesterolemia. Special drug-delivery technologies (DDT) were used for nighttime theophylline and β-adrenergic tablet and capsule formulations to enhance protection against nocturnal asthma. Today more advanced DDT are used to improve the control of essential hypertension by proportioning medication levels in time to the circadian rhythm of blood pressure, and programmable ambulatory infusion pump devices are used for the chronotherapy of colorectal and other cancers to reduce drug toxicities and to achieve greater dose intensity. New microparticle DDT will make possible new-generation chronotherapies that anticipate varying-in-time medication requirements and host tolerance.

Key words: Biological Rhythms, Chronopharmacology, Chronotherapeutics

S53.2

Chronotherapy of Bone Diseases

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Bone fracture is a serious problem and impairs the quality of life. Several drugs are used for the prevention of such the episode, but are sometimes withdrawn by severe adverse effects. To establish a regimen with less adverse effect, we performed the following studies; Calcitriol (1,25(OH)2 vitaminD3) was given to 5/6 nephrectomized rats, a model of renal osteodystrophy, and strok-prone spontaneously hypertensive rats, a model of osteoporosis. Animals were maintained under a 12-hours light-dark cycle, and drug was given, once daily at 2 or 14 HALO (Hours After Light On) for 12 weeks. These studies showed that calcitriol-induced hypercalcemia was greater in the 2 HALO trial while the increase in bone density was greater in the 14 HALO trial. Next, calcitriol was given to patiens with renal osteodystrophy at 8AM or 8PM for 12 months by a cross-over design. The study demonstrated that the elevations in blood calcium and bone density were greater after dosing at 8AM and 8PM, respectively. Thus, toxicity and efficacy of calcitriol depend on its dosing-time. Regimen based on these data will improve the outcome of patients treated with calcitriol.

Key words; vitaminD3, bone disease, chronotherapy

S53.3

Chronopharmacology of Nonsteroid Anti-inflammatory Drugs

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Many patients with rheumatoid arthritis and some patients with osteoarthritis have predominantly nocturnal or morning pain. Previous studies showed that the evening dosing of flurbiprofen and indomethacin was preferable for the treatment of patients with rheumatic disorders. We showed that the kaolin-induced writhes have the daily variation with a peak at the end of the resting period (14:00-18:00) in mice under light from 07:00 to 19:00 and evaluated chronopharmacodynamic profiles of indomethacin using this model. Its suppressive effects during this period were relatively small after other dosings. These data suggest that the analgesic effects of indomethacin are greater after dosing at early resting period in mice with the kaolin-induced writhes, which is similar to that in patients with nocturnal pain. Mechanism of chronotoxicity of indomethacin was also examined in Wistar rats. Percent reduction in gastric prostaglandin E2 content was significantly greater after dosing of the agent at 00:00. These results suggest that the dosing-time-dependent change in the reduction of gastric prostaglandin E2 may be involved in the chronotoxicity on gastric mucosa of indomethacin.

S53.4

Cancer chronotherapeutics

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Chronotherapeutics improve cancer treatments through drug delivery based upon interacting circadian clock and cell division cycle. Tolerability and efficacy of 35 anticancer drugs vary 2 to 10-fold according to dosing time in rodents, through circadian clock control of drug metabolism, detoxication and molecular targets. Safety and activity of oxaliplatin against metastatic colorectal cancer (MCC) were first shown through chronotherapeutic development, once conventional trials failed. A 5-fold reduction in mucosal toxicity, a 2-fold decrease in sensory neuropathy and a ~doubling of antitumor activity resulted from chronomodulated 5-Fluorouracil-Leucovorin-Oxaliplatin (chronoFLO) vs constant rate infusion in 278 MCC patients. In 564 subsequent MCC patients, gender was an essential determinant of tolerability, disease control and survival on chronoFLO, while the persistence of circadian rhythms significantly predicted for best outcome. Gathering dynamic information on host and tumor circadian timing systems with dedicated technology allow to model optimal drug delivery schedules. Chronotherapeutics can

enhance efficiency of anticancer drug development.

Key words: Circadian, cancer, drug development, drug delivery

S53.5

Radiotelemetry in Cardiovascular Chronopharmacology in Transgenic and Knock-out Rats and Mice

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In freely moving rodents radiotelemetry has improved knowledge on circadian regulation of cardiovascular system (blood pressure BP, heart rate HR, electrocardiogram ECG, activity MA, body temperature BT). Radiotelemetry (Data Sciences) was used in strains of normotensive (Sprague-Dawley; Wistar-Kyoto), hypertensive/transgenic (SHR; TGR(mREN2)27) rats, C57 wild type and eNOS-/- knock out mice. Experiments were performed under 12:12h light:darkness (LD), total darkness (DD, i.e. free-run), and after phase-shift of LD cycle by +6 hrs. Rhythm data was analysed with Chronos-Fit (Zuther & Lemmer, 2004), fitting Fourier series to data of single/grouped animals, Power spectrum and actogram are implemented. Significant circadian rhythms were present in all rat/mouse strains. Since rodents are night-active peak values in BP, HR, ECG, MA and BT were at night, except in TGR (with additional mouse renin gene) with peak in BP in rest phase. In rats and mice rhythms persisted in DD, indicating an endogenous rhythm, in rats rhythms were abolished after lesioning of master clock in nucleus suprachiasmaticus. Data evidence that the cardiovascular system is under control of a circadian clock.

Telemetry Rat Mice Circadian

S53.6

CHRONOTHERAPY WITH BLOOD PRESSURE LOWERING MEDICATIONS.

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The extent of the nocturnal blood pressure (BP) decline is deterministic of cardiovascular injury and risk. Accordingly, there is growing interest in how to tailor the treatment of hypertensive patients according to their circadian BP pattern. Several trials have documented differences in efficacy depending on the time of day of drug administration. Thus, bedtime dosing with nifedipine is more effective than morning dosing, while also reducing secondary effects. The dose-response curve, therapeutic coverage, and efficacy of doxazosin are all dependent on the circadian time of drug administration. Moreover, valsartan dosing at bedtime as opposed to upon wakening results in improved day/night BP ratio, an increase in the percentage of controlled patients after treatment, and a significant reduction in urinary albumin excretion. Normalization of the circadian BP pattern is considered to be an important clinical goal of pharmacotherapy because it may slow the advance of cardiovascular and renal injury. Chronotherapy provides a means of individualizing treatment of hypertension according to the circadian BP profile of each patient, and constitutes a new option to optimize BP control and to reduce risk.

S54.1

The molecular structure of biogenic amine transporters

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The biogenic amines transporters belong to the SLC6 gene family of Na^+/Cl^--coupled transporters and include the transporters for serotonin (SERT), dopamine (DAT) and norepinephrine (NET). The transporters mediate rapid reuptake of the biogenic amines from the synaptic cleft, as well as they are targets for antidepressants and for psychostimulants, such as cocaine and amphetamine. A major goal in our laboratory is to gain insight into the molecular basis for the action of these drugs and to understand the molecular processes responsible for the substrate translocation mechanism. Until recently, these efforts have been hampered by the lack of high-resolution structural information; however, the crystallization of a bacterial homolog (LeuT) has provided the first detailed insight into the tertiary structure of this transporter class and allowed generation of the first reliable structural models. Currently, we use these models in conjunction with engineering of metal ion binding sites, cysteine substitutions and fluorescence-based technologies to study the dynamics of the transport process and thus to map substrate and inhibitor induced conformational changes in the transport proteins.

S54.2

Regulation of Serotonin Transporters via Cell Signaling Pathways and Interacting Proteins

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The antidepressant-sensitive, serotonin (5HT) transporter (SERT) is a major determinant of 5HT signaling capacity. SERT proteins are sensitive to intracellular signaling pathways, providing mechanisms by which 5HT neurons can adjust 5HT signaling in parallel with modulation of 5HT release. We hypothesize that abnormal regulatory control of SERT through these pathways may elevate risk for disorders linked to altered 5HT signaling. Chief regulatory pathways for SERTs include those linked to PKC, PKG and p38 MAPK. These regulatory pathways appear to influence both SERT trafficking and intrinsic activity, likely controlling a set of SERT-interacting proteins such as syntaxin 1A, PP2A, and Hic-5. Recently, we and others have identified mutations in human SERT that establishes constitutively altered levels of SERT activity and which eliminate regulation through PKG and p38 MAPK pathways. These findings reveal new opportunities to link disrupted 5HT signaling to neuropsychiatric disorders and point to, as yet, poorly studied signaling pathways that may bear additional risk determinants for mental illness as well as opportunities for novel therapeutics.

Supported by NIH Award DA07390.

S54.3

Genetic Perspectives on SRI Actions

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Serotonin transporter (SERT) gene polymorphisms and functional mutations are predictive of reduced efficacy or greater side effects with serotonin-reuptake inhibiting (SRI) antidepressants, as are variants in TPH, MAOA and HTR2A receptor genes. Studies in gene knockout mice models are providing parallel results, revealing reduced or absent responses to SRIs in heterozygous (SERT +/-) or homozygous (SERT-/-) knockout mice, respectively, in forced swim test or tail suspension models of antidepressant drug efficacy, depending on mouse strain genetic background. In addition, in some measures such as voltammetry (hippocampal 5HT clearance), locomotor stimulation (alcohol-elicited), place preference (cocaine), and temperature, hormonal and behavioral stress responses to 5HT receptor agonists (e.g., DOI, 8-OH-DPAT and MDMA), SERT +/- and -/- mice show profoundly exaggerated or reduced responses. These may provide models for some SRI side effects in bipolar and suicidal subgroups, as well as epistatic and other pharmacogenomic contributions to understanding drug-induced weight gain or SRI drug interactions such as the serotonin syndrome.

Key words: SERT, knockout mice, serotonin syndrome, antidepressants

S54.4

Early Life Exposure of Antidepressants on Neuronal Morphogenesis and Mental Development

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Depression during pregnancy affects about 10-20% of women, some of whom require treatment with antidepressants. It is important to study the safety of this particular class of drugs to ensure the optimal treatment of the mother while protecting her unborn child. There are observations that antidepressant medication during pregnancy is associated with persistent pulmonary hypertension of the newborn, but its the long-time consequences on neuronal and mental development are unknown. Here, we reported that early life of daily exposure to antidepressant, mimicking the third trimester of human pregnancy, impaired the development of pyramidal neurons of CA1 in hippocampus, a brain structure which is considered to be critical for learning and memory. The spine density of CA1 neurons was de-

creased after daily injection of antidepressant. Furthermore, this early life exposure to antidepressants impaired contextual fear conditioning in adulthood. We thus conclude that maternal use of antidepressants has profound long-term side-effects both on neuronal morphology and learning behavior, and raising the issue of cautious use of antidepressants especially in pregnant women.

S54.5

Escitalopram, a novel antidepressant with allosteric interaction on the serotonin transporter- molecular mechanisms and therapeutic potential

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Citalopram is a racemic mixture of S-citalopram (escitalopram) and R-citalopram. In studies of the individual enantiomers, escitalopram is found to account for essentially all the 5-HT uptake inhibition. However, clinical studies consistently show superiority of escitalopram compared to citalopram and pre-clinical in vivo studies show that R-citalopram antagonizes the effects of escitalopram. In vitro association studies demonstrate that R citalopram reduces the association rate of escitalopram to the 5-HT transporter (SERT). Furthermore binding kinetics studies and site-directed mutagenesis studies demonstrate the existence of an allosteric modulator site on the SERT distinct from the primary (5-HT uptake inhibition) site. These observations form the basis of our current understanding of why R-citalopram inhibits escitalopram. The hypothesis is that the improved efficacy of escitalopram is ascribed to a combined 5-HT reuptake inhibitory and allosteric modulatory effect on the SERT and that R-citalopram attenuates the latter effect.

S55.1

Pharmacology and Toxicology of Peroxisome Proliferator Activated Receptor Agonists: Differential Apoptosis of Troglitazone and Rosiglitazone

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Insulin and its analogues, sulfonyl ureas, biguanides, alpha-glucosidase inhibitors, and ATP-dependent K^+ channel inhibitors have been used as antidiabetic agents with varying degrees of success. Thiazolidinedione PPAR agonists are useful, although their toxicologic mechanisms need to be defined. Thus, the purpose of the presentation was to clarify the molecular mechanism(s) of PPAR agonist toxicities. Percentages of HepG2 cells undergoing apoptosis were determined by Cell Quest software program. Troglitazone damaged the cell drug-dose dependently while rosiglitazone did not, even at 0.1 mM concentration. The percentage of HepG2 cells in the sub-G1 (apoptotic cells) phase significantly increased as the concentration of troglitazone increased. Sub-G1 cell populations after troglitazone exposure are remarkably different from that of control. Troglitazone also increased mRNA of various transcription factors, which indicate its hepatotoxicity is, in part, due to its effects on cell cycle intermediates. Key words: Troglitazone, Hepatotoxicity, and Rosiglitazone.

S55.2

A Novel Ligand with PPAR-g agonistic activity.

Sung-eun Yoo; The Center for Biological Modulators; 21c Frontier Research Project; Korea Research Institute of Chemical Technology

The most effective therapy currently available for the treatment of type II diabetes is thiazolidine type of PPAR-g agonists, rosiglitazone and pioglitazone.

Despite of its good therapeutic efficacy in lowering the blood glucose level, TZD type of antidiabetic agents have various adverse side effects, such as liver toxicity, edema and cardiomegaly, etc.

Since it might be difficult to avoid such side effects with TZD type of PPAR-g agonists, we initiated a research project to identify novel scaffolds. From this effort we have found a novel structure which has a good PPAR-g agonistic activity with a different side effect profile.

The x-ray structure of the co-crystal of this ligand and the protein revealed that the ligand binds to the protein in a different mode from TZD. This difference in binding mode might explain the difference in recruiting coactivators and repressors and thus explains the different side effect profile between our ligand and TZDs.

S55.3

Anti-atherosclerotic Activities of PPAR Gamma Agonists

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Type 2 diabetes patients are at high risk of cardiovascular event, which is the major reason for their decreased life expectancy. It has been suggested that activation of peroxisome proliferator-activated receptors (PPARs) led to favorable effects against the atherosclerosis, in addition to the effects on metabolic disorders. We have demonstrated that pioglitazone, a PPARgamma agonist which has been clinically used for type 2 diabetes, enhanced cholesterol efflux via mRNA inductions of anti-atherogenic factors in THP-1 macrophages. Moreover, pioglitazone ameliorated the vascular lesions in animal models such as apo E deficient mice. Recently, beneficial effects of pioglitazone on macrovascular event were demonstrated clinically in PROactive study. Thus, these pleiotropic effects of PPAR gamma agonist, those might be independent of regulatory effects on metabolic disorder, may contribute to the clinical benefit against cardiovascular disease.

S55.4

Rosiglitazone ameliorates abnormal expression and activity of protein tyrosine phosphatase 1B (PTP1B) in skeletal muscle of type 2 diabetic rats

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PTP1B acts as a physiological negative regulator of insulin signaling by dephosphorylating the activated insulin receptor (IR). Here we examine the role of PTP1B in the insulin-sensitizing action of rosiglitazone (RSG). Ten-week-old, fat-fed, STZ-treated rats, were treated with RSG (10 μmol kg-1 day-1) for 2 weeks. After RSG treatment, the diabetic rats showed a decrease in blood glucose and improved insulin sensitivity. Diabetic rats showed increased levels and activities of PTP1B in muscle and liver. We found that 55%, 48%, and 39% decreases in insulin-induced glucose uptake, tyrosine phosphorylation of IR β subunits, and IRS-1, respectively, in muscles of diabetic rats were normalized after RSG treatment. These effects were associated with 34% and 30% decreases in increased PTP1B levels and activities, respectively, in muscles of diabetic rats. In contrast, RSG did not affect the increased PTP1B levels and activities or the reduced insulin-stimulated glycogen synthesis and tyrosine phosphorylation of IRβ subunits and IRS-2 in livers of diabetic rats. These data suggest that RSG enhances insulin activity in muscle of diabetic rats by ameliorating abnormal levels and activities of PTP1B.

S55.5

Relevance of Nonclinical Toxicology Data for Predicting Adverse Effects in Humans Treated with PPAR Agonists.

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Peroxisome proliferator activated receptors (PPARs) of the alpha, gamma, and beta/delta types are important targets for drugs indicated to treat insulin resistance and dyslipidemia in Type 2 diabetes. Nonclinical studies have revealed class toxicities in specific target organs (liver, heart, and hematopoietic systems) as well as carcinogenic potential. Previously, hepatotoxicity was a major concern due to severe liver toxicity in dogs and liver failure in humans treated with troglitazone. However, subsequent experience has generally not pointed to the liver as a key clinical concern. In contrast, nonclinical effects on the cardiovascular and hematopoietic systems have often been paralleled by similar findings (e.g., signs of congestive heart failure and anemia) in humans. Recently, PPAR carcinogenicity in rodents has emerged as a major concern, with transitional cell carcinomas (rats) and sarcomas (rats, mice, hamsters) being common findings. In some cases, mechanistic studies helped guide decisions on the clinical relevance of rat bladder tumors. In all cases, close cooperation with regulatory scientists is recommended to ensure patient safety in drug development.

Key words: PPAR, diabetes

POSTER ABSTRACTS

P01. Antiviral Agents

P010001

THE STUDY OF BLOOD CADMIUM CONCENTRATION IN HYPERTENSIVE AND NORMOTENSIVE ADULTS IN TEHRAN'S HOSPITALS

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Hypertension is a very common and important disease. There is conflicting report about cadmium, a trace element in the genesis of hypertension. In this study we examined the relationship between blood cadmium level and hypertension prevalence in a population - based sample of hypertensive and normotensive patients in the Shariate and Imam Khomeini hospitals in Iran. Cross sectional samples of 370 patients (age: 40 - 70), who participated in a physical examination from these hospitals' survey conducted in 2004.

The range of blood cadmium levels from patients was 0 to 69.45 μg/l. The mean blood cadmium levels of normotensive patients (42.05 ±2.52 μg/l) were higher than hypertensive patients (26.26 ±3.62 μg/l). There was a significant difference in mean blood cadmium levels of normotensive men (43.25 ±2.65 μg/l) and hypertensive men (27.01 ±4.29 μg/l) in this study ($P < 0.0001$). The comparison of blood cadmium levels of normotensive women (30.76 ±6.56 μg/l) and hypertensive women (24.81 ±6.56 μg/l) did not show a significant difference. This difference were not affected by age sex, smoking. In this population we concluded there is no positive relationship between the concenteration of blood cadmium and hypertension.

Key word: cadmium, hypertension, cardiovascular disease.

P010002

Antibiotic Resistance and gyrA, parC genes Mutations in Pseudomonas aeruginosa

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OBJECTIVE To observe the relationship between gyrA, parC gene mutations and quinolone resistance of Pseudomonas aeruginosa. METHODS MIC values of 16 clinical isolates of Pseudomonas aeruginosa were determined. The quinolone - determining region (QRDR) of the gyrA and parC genes were amplified by PCR. The gyrA - PCR products were digested with enzyme Sac II. The gyrA and parC genes were sequenced and analysed with PCR - SSCP. RESULTS By DNA sequencing, the gyrA genes of 6 quinolone - resistant strains had an amino acid substitution of Thr - 83 - Ile (ACC - to - ATC). At the same time, a silent mutation (CAC - to - CAT) in codon 132 of gyrA gene and a silent mutation (CCT - to - CCA) in codon 105 of parC gene occurred, which did not lead to amino acid change. The results of PCR - RFLP and PCR - SSCP were consisterted with DNA sequencing. CONCLUSIONS The mutation of gyrA gene is one of mechanisms which response for fluroquinolone resistance in Pseudomonas aeruginosa, the Thr - 83 - Ile mutation was the most frequent.

Key words: Pseudomonas aeruginosa; gyrA gene; parC gene; RFLP; SSCP;

P010003

A study on the TEM - 29 Extended - Spectrum β - Lactamase Produced by a Clinical Isolate of Pseudomonas aeruginosa

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Objective To investigate the properties of ESBLs of Pseudomonas aeruginosa clinical isolates Pa03 - 104. Methods blaSHV, blaCTX - M and blaTEM genes was amplified by PCR and sequenced after being subcloned into pUCm - T vector. The gene of β - lactamase was cloned into pBK - CMV vector and expressed in E. coli JM109. The phenotype was determined by three - dimensional test. The isoelectric point (pI) of the recombinant protein was determined by isoelectric focus. Results The encoding gene of β - lactamase was belonged to TEM type. The PCR product of the strain had 861 nucleotides. The sequence of β - lactamase produced by Pa03 - 104 was the same as TEM - 29 (GenBank Y17584) produced by Escherichia coli. The enzyme was characterized as ESBL by three - dimensional test with pI of 5.4. Conclusions It was the first report of TEM - 29 type extended spectrum β - lactamase produced by Pseudomonas aeruginosa in China.

Key words: Pseudomonas aeruginosa; TEM - 29; Sequence analysis; Prokaryotic expression

P010004

Immunity Modulating of Arbidol in mice

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Objective: To observe the effects of Arbidol (Ar) on interferon induction and immunity regulation in mice. Method: The mice were intragastrically given Ar of 100, 50 and 25 mg·kg^{-1}. Then the serum interferon was timely determined after a single Ar administration. The rate of abdominal phagocytosis of macrophage in normal mice, carbon particles clearance index and delayed type hypersensitivity in immune - suppressed mice induced by Hydrocortisone (Hc), and the contents of serum hemolysin both in normal and immune - suppressed mice induced by Cyclophosphamide (Cy) were detacted. Results: The interferon contents were detected in 6 ~ 24h after Ar administration, and the maximum was in 18h. An increased phagocytosis of peritoneal macrophage in normal mice, the enhanced carbon particles clearance index and delayed type hypersensitivity in immune - suppressed mice induced by Hc and the higher both in normal and immune - suppressed mice induced by Cy were observed with the administration of Ar for 5d. Conclusion: Ar exerts enhancing effects on interferon induction in vivo and immunity function both in specific and nonspecific one.

P010005

Interface targeting peptides as inhibitors of HIV - 1 protease mutants

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The terminal segment peptides of HIV protease (PR) dissociate the PR dimer into inactive subunits. From these peptides, highly active dimerization inhibitors were developed by CAD [1]. The best 3 - mer inhibitors have C - terminal thyronine (T0) or thyroxine (T4). Palmitoyl - YE (Tx) - OH shows Ki ~5 nM. (Tx) is anchor and receptor targeting group. The PIs are the most potent reagents for protein/protein dissociation and should also inhibit mutants. Pam - YE (T0) - OH abrogates viral replication. It is possible to convert the peptides into more stable mimetics (peptoids, ester prodrugs, retro - inverso, cyclic and D - peptides [3]). Some triterpenes and steroids with low toxicity (ursolic, ursodesoxycholic acid) also inhibit PR [2] and may be used in urgently needed anti - AIDS cocktails. Some PIs interact with Alzheimer aggregates. Serpin beta - sheet insertion peptides (Ac - AMFLEAIP - Nle - E from α1 - AT) also inhibit PR. This suggests that endogenous proteins may interfere with PR or other dimeric HIV proteins and in this way modulate disease progress. Similar PR inhibitory sequences occur in virus and cell proteins (p6*, Q8NA00, FLJ360).

P010006

Antivirals with immunostimulatory properties: acydic nucleoside phosphonates

Zidek Zdenek[1*], Cesnek Michal[2], Doláková Petra[2], Krecmerová Marcela[2], Potmesil Petr[1], Kmonickova Eva[1], Hoby Antonin[2]. 1. Inst. Exp. Medicine, Acad. Sci.. 2. Inst. Org. Chem. Biochem., Acad. Sci.. Acyclic nucleoside phosphonates (ANPs) are antivirals effective against both DNA - viruses and retroviruses. Most of them are derivatives of adenine (A) and 2,6 - diaminopurine (DAP), containing 2 - (phosphomethoxy) ethyl or 2 - (phosphomethoxy) propyl moieties at the N9 - side chain. Parent agents PMEA (Adefovir) and PMPA (Tenofovir) are used for treatment of hepatitis B and AIDS, resp. We synthesized new derivatives comprising alterations in both 9 - side chain and 6 - amino group of the heterocyclic base. They were screened for possible immunobiological activities using in vitro system of mouse macrophages and lymphocytes. Factors that are known to interfere with either virus replication, such as production of nitric oxide and cytokines (TNF - α, IL - 10), or with HIV penetration in cells (secretion of chemokines RANTES, MIP - 1a, MCP - 1 - 5), have been investigated. In this respect, several of the newly developed ANPs possess outstanding immunostimulatory and immunomodulatory potential. The effects have been found to depend on activation of MAP kinases, and transcriptional factor NF - kappaB.

Key words: virostatics, immunostimulation

The study was supported by Centre for New Antivirals and Antineoplastics (1M6138896301).

P010007

Dependence of immunostimulatory effects of antiviral acydic nudeoside phosphonates on purine P1 receptors.

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Acyclic nudeoside phosphonates (ANP) are analogues of nucleotide monophosphates. The purine derivatives represent counterparts of AMP, and mono- and diphosphorylated ANPs are analogues of ADP and ATP, resp. Similar to natural nucleotides, also ANPs are endowed with immunobiological potential. We found that secretion of various cytokines induced by ANPs in mouse macrophages and lymphocytes depends on activation of P1 purinoceptors. All adenosine A1, A2b (not A2a), and A3 receptors are involved in cell responses to ANPs, though different cytokines (e.g. TNF-a, IL-10) and chemokines (e.g. RANTES, MIP-1a) have distinct requirements for activation of individual members of P1 family of purinoceptors. Correspondingly, ANPs modulated stimulation of inducible nitric oxide synthase (iNOS) and subsequent enhancement of NO production in macrophages are controlled by adenosine A1 receptor. It may be suggested that acydic nudeoside phosphonates are nonspecific ligands for purine P1 receptors. The findings can be used for development of new pharmacologically prospective compounds.

Key words: virostatics, adenosine receptors

The study was supported by Centre for New Antivirals and Antineoplastics (1M6138896301).

P010008

Anaferon, an oral anti-interferon gamma antiviral: clinical efficacy in common paediatric viral infections

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Specific therapy for common paediatric viral infections (PVI) remains an unmet need despite vaccination and existing antivirals. A series of randomised controlled clinical trials (RCT) has been performed in Russia to study the efficacy of a novel drug approved for prevention and treatment of common PVI. Anaferon (AF) contains antibodies to interferon gamma (ultra-low doses for oral use). A placebo-controlled RCT in laboratory-confirmed influenza involved 105 non-vaccinated children (1-10 yrs). They received AF (oral tablets/water solution) 3-8 times daily for 7-9 days with symptomatic therapy. AF reduced time to no fever and duration of cough by 1.5 days, the incidence of purulent rhinitis from 15.6% to 3.3%, and was safe. A placebo-controlled RCT in varicella involved 236 children (1-17 yrs). Treatment with AF reduced time to no fever (by 2.7 days), to no new lesions (by 3.3 days) and to no itching (by 4.2 days). The use of AF also reduced the development of pustules (6.5-fold) and the need in additional antibiotics (9.1-fold). Taking into account that the drug is effective and safe irrespective of aetiology of the viral infection, Anaferon can be regarded as a choice treatment for common PVI.

P010009

Production of Poxvirus Neutralising Antibody in Hen's Eggs and Evaluation of Antibody Effect

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Aimed to develop rapid reliable diagnostic and therapeutic tool, specific anti-poxvirus-chicken antibodies were produced and evaluated. 3 chickens were immunised and boosted (i.m.) with 3 species of inactivated poxviruses. Egg yolk antibodies (IgY) were purified with improved polyethylene glycol precipitation. The reactivity of specific IgY and the development of IgY titers was confirmed by immunofluorescence (IFA), neutralisation test (NT) and immunoelectron microscopy (IEM). Cross-reaction ability of IgY antibodies with cells infected with various poxvirus strains was also investigated (IFA, NT). The results show that specific IgY could appear positive reactions even in very high dilution levels and the plateau levels could persist for long time. Accordingly, satisfying neutralising reaction and ultra-structural detection of antibody labelling with antigen was observed. Strong cross-reaction activity of different poxviruses and IgY was found. Specific binding of IgY to the respective proteins of viruses were shown in western blot. This study suggest that anti-poxvirus IgY could serve as a possible alternative to diagnosis and treatment of poxvirus diseases. The yield of specific IgY is remarkable.

Keywords: Pox virus, Egg yolk antibody (IgY), neutralising ability

P010016

HIV PROPHYLAXIS IN AN EMERGENCY DEPARTMENT FOLLOWING NON-OCCUPATIONAL

EXPOSURE: observance and tolerance of triple therapy, 1 month after start of treatment

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Thanks to their full time availability and in compliance with French and international recommendations, emergency departments are in the frontline when it comes to starting the initial treatment of people who have been exposed to HIV. This project aims at assessing the tolerance as well as the observance of triple therapy over a period of 1 month (lamivudine 150 mg + zidovudine 300 mg - Combivir(R) - and nelfinavir 250 mg - Viracept(R) -). Methods: Prospective and descriptive study with clinical and biological follow-up (3 biological check-ups). Findings: 50 patients were included. The main side effects were digestive disorders (66% at the start of treatment and 42% by the end of treatment), neurological disorders (36%), asthenia (36%), respiratory disorders (16%) and rheumatological disorders (8%). One patient suffered a mild biological pancreatitis 344 UI.L-1 lipasemia), requiring a change in triple therapy. 12% of patients had developed cytolysis (this disorder getting back to standard levels one month after start of treatment). The tolerance of Combivir(R) - Viracept(R) therapy is satisfactory (only one change in treatment, no serious side effect).

P010021

The inhibitory effect of Compound Liuyuexue on DHBV DNA

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Objective: To study the inhibitory effect of traditional Chinese medicine Compound Liuyuexue (CLYX) on duck hepatitis B virus (DHBV) DNA, and provide an experimental basis for developing a new drug for the clinical treatment of patients with hepatitis. Methods: Positive ducks were detected by FQ-PCR at 13 days after the infection of DHBV, and were randomly divided into five groups: the high dose group, middle dose group and low dose group of Compound Liuyuexue (CLYX), model group (saline control), aciclovir control group, Every group had 10 ducks. CLYX was given, i.g., for 14 days, and the content of DHBV DNA in serum was measured by FQ-PCR. Results: The serum DHBV DNA content was decreased significantly by the treatment with CLYX. The high dose group and middle dose group of CLYX could significantly inhibit DHBV DNA replication in vivo ($P < 0.05$ or $P < 0.01$). DHBV DNA content in serum in high dose group did not return significantly 3 days after stopping treatment, and its inhibitory effects were dose- and time-dependents. Conclusions: CLYX could inhibit significantly DHBV DNA.

Key Words: Compound Liuyuexue (CLYX); Duck Hepatitis B Virus; FQ-PCR.

P010022

Antiviral effects of the XYW injection against 8 viruses

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In order to study the antiviral effects of the XYW injection (XYW, a new structural Chinese material medca), we observed the antiviral effects of the XYW on MDCK or Vero or Hep-2 cells infected with 8 viruses as well as its protective effects on pneumonia mice infected by mouse influenza virus via nasal dripping. 100 TCID50 virus (ADV3, ADV7, ADV11, RSV, COXB, HSV1, influenza H1N1, H3N3) was inoculated in MDCK or Vero or Hep-2 cells. The minimal effective concentrations of XYW against these 8 viruses were 1.6, 3.2, 3.2, 1.6, 0.8, 6.4, 1.6, 1.6 mmol/L, respectively. The infectious therapeutic indices of XYW to these viruses in MDCK or Vero or Hep-2 cells were 23.3, 11.7, 11.7, 23.3, 46.7, 5.8, 16.7, and 16.7, respectively. Mouse influenza virus were dropped nasally in BALB/c mice (Grade II). The XYW prolonged the life span of mice infected with pneumonia by influenza virus to 43% ~100%. It inhibited the inflammation and decreased the virus titer. The inhibitory rats of XYW to pulmonary index were 21% ~50%. In our study, it showed that the XYW inhibited the proliferation of these viruses and improved the symptom of mouse pneumonia caused by influenza virus.

Key words: antiviral agents; XYW injection

P010023

In vitro antiviral activity of different parts of Juniperus communis subsp. Hemisphaerica and Juniperus oblonga against HSV-1

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The KOS strain of HSV-1 and Hela cell line were used for survey of antiviral activity of Juniperus communis subsp. Hemisphaerica and Juniperus oblonga, the two species of Iranian native conifers. The infected cells were counted according to cytopathic effects of virus on cells, and based on non-infected cells. Protection percent of any extract as antiviral activity were calculated.

Due to this study the extract of leaves of male J. communis subsp. Hemisphaerica had the most antiviral activity, although all extracts had antiviral activity in comparison with positive control.

Key words: antiviral activity, juniperus, HSV-1

P010024

Glutaminase mediated glutamate neurotoxicity by HIV-1 infected macrophage

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Mononuclear phagocyte (MP, macrophages and microglia) dysfunction is thought to play a significant role in the pathogenesis of neurodegenerative disorders including HIV-1-associated dementia (HAD). Glutamate is known to be elevated in HAD, and previous studies have reported HIV-1-infected human monocyte derived macrophage (MDM) increase the production of glutamate in a glutamine dependent manner. The enzyme glutaminase converts glutamine to glutamate in an energy free reaction. In this report, we demonstrate that HIV-1 infected MDM condition media (MCM) induces neurotoxicity. Three glutaminase inhibitors and one control designed by MGI Pharma were blindly tested, with glutamate production measured by reverse-phase high performance liquid chromatography (RP-HPLC). Two of the GPI inhibitors nearly abrogated the increased production of glutamate by HIV-infected MDM when delivered at micromolar concentrations (19560 and 14256); the inhibitors did not directly affect cell viability or alter the progression of HIV infection. Glutaminase its and specific inhibitors may be important as a potential target for therapy in HIV-1 mediated neuronal injury during HAD.

P010025

Activation of AMPA receptors on human neural progenitor cells

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Neural progenitor cells (NPC) are capable of proliferating and differentiating into new neurons and astrocytes through neurogenesis. Stimulation of neurogenesis would be vital to the recovery from neurodegenerative disorders, such as HIV associated dementia (HAD), where impairment results form neuronal injury and apoptosis. Previously, we demonstrated increased production of glutamate from HIV infected macrophages leading to neuronal injury. Also, glutamate has been proposed to mediate neurogenesis through processes resulting from the activation of the AMPA receptor, an ionotropic glutamate receptor. In this study we propose that the activation of AMPA receptors on human NPC results in calcium influx, activating pathways linked to neurogenesis. AMPA receptor mRNA and protein were demonstrated on human NPC and differentiated cells.

Calcium influx due to AMPA receptor activation was measured using Fura-2 AM. Our data suggests that calcium influx in NPC occurs through AMPA receptors mediated mechanisms. Calcium influx was stimulated by glutamate, potentiated by an AMPA specific potentiator, and blocked by Joro Spider toxin. This result demonstrated that AMPA receptors are functional on human NPC.

P02. Antimicrobial Agents

P020001

Screening of Chinese herbs for anti-Helicobacter pylori activity

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Background Helicobacter pylori (H. pylori) is a bacterium implicated in the etiology of stomach cancer and ulcers. Epidemiology of the antibiotic resistance of Helicobacter pylori appears to be higher. In search of efficiency substance of Chinese herbs, which could be used in preventing and treating Helicobacter pylori diseases. Methods Extracted efficiency substance from Chinese herbs and Identified by antimicrobial sensitivity tests performed on 96 microwell plate. Results Its showed that all except 15 extract from 40 plants showed bactericidal activity against the microorganism, but the most active extracts were those from Gallnut (cocoon) of Chinese Sumac (MIC: <1:512), Clover Flower Bud (MIC: 1:256), Houtuynia (MIC: 1:512), Agrimony(MIC: 1:256) Coptis Rhizome (MIC: <1:512). Conclusion Amongst the active plants the inhibitory properties of Helicobacter pylori were found prominent. Gallnut (cocoon) of Chinese Sumac, Clover Flower Bud, Houtuynia, Agrimony and Coptis Rhizome are efficient for inhibition Helicobacter pylori.

P020002

Antibacterial Drugs Usage in Poultry and Dairy Cattle Farms and Public Health Hazard in Qum Province

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According to the World Health Organization report, the presence of antibacterial drugs residues in food products include bacterial drug resistance through transference of resistance factor that it can conceivably complicate treatment of human, as well as animal diseases. The purpose of the present study was to survey on antibacterial drugs usage in poultry and dairy cattle farms in Qum province.

The broiler farms in Qum province were divided by six regions (138 farms) and the dairy cattle farms were divided by four regions (100 farms). According to the performed studies, it was determined that the minimum usage of antibacterial drugs in broiler farms were in the North, South and the East regions, and in dairy cattle farms was in the South region of province. The reason can be the less number of farms and the climate condition of these areas. The maximum usage of the drugs in broiler farms was in the West, South-west and the South-east regions, and in dairy cattle farms was in the central region of the province. The reason can be the more concentrated number of farms and very weak hygienic and management situations in some farms in these areas.

P020004

A study on the encoding genes of Extended-spectrum β-lactamases in Enterobacter cloacae isolates

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OBJECTIVE To identify the prevalence of ESBL genes in E.cloacae strains isolated from the first teaching Hospital of North Sichuan Medical College. METHODS Antimicrobial susceptibility, plasmid characterization (isolation, PCR, cloning and nucleotide sequencing), and β-lactamase assays were used. RESULTS Of 59 clinical isolates of E.cloacae, 18 isolates were shown to be resistant to oxyimino cephalosporins and aztreonam. Plasmids were isolated from the 18 isolates and were used as templates by PCR with the primers for blaSHV, blaTEM or blaCTX-M. The PCR results revealed that 3 plasmids contained SHV genes (SHV-5, SHV-12 and SHV-70, respectively). 2 plasmids contained TEM genes(variants of TEM-28 and TEM-116), and the blaCTXM-22 genes were found to coexist with blaTEM-1 genes on the same plasmids in 13 isolates. The transformants producing CTX-M-22 with PI of 8.7 were resistant to most beta-lactams, which were much more resistant to cefotaxime than to ceftazidine. CONCLUSION CTX-M-22 is the most common genotype in plasmid mediated ESBLs produced by the 18 resistant E.cloacae isolates in the teaching Hospital.

Key words: Enterobacter cloacae; Extended-spectrum β-lactamase; Sequence analysis

P020005

Experimental Study of Chrondogical Dosage Regimens on Gentamicin(GTM) in rats

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Aim: To study the effects of different administration time of GTM in rats. Method: 108 rats were divided into 6 groups: control group; once-daily dose groups(N100 and D100 group, which were intramuscularly administrated 100 mg/kg GTM at 1:00 or 13:00 respectively); twice-daily dose groups(N90+D10, N70+D30, N50+D50 group, in which 100 mg/kg/d GTM were given at 1:00 and 13:00). The Wt, BUN and Cr were observed, GTM concentrations were determined, C-T curves were profiled and the pharmacokinetic parameters were calculated at the 1st, the 10th, and 20th day after administrations. Results: nephrotoxicity: At the 20th day after administration, N100 group had the lowest Cr, BUN level and Wt decrease. GTM Concentration: Significant difference of peak concentrations existed between the once-dose daily groups (N100 < D100). The peak concentration of N50+D50 group was higher than that of other twice-daily dose groups. Pharmacokinetic parameters: N100 group had the lowest AUC, the shortest T1/2 and the largest CLs. Conclusions: Once-daily dosing in activity period is the best chrondogical dosage regimens from the view of nephrotoxicity, concentration and pharmacokinetic parameters.

Keywords: GTM; Chrondogical Dosage Regimens; Rat

P020006

In-vitro and in-vivo activity of Marine Lysozyme, a new antimicrobial agent for treating vaginitis

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In our study, we investigated the in vitro and in vivo activity of Marine Lysozyme (ML), a novel antimicrobial agent extracted from Musculous Ostrea for treating vaginitis. The activity of ML was tested against C. albicans, C. sporogenes, S. aureus, S. epidermidis, Enterococcus spp., E. coli, P. aeruginosa, P. vulgaris, K. pneumoniae and Acinetobacter spp. and against experimental bacterial vaginosis (BV) caused by the combination of SA and E. coli. With the transmission electron microscope, we also observed the mechnism of its effect. In our study, the MIC rang of ML against most strains was 0.125-32 μg/mL, MIC_{50} was 1-8 μg/mL and MIC_{90} was 4-64 μg/mL. MBCs and MICs of ML against tested bacteria were close. In addition, ML (10 mg/kg, 5 mg/kg, 2.5 mg/kg) showed therapeutic effects on BV, which the cure rates were 92.9%, 82.1% and 64.3%. Moreover, after treated with ML, the cell walls of pathogens showed great morphologic changes. These results suggest that ML has potent antimicrobial activity and broad antibacterial spectrum and may be a promising therapeutic agent for the treatment of Candida vaginitis and BV. And the effective localization of ML on bacteria is their cell walls.

Key Words: Marine Lysozyme; antibacterial activity; vaginitis

P020007

Resistance Mechanisms To Imipenem In Clinical Isolate Pseudomonas Aeruginosa.

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OBJECTIVE: To study resistance mechanisms to imipenem in clinical isolates of PA. in chengdu

METHODS: The MICs to imipenem were detectde by an agar dilution metheod;. PCR method was used to detect the losing of Oprd2 in resistance strains; Using imipenem-Mercaptoacetic acid Double-Disk synergy test to evaluate producing Metallo-β-lactamase bacteria in resistance strains; Using CCCP to inhibitate the efflux pump. The levels of mRNA expression of OprM and OprN in clinical PA was determined by RT-PCR. RESULTS: In 62 clinical isolate strains, 28 strains were resistant to imipenem, 4 strains were middle susceptible, and 30 strains were imipenems susceptible; Four imipenem resistance strains have the outer membrane protein losing; Only one resistance strain produced Metallo-β-lactamase; Efflux pump inhibitors CCCP can reduce MICs of resistance strains; The levels of mRNA expression of OprM and OprN in resistant strains are higher than those in susceptible ones. CONCLUSIONS: The study indicated that outer membrane protein losing production of metallo-β-lactaase and the higher expression of efflux pump are the important mechanisms of imipenem resistance in PA.

Key words: Pseudomonas aeruginosa PA imipenem; resistance

P020008

Effect of fatty acid binding protein on Chlamydia Trachomatis L2 growth

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Chlamydia require host ATP for their growth. Liver-Fatty Acid Binding Protein (L-FABP) plays an important role in cellular long chain fatty acid (LCFA) uptake and energy metabolism. We explored the effect of FABP expression in host cells on Chlamydia growth. METHODS: Chlamydia trachomatis L2 was used to infect LFABP transfected Chang liver cells. The status of chlamydial infection in parallel cultures was monitored by immunofluorescence microscopy. Host [3H]-palmitate uptake was detected by measuring radioactivity in cell lysates from infected or mock-infected cultures. FABP expression was detected by Western blot and RT-PCR. RESULTS: Chlamydia L2 infection caused a 23% increase in fatty acid uptake in Chang liver cells compared to mock-infected cells. L-FABP expression did not change Chlamydia infection rate in Chang cells, but promoted Chlamydia growth by significantly increasing inclusion-forming units, inclusion size, and inclusion density. This promotion effect was not observed in culture conditions devoid of LCFA. CONCLUSION: L-FABP and LCFA may play a pivotal role during the process of bacterial infections. This study was supported by a grant from the CIHR (Canada) and NIH (USA).

P020009

In Vitro Inhibitory Activity of Antibacterial Polysaccharide from Durian-rinds Against Field Isolates of Mastitis Causing Bacteria in Dairy Cows

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Mastitis is a contagious disease causing high economical loss in dairy farms each year. Searching new antibacterial agents from plant sources to replace and limit antibiotic uses is interesting. Field isolates of mastitis causing bacteria including 42 isolates of Staphylococci, 31 isolates of Streptococci, 15 isolates of Pseudomonas, 13 isolates of Escherichia coli and 5 isolates of Klebsiella pneumoniae were evaluated their susceptibility *(in vitro)* to antibacterial polysaccharide gel (PG) isolated from fruit-rinds of durian *(Durio zibethinus Murr.)*. MIC and MBC of PG were determined by broth microdilution method and viable bacteria were examined by streak plate method. The results demonstrated that values of MIC (MBC) of PG were 3.12-25 (6.25-50), 3.12-25 (6.25-50), 3.12-12.5 (6.25-25), 6.25-25 (12.5-50) and 6.25-12.5 (12.5-25) mg/ml of PG against most of isolates of Staphylococci, Streptococci, Pseudomonas, E. coli and K. pneumoniae, respectively. In conclusion, PG of durian-rinds had potentially benefit for preventing mastitis bacteria. Further studies for clinical uses of PG in dairy cows are under way.

Keywords: Durio zibethinus, antibacterial polysaccharide

P020010

Novel phenacylhomoserine lactones: microwave synthesis and structure activity evaluation in bacteria and cancer

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A number of gram-negative bacteria utilize acylhomoserine lactones (AHLs) to sense population density and direct expression of genes controlling virulence and biofilm formation through quorum sensing (QS).

Interestingly, the predominant AHL produced by Pseudomonas aeruginosa, 3-oxo-dodecanoyl AHL (ODHL), also elicits anti-cancer effects in cancer cell lines. A library of analogs was rapidly synthesized using microwaveassisted organic synthesis. Anti-cancer activity was examined using the sulforhodamine-B cell growth assay. QS modulation activity was explored using a competitive-binding green fluorescent protein reporter assay. Several compounds significantly inhibited growth in three cancer cell lines, whereas, a number of compounds both agonized and antagonized bacterial QS. Through this study, we have established a quick and simple method for AHL synthesis and discovered analogs that have potential to modulate QS and disrupt cancer. Also, this study has opened the possibility for bifunctional agents useful for treating cancer and associated infections simultaneously. Keywords: Microwave, bacteria, AHL, cancer. Supported by NIH/NCI Training Grant CA009072, Woodburn Presidential Graduate Fellow-

ship .

P020011

Comparison of metronidazole and ceftizoxim in prophylaxis of post - hysterectomy infections

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Ceftizoxim is the standard agent used in prophylaxis of infections after abdominal hysterectomy . Metronidazole could be used instead of ceftizoxim for this matter . To compare these two drugs , in a randomized clinical trial , 34 patients received metronidazole suppositories (1g) and 34 patients received intravenous ceftizoxime (1g) before surgery . There were not any significant demographic (age , weight , pariety , hospitalization duration , preoperation hemoglobin) difference between two groups . Also , the incision type and post - operation bleeding were the same in two groups . The complications after abdominal hysterectomy such as febrile morbidity , urinary tract infections and wound infections were not significantly different between two treatment groups . These results indicate that a single dose metronidazole has the same effect as ceftizoxim in infection prophylaxis of posthysterectomy infection .

P020012

Molecular functions of transcription factor Cap1p in the development of drug resistance in Candida albicans

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This study is designed to clarify the functions of Cap1p in the development of drug resistance in C. albican . MICs determining , RT - PCR, flow cytometery and virulence test were carried out in this study . The mRNA level of CDR1 and MDR1 in wild - type and CAP1 over - expressive strains , which had been incubated in the presence of fluconzole , increased significantly , but not in CAP1 deficient strains . With the deficiency of CAP1 , the transcription of ERG7 , ERG9 , ERG11 could not be detected, but developed again after incubating in the presence of fluconzole . With the deficiency of CAP1 , cells wrinkled and contracted , infection ability weakened and growth cycle prolonged . Cap1p can affect drug resistance genes CDR1 and MDR1 as well as azole antifungal target genes ERG7 , ERG9 and ERG11 , although it is not the unique transcription factor of ERG7 , ERG9 and ERG11 . CAP1 was involved in the morphology , growth cycle as well as virulence condition of the strains . Cap1p is an important transcription factor in the development of drug resistance in C. albicans .

C. albicans , resistance , Cap1p .

This work was partially supported by the S&T Fund of Shanghai (grants 04JC14003 and 05QMX1470 to C. YB) .

P020014

Identification of astemizole as an anti - malarial agent by screening a clinical drug library

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The high cost and protracted timeline of new drug discovery is a major roadblock to creating therapies for diseases found primarily in the developing world . To accelerate drug discovery for neglected diseases we created a library of 2 ,687 existing drugs and in a test of concept screened for inhibitors of the human malaria parasite P. falciparum. The non - sedating antihistamine astemizole and its principal human metabolite desmethylastemizole were identified as potent inhibitors of chloroquine - sensitive and - resistant parasites . Astemizole , like the quinoline antimalarials , inhibits heme crystallization , concentrates within the P. falciparum food vacuole , and copurifies with hemozoin from chloroquine - sensitive and - resistant parasites . In mice infected with chloroquineresistant P. yoelii astemizole and desmethylastemizole reduced parasitemia with an apparent IC_{50} of 15 mg/ m^2 , which is near the dose used to treat allergic rhinitis . These results suggest astemizole is promising for the treatment of malaria , and highlight the potential of finding new treatments for diseases of the developing world by screening libraries of existing drugs .

P020015

In vitro antiplasmodial activity of corrinoid derivatives

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Ferriprotoporphyrin is toxic to the malaria parasite but is converted to an inert haemozoin crystal within the parasitic food vacuole . It is proposed that corrinoids which structurally resemble the porphyrins could interfere with the formation of haemozoin crystals and cause parasite death . The effect of five corrinoids were tested on the in vitro growth of Plasmodium falciparum using the [3H] - hypoxanthine incorporation assay , and the ferriprotoporphyrin biomineralisation inhibition test was carried out under acidic conditions to mimic the process of haemozoin formation in the parasitic food vacuole . Adenosylcobalamin and aquocobalamin were the most active in inhibiting parasite growth . In combination , adenosylcobalamin displayed an additive/slightly antagonistic interaction with 8 - aminoquinolines . All the corrinoids , except dicyanocobinamide were approximately 40 times more potent than the 8 - aminoquinolines in inhibiting β- haematin formation . The low toxicity and antimalarial activity of these corrin - ring containing compounds highlights their potential as templates for further investigation .

malaria , corrinoid

We acknowledge the Belgian Technical Cooperation and University of the Witwatersrand .

P020016

IN VITRO ANTIPLASMODIAL ACTIVITY AND CYTOTOXICITY OF NEW N- ALKYL AND NBENZYL 1,10 - PHENANTROLINES DERIVATIVES

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Previous study showed that 1 ,10 - phenantroline skeleton was active in vitro on chloroquine - resistant and sensitive strain of Plasmodium falciparum. Based on the skeleton , 8 derivatives of N - alkyl and N - benzyl 1 ,10 - phenantrolines have been synthesized . This study was conducted to evaluate in vitro antiplasmodial activity and cytotoxicity of these compounds . The in vitro antiplasmodial on chloroquine - resistant P. falciparum strain (FCR - 3) , chloroquine - sensitive P. falciparum strain (D10) and cytotoxicity test on Vero cells were determined by radioactive method after 24 and 72 h incubation periods , and were expressed by the 50 % concentration inhibiting of the parasite or cell growth (IC_{50}) . Cytotoxic/ antiplasmodial ratio was calculated to evaluate its safety . The highest antiplasmodial activity was observed for (1) - N- benzyl - 1 ,10 - phenantrolinium iodide with IC_{50} 0 .08 - 0 .59 uM, IC_{50} on Vero cells was 2207 .77 - 126631 . 51 uM, and cytotoxic/ antiplasmodial ratio showed that this compound was safe (9199 .04 - 214629 .67) .

Key words : 1 ,10 - phenantroline , P. falciparum, antiplasmodial , cytotoxicity

P020017

In Vivo Antiplasmodial Activity and Acute Toxicity of N- alkyl and N- benzyl - 1 ,10 - Phenanthroline Derivatives

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Previous study on in vitro antiplasmodial activity of diaza analogs of phenanthrene led to return the 1 ,10 - phenanthroline skeleton as a potential antimalarial leader compound . Based on the skeleton it has been synthesized six derivatives of N- alkyl and N- benzyl - 1 ,10 - Phenanthroline and its in vitro antiplasmodial activity have also been evaluated .

This study was performed to evaluate in vivo antiplasmodial activity of 1 ,10 - phenanthroline derivatives by the classical 4 - day suppressive test against P. berghei . Acute toxicity of each drug was determined after a single injection of drug on Swiss mice .

The 50 % effective dose (ED50) after intraperitoneal administration ranged from 2 .08 to 50 .93 mg/ kg of body weight , and the therapeutic indices (TIs) were ranged from 2 .06 to 7 .57 except (1) - N- benzyl - 1 ,10 - phenantrolinium iodide was 58 .38 . All of the 1 ,10 - phenanthroline derivatives have antiplasmodial activity and (1) - N- benzyl - 1 ,10 - phenantrolinium iodide was the most po-

tential.

Key words: 1,10-phenanthroline derivatives - in vivo antiplasmodial - acute toxicity - therapeutic indices.

P020018

Antimalarial artemisinin synergies antibiotics to protect against lethal live Escherichia coli by decreasing proinflammatory cytokine release

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In this study, CpG ODN, LPS, heat-killed and live Escherichia coli 35218 (E. coli), were used to induce sepsis in animal models. We found ART protects mice from a lethal challenge by CpG ODN, LPS and heat-killed E. coli in a dose-dependent manner, and the protection is related to reduction of serum TNF-α. More significantly, combination of ART and ampicillin or unasyn protect mice challenged with lethal live E. coli, suggesting that ART protection is due to its anti-inflammatory effect, not an antimicrobial effect because ART cannot inhibit bacterial growth. Using RAW264.7 cells, pretreatment with ART potently inhibited the release of TNF-α and IL-6 induced by CpG ODN, LPS or heat-killed E. coli. Using affinity sensor technology, we found no direct binding between ART and CpG ODN or LPS. Flow cytometry showed ART influenced neither CpG ODN binding to cell surfaces nor internalization of CpG ODN. In addition, the up-regulations of TLR9 and TLR4 mRNA were not down-regulated by ART. However, ART blocked the NF-κB activation induced by CpG ODN, LPS or heat-killed E. coli. Therefore, our findings show ART may be an important potential drug for treating sepsis.

Key words: Artemisinin; Ampicillin; E. coli

P020019

Effects of fluoroquinolones on the cardiovascular system in telemetered conscious dogs.

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Balofloxacin and gemifloxacin are two new fluoroquinolones, which are highly effective against infectious enteritis and against respiratory tract infections, sexually transmitted diseases and urinary track infections respectively. Some fluoroquinolones have been reported to induce QT interval prolongation associated with the onset of Torsades de Pointes (TdP), resulting in a life-threatening ventricular arrhythmia. In this study, we investigated effects of balofloxacin, gemifloxacin, levofloxacin, and enoxacin on electrocardiograms and hemodynamic parameters in conscious telemetered dogs. Single administration effects were tested during 24 hours for each test drug at does 10 mg/kg, 30 mg/kg, 100 mg/kg. We monitored QT, QTc, heart rate, blood pressure and body temperature after administering test drugs. In conscious telemetered dogs, balofloxacin significantly prolonged QTc at 100 mg kg-1, with mean serum Cmax of 22.3 g ml-1. Other drugs do not affect QT, QTc and other hemodynamic parameters.

Key words: fluoroquinolones, ventricular arrhythmia, telemetered dog, QT prolongation

P020020

Biological functions of transcription factor Cap1p in Candida albicans

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Objective: To provide new insight into the biological functions and the regulation network of Cap1p, a transcription factor in Candida albicans related to oxidative stress tolerance and drug resistance. Methods: With the CAP1 deletion strain CJD21 and its parental strain CAI4, microarray analyses, CAP1 over-expression, Western bolt, Real time RT-PCR, bioinformatics and efflux analyses were used. Results: The identified 65 Cap1p-dependent oxidative-stress-responsive genes could be functionally classified into five categories, including drug resistance pathway. Under the stress-absent condition, CAP1 deletion resulted in the differential expression of genes functionally related to redox, energy metabolism, substance transport and some others. Efflux analyses indicated that CAP1 was involved in energy-driven drug efflux. Conclusions: Cap1p plays important regulation roles under both oxidative stress condition and stress-absent condition.

Key words: Candida albicans, Cap1p, Transcription factor, Microarray

Acknowledgements: Thanks the National Natural Sciences Fund of China (30200012 and 30500628) and the Science and Technology Development Fund of Shanghai (02QMA1408).

P020021

Comparison of clindamycin and metronidazole in the treatment of bacterial vaginosis

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Metronidazole is drug used for treatment of bacterial vaginosis. We compared clindamycin and metronidazole effects in 123 patients with vaginal discharge (thin and homogenous discharge, vaginal pH>4.5 and positive amine test). In a double-blind randomized trial, the patients were assigned randomly to clindamycin (300 mg twice daily for 7 days in 62 patients) or metronidazole (500 mg twice daily for 7 days in 61 patients). One to two weeks after last day of treatment, the patients were examined for vaginal discharge, vagina pH and KOH test. A significant reduction in the frequency of mal odor, vagina pH and the amine test intensity was observed in both treatment groups. The cure rate was the same in both groups (clindamycin:90.32 %, metronidazole:88.52 %). There was no significant difference in side effects between two groups except for metallic taste reported in metronidazole group. Patient acceptance for clindamycin was significantly more than that of metronidazole due to the metallic taste experienced in metronidazole taking. Overall, clindamycin is effective as metronidazole in the treatment of women with bacterial vaginosis and it is also more converient to use by patients.

P020022

Antibacterial Effects of Ampelopsin From Teng Cha (*Ampelopsis grossedentata*)

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To investigate the antibacterial effects of Ampelopsin (APS), a monomer extract from the Chinese herb Teng Cha(*Ampelopsis grossedentata*), we studied the antibacterial activities in vitro. Compared with berberine chloride, MICs were determined by the agar dilution method. APS had good antibacterial activities against Staphylococcus aureus、meticillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Escherichia coli, β-hemolytic streptococcus, Shigella flexneri, Staphylococcus albus, Neisseria, Candida albicans, Bacillus subtilis: MICs were 0.078, 0.078, 1.25, 0.31, 2.5, 1.25, 0.625, 2.5, 5, 1.25 mg/ml, respectively. MBCs against S. aureus, MRSA, P. aeruginosa, E. coli, Shigella flexneri were 0.312, 0.312, 6.25, 1.55, 5 mg/ml, respectively. The rabbit serum which contained APS against S. aureus、MRSA and P. aeruginosa were determined by a time-effect study. Time-effect curves showed the bactericidal effects at concentration above the MICs, although the bactericidal activity against P. aeruginose was weak at four times the MIC; time-effect curves of rabbit serum contained APS showed obvious bactericidal activity by decreasing the number of viable cells during an incubation period of 2 to 4 hours. The resistant induced test suggested that APS was not liable to produce resistance for S. aureus. These results are promising, showing APS is biologically active against Gram-negative bacteria and Gram-positive bacteria, especially showed high activity against MRSA.

P020023

BACTERICIDAL EFFECT OF PEROXYNITRITE ON HELICOBACTER PYLORI: A MORPHOLOGICAL STUDY

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INOS activity is elevated in the antrum and fundus of duodenal ulcer patients infected with Helicobacter pylori. We aimed to investigate the time- and concentration-dependent bactericidal and morphological effects of peroxynitrite (PN; $ONOO^-$) on H. pylori.

Authentic PN was synthesized as quenched-flow method. A stock culture of H. pylori NCTC 11637 was exposed to different concentrations of PN ($4x10^{-2}$-10^{-4}) or decomposed PN or fresh medium. Samples were taken at 0, 15, 30, 60, and 120 minutes, for the evaluation of viable bacteria, bacterial morphology with gram strain and transmission electron microscopy.

After PN exposure the number of viable bacteria decreased within the first 15 minutes. The morphological conversion of replicating spiral form to viable but non-replicating coccoid form, and bacterial lysis were found to be concentration dependent. Decomposed PN showed no bactericidal activity against H. pylori.

Key words: peroxynitrite, Helicobacter pylori

Supported by B.U. Research Grant (DA 05/31).

P020024

IN VITRO ACTIVITY OF FLUOROQUINOLONES AGAINST S. INTERMEDIUS AND S. SCHLEIFERI.

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TALY

The purpose of the present study was to determine the antimicrobial sensitivity to fluoroquindones (FQs) of 110 strains of S. intermedius and 8 strains of S. schleiferi isolated from 270 dogs during 2005 in Italy. Sensitivity to 14 FQs (ciprofloxacin, danofloxacin, difloxacin, enoxacin, enrofloxacin, flumequine, gatifloxacin, lomefloxacin, marbofloxacin, norfloxacin, ofloxacin, orbifloxacin, pefloxacin, trovafloxacin) was tested by the agar disk diffusion test, according to National Committee for Clinical Laboratory Standards. The results of the present study indicate that FQ resistance among S. intermedius isolates is still rare (less than 2%) in dogs as 108 out 110 isolates were susceptible to all FQs. Fully susceptibility to FQs was on the contrary observed in 3 out 8 of S schleiferi isolates only. Resistart strains of S. intermedius (n = 2) and of S. schleiferi (n = 5) showed a pattern of dichotomrous resistance: they became resistant to most FQs (12 out 14) but maintained sensitivity to the newer FQs gatifloxacin and trovafloxacin.

P020025

Effect of DADAG on expression of protein Bcl-2 and Bcl-xL in leukemic L1210 cells

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Objective To investigate the effect of diacetyldianhydrogalactitol (DADAG) on expression of protein Bcl-2 and Bcl-xL in mouse leukemia L1210 cell. Methods The cytotoxic effect of DADAG on L1210 cells was determined using MTT assay; DADAG-induced apoptosis in L1210 cells was identified by flow cytometry and electron microscopy; the levels of Bcl-2 and Bcl-xL protein were examined with Western blotting analysis.

Results Compared with control, DADAG could dose-dependently decreased the survival rates of L1210 cells. Apoptotic peaks were detected in the cycle analysis by flow cytometry when DADAG was used for 24 h at the concentration of 12.0 mg/L、17.2 mg/L、24.5 mg/L、35.0 mg/L and 50.0 mg/L. The cytoplasm was shrinked and the chromatin of cells became condensed and marginated after DADAG 50.0 mg/L treatment for 24 h. During the apoptotis induced by DADAG, the expression of Bcl-2 and Bcl-xL protein was down-regulated in ati me-dependent manner. Conclusion DADAG induced apoptosis of L1210 cells through inhibiting the expression of Bcl-2 and Bcl-xL protein.

P020026

Antimicrobial Activity of Acne-lotion Product from Antibacterial Durian Polysaccharide and Betel Vine Oil

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The objective of this study was to develop acne-lotion from anti microbial agents from plants. Antibacterial polysaccharide gel (PG) from fruit-rinds of durian *(Durio zibethinus Murr.)* and betel vine oil (BO) from Piper betle L. were examined their anti microbial activity against bacteria and fungus causing skin infection, such as Staphylococcus aureus, S. epidermidis, Propionibacterium acnes and Candida albicans. The susceptibility of microorganisms were determined by agar diffusion test. Broth macrodilution test was used to determine MICs and MBCs of the PG and BO. An acne-lotion was developed using 2.5% w/v PG as antibacterial and gelling agent, 2% v/v BO as a second antibacterial agent and other ingredients were used as necessary for topical skin lotion. The results showed that 0.31% v/v BO and 0.63% w/w PG inhibited growth of tested microorganisms. MICs and MBCs were 0.039 and 0.078% BO; and 1.25 and 2.5% PG, respectively. Acne-lotion product at 1:5 dilution inhibited all tested bacteria. In conclusion, acne-lotion using plant anti microbial agents including 2.5% PG and 2% BO effectively killed acne causing bacterium *(in vitro)*.

Keywords: antibacterial, polysaccharide, Durio zibethinus

P020027

ANTIBIOTICS CONSUMITION IN CLINICAL CENTRE NIS

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Antibiotics are among the most frequently used medications and constitute 25,7% of the total material cost for drugs in Clinical Centre Nis. The aim of our work to analyze the hospital antibiotic utilization during the period of 2003-2005. Using the ATC/DDD methodology, we analyzed the antibiotic consumption, and results were presented as defined daily doses (DDD) per 100 bed days. Total utilization of antibiotics had a significant decrease in 2005 (62,22 : 32,6 DDD/100 BOD, p < 0,01). The most utilizated antibiotics were aminoglikosides (13,46 : 11,79 DDD/100bod). The next most used antibiotics were cephalosporines, especially cephtriaxone (8,18 : 3,86 100/DDD bod). Cephalosporines III generation were used irrationally, much more then in Surgery during 2003 year (cephtriaxone consumed around 20% of drug budget). After the active management program was implemented, usage of all antibiotics decrease by 38.23% compared to 2003(p < 0,05, ceftriaxone decreased by 43.75% and gentamycin 38.4%. Expenditure decreased by 28.4%. It is important to emphasize that almost all antibiotics were on the positive drug list. This analysis pointed to significant therapeutical irrationalities, which shows an improvement by targeted education of prescribes.

P020028

Inhibitory Action of Penicillin Antibiotics on the Enkephalinase Enzyme in the Guinea Pig Ileum

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It has been shown by biochemical enzymatic study that Penicillin antibiotics are able to act as competitive reversible inhibitors of enkephalinas enzyme. In this study we evaluated the effect of Penicillin antibiotics on the enkephalinas enzyme in the guinea pig ileum.

Guinea pig ileum was used in normal Tyrode solution. The ileum was stimulated at 0.1 HZ frequency and the isotonic contraction of this muscle was recorded by a Narco physiograph. Stimulation of guinea pig ileum at 10 HZ resulted in Naloxone sensitive depression of the twitch contractions of this muscle which shows the release of endogenous opioid peptides. After several minutes this depressive effect was reversed by enkephalinas enzyme.

Addition of Penicillin antibiotics during the 10 HZ stimulation potentiated the depressive effect of endogenous opioid peptides in a dose dependent manner. IC_{50} of Ampicillin, Nafcillin and Cloxacillin was calculated as 4.8×10^{-8} M, 1.4×10^{-8} M, 7.4×10^{-9} M respectively.

Our result shows that the Penicillin antibiotics potentiate the depressive effect of 10 HZ stimulation of guinea pig ileum by inhibition of enkephalinas enzyme.

Key Word: Penicillin, Enkephalinas, Ileum, Opioid Peptides.

P020029

ANTIBIOTIC UTILIZATION IN NIS REGION OF SERBIA AND MONTENEGRO

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Pharmaco-epidemiological analyses present the basis for the evaluation of the therapy rationalization within a certain population. Antibiotics are among the most often prescribed medications in primary health care. In total expenditure, antibiotics amount to 16,15% of the total remedy budget on the territory of Nis.

The aim of our work was to monitor and analyze the out-patient usage of antibiotics in Nis area, in the period of 2003 to 2005. By using the ATC/DDD methodology, we analyzed the expenditure of the antibiotics and presented the results as the defined daily doses (DDD) for 1000 citizens per day.

Results and discussion: The total usage of antibiotics increased in 2005 (22,83 : 25,96 DDD/1000/a day, p < 0,05). The most frequently prescribed antibiotics are half-synthetic penicilins (9,67 : 10,0 DDD/1000/a day), then follow macrolides with a significant tendency of growth (3,05 : 4,9 DDD/1000/a day, p > 0.05). The biggest growth has been registered with the usage of azithromycin (0.26 : 0.7 DDD/1000/a day), the number of prescribed recipes shows the growth of 164%. The usage of antibiotics shows the growth of 14% in the year 2005 regarding 2003. By analyzing the number of prescribed recipes, the growth of 3,2% in the prescribing of the antibiotics has been registered. It is important to point out that all the prescribed antibiotics were on the positive drug list. This analyses showed an irrational usage of the antibiotics in primary health care in Nis area, which requires additional application of educative programs.

The cited results will be the basis for further evaluations of the rationality in the usage of antibiotics in primary health care.

P020030

In vitro activity of Cefepime combined with Sulbactam against Clinical Isolates of Carbapenem Resistance Acinetobacter.spp

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The aim of this study was to assess the in vitro activity of Cefepime combined with Sulbactam against Carbapenem- resistant strains of Acinetobacter.spp clinical isolated. We used the checkerboard method to determine whether combinations act synergistically against these strains. 23 A. baumannii and one A.junii strains that were found to be Carbapenem- resistant were included the study. Isolates were collected from the specimens, blood, urine, sputum of patients from 2004 to 2005. All isolates were identified by VITEK- 2 system and stored at - 70 °C until use. The susceptibility results for Cefepime and sulbactam were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. P. aeruginosa ATCC 27853 and E. coli ATCC 25922 were used as quality control strains. The combination of Cefepime and sulbactam demonstrated 33.3% (8/24) synergism, 58.3% (14/24) partial synergism, 4.2% (1/24) additive, 4.2% (1/24) indifference, and no antagonism (Sigma FIC(min) = 0.25 and Sigma FIC(max) = 1.5)

According to our in vitro study results, Combinations of Cefepime with Sulbactam has moderate synergistic activity against some Carbapenem- resistant strains of Acinetobacter.spp which could be likely to prove beneficial for the treatment of infections due to multidrug- resistant strains of Acinetobacter.spp.

Key words: Cefepime, Sulbactam, antimicrobials; Acinetobacter.spp; Carbapenem- resistant; synergy

P020031

The Tolerance of Gatifloxacin Mehanesulfanae after Single- Dose Intravenous Infusion in Chinese Healthy Volunteers

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OBJECTIVE To evaluate the safety and tolerance of gatifloxacin mehanesulfanae in Chinese healthy volunteers treated by single- dose intravenous infusion. METHODS The clinical trial protocol was designed according to the GCP principle after ethics committee passed. After physical examination and laboratory tests were performed, 48 healthy volunteers in 18～50 years old were divided into 100 mg、200 mg、300 mg、400 mg、500 mg、600 mg、700 mg and 800 mg groups respectively by Latin method. Clinical symptoms, vital signs, blood routine et al were observed or examined before and after single- dose intravenous infusion of gatifloxacin mehanesulfanae. RESULTS It has shown that after single- dose intravenous infusion from 100 mg to 800 mg of gatifloxacin mehanesulfanae in the volunteers, the vital signs, clinical symptoms and laboratory tests were mainly in the normal range, only 3 cases of ADRs were found involved in the drug, such as pruritus, rash, GOT or GPT increasing slightly. CONCLUSION Chinese healthy volunteers treated by single- dose intravenous infusion up to 800 mg of gatifloxacin mehanesulfanae were safe and tolerable.

P020032

Research of target genes mutant site of E.coli mutants selected in the MSW

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Objective To investigate the effect of drug concentration、drug structure of fluoroquinolones on the resistant gene of E.coli mutants selected in the mutant selection window (MSW). Methods The target genes, gyrA and parC of E.coli mutants selected in the MSW were obtained by PCR method and sequenced by DNA sequencing. The agar dilution method was carried out to determine MIC of E.coli mutants. Results Among 53 mutants selected by five fluoroquinolones, 79% had a Ser- 83—Leu mutation detected in the quinolone resistant determining region of the gyrA gene, 19% from Asp to a Asn residue at position 87, 2% from Gly to a Cys residue at position 81, and no parC mutation was detectable. MIC of mutation at position 83 was 2～8 fold larger than that at position 81 and 1～2 fold larger than that at position 87. Mutation at position 83 was the most important factor to influence the sensitivity of E.coli. DNA gyrase is the primary target, mutation at position 83 and 87 was the most frequent and no- target mutation was also involved in the resistance.

Conclusion DNA gyrase is the primary target of five fluoroquinolones against E. coli, mutation at position 83 and 87 was the most frequent.

P020033

The study on characteristics and dynamics of Escherichia coli during PAE determined by flow cytometry

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Objective The change of sizes and nucleic acid contents of Escherichia coli were studied during the Postantibiotic effect after exposure to gatifloxacin and ciprofloxacin in order to investigate the mechanism of PAE. Methods The aliquots were taken from the bacterial culture at regular interverals during postantibiotic effect after exposure to gatifloxacin and ciprofloxacin. The dynamic change of sizes and nucleic acid contents of Escherichia coli were determined by flow cytometry in conjunction with fluorescent probes. Results The sizes of Escherichia coli were different from those of the control population. In parallel, an increase in nucleic acid contents was still noted at the end of the experiment. This change was inhibited by the protein synthesis inhibitor Chloramphenicol and the RNA synthesis inhibitor Rifampicin. Conclusion: Gatifloxacin and Ciprofloxacin induced filamentation and the increase of nucleic acid contents of Escherichia coli was inhibited by the protein synthesis inhibitor and the RNA synthesis inhibitor. Flow cytometry is an ideal methodology for study of the PAE.

P020034

Mutant prevention concentration for four fluoroquinolones with Staphylococcus aureus and Escherichia coli

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OBJECTIVE: The mutant prevention concentration (MPC) and MIC against S. aureus and E. coli of ciprofloxacin, pazaufloxacin, gatifloxacin, moxifloxacin were determined and their potent to resitrict resistant mutants was compared. METHODS: For MPC testing, 1010 cells were applied to agar plates containing drug and incubated at 35 °C for 48～72h, the lowest concentration inhibiting mutant was difined as MPC. MPC90 was the concentration inhibiting 90% of mutant. RESULTS: MPCs of moxifloxacin, gatifloxacin, pazaufloxacin and ciprofloxacin to S.aureus ATCC 25923 were 0.18, 0.3, 0.75, 1.8ug/ml and MPC90 to clinical isolates of S. aureus of the four drugs were 1、1、4 and 8ug/ml respectively. MPCs to E.coli ATCC25922 of moxifloxacin, gatifloxacin, pazaufloxacin and ciprofloxacin were 0.072, 0.048, 0.09, 0.06ug/ml and MPC90 to clinical isolates of E. coli (n = 20) were 1, 2, 1, 2ug/ml. MPCs of moxifloxacin and gatifloxacin against S. aureus and E. coli were 2～4 fold less than pazaufloxacin and ciprofloxacin CONCLUSION: The results suggested that moxifloxacin and gatifloxacin would be more effective to prevente selection of resistance mutant of S. aureus and E. coli than pazaufloxacin and ciprofloxacin.

P020035

Anti- Helicobacter pylori activity of three spcies of Lamiaceae family

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In this study, the anti- Helicobacter pylori activity of three species of Lamiaceae family, namely Zizphora clinopodides, Thymus trancaspicus and Zataria multiflora grown wild in Iran against clinical isolates were investigated using hole plate method. The results indicated that the extracts exhibited inhibitory activity against most isolates. The activities are dose dependent and approaches that of metronidazole at about 200 mg/ml.

P020036

Synthesis of conformationally restricted analogues of pentamidine as antileishmanial agents

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Our groups are interested in the design and evaluation of novel bisbenzamidines that are more efficient and less toxic than the parent compound, pentamidine(1) with oral bioavailability. With that goal in mind and based on previous works we considered 1 as a bisbenzamidine in which both benzamidine moieties are linked by a flexible pentamethylene chain and activated by electron-donating ether functions. We studied the influence of the linking chain by reducing its flexibility. We also replaced the strong electron-donating ether functions present in 1 with poor electron donating groups, namely amides. So, series of conformationally restricted analogues of pentamidine in which the flexible central bridge has been replaced by pyridinyl-3,5-dicarbamido, or pyrazolyl-3,5-dicarbamido groups were synthesized. Treatment of 4-aminonenzamidine with pyrazole or pyridine 3,5-dicarbonyl halides afforded the title compounds (2,3). The synthesized compounds pKa compared to 1 is greater and so better penetration through cell membranes are expected. As amastigote forms of leishmania parasite are intracellular, we suppose more potency and better oral absorption for the title compounds.

P020037

Restoration of antibiotic susceptibility of methicillin-resistant Staphylococcus aureus **by blocking blaR1 with a DNAzyme**

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AIM: To investigate the effects of DNAzyme inhibiting Methicillin-resistant Staphylococcus aureus (MRSA) drug-resistant gene blaR1 on the expressions of MRSA drug-resistance. METHODS: Specific DNAzyme to blaR1 mRNA was designed and synthesized. After DNAzyme was introduced into MRSA, drug-resistant characters of MRSA were evaluated by plate cloning formation experiment. The inhibition effects of DNAzyme on the expressions of drug-resistant gene blaR1 and its downstream gene blaZ were observed by real time RT-PCR. RESULTS: Colony forming units(CFU) of MRSA incubated with DNAzyme on the M-H agar added oxa(6 mg/l) were less than those of control group($P < 0.01$). Levels of blaR1 and blaZ mRNA of the DNAzyme groups were lower than those of the control group. CONCLUSION: Antibiotic sensitivity on MRSA may be partially restored by DNAzyme which blocks the expressions of drug-resistant genes blaR1-blaZ. This provided a new idea for development gene drugs to resist other drug-resistance bacteria and diseases.

Key words: Methicillin-resistant Staphylococcus aureus (MRSA); DNAzyme; drug-resistance; real-time fluorescence quantitation.

Acknowledgement: This work was supported by grants from The National Natural Science Foundation of China (30271556).

P03. Cancer Chemotherapy

P030001

Protective effects of L-arginine against cisplatin-induced renal oxidative stress and toxicity: Role of nitric oxide.

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Nephrotoxicity is a dose-limiting factor in clinical use of cisplatin. The aim of the present study was to investigate the effect of modulation of nitric oxide on cisplatin-induced Nephrotoxicity in a rat model. A nitric oxide precursor, L-arginine and a competitive inhibitor of NO synthase, L-NAME were used. Six days after cisplatin injection, acute nephrotoxicity was demonstrated by a marked increase in serum creatinine and blood urea. Histological examination confirmed the occurrence of renal damage. Moreover, cisplatin induced an increase in lipid peroxides and oxidized glutathione and a depletion of reduced glutathione. Activities of antioxidant enzymes glutathione peroxidase and superoxide dismutase were lowered. Besides, there was a reduction in kidney total nitrate/nitrite levels. L-arginine attenuated the oxidative stress and the nephrotoxic effect of cisplatin while. L-NAME aggravated cisplatin nephrotoxicity. In conclusion, the decrease in kidney nitric oxide level contributes, at least in part, in the mechanism underlying the nephrotoxicity of cisplatin. Furthermore, Larginine provides nephroprotective effects and might be useful in improving the therapeutic index of cisplatin.

P030002

Antiproliferation in human EA.hy926 endothelial cells and inhibition of VEGF expression in PC-3 cells by topotecan

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To investigate the mechanism of the antiangiogenesis activity of TPT, series of experiments were performed. We found that TPT inhibited proliferation of human EA.hy926 endothelial cells (IC_{50} value was 0.13 μM in MTT assay), and exhibited high inhibitory activity of angiogenesis in chick embryo chorioallantoic membrane assay. DNA analysis confirmed that TPT could trigger EA.hy926 cells apoptosis in a dosed-dependent manner, and cause disturbance of cell cycle, inducing G2/M phase accumulation at a dose of 0.05 μM, G1/G0 phase accumulation at a dose of 5.0 μM, and S phase accumulation at a dose of 0.5 μM. Western Blotting showed that overexpression of p53 and downregulation of ERK caused by TPT were observed in EA.hy926 cells, and the VEGF expression of PC-3 cells was inhibited by TPT in hypoxia. Altogether, inhibiting proliferation of endothelial cells and down-regulating the expression of VEGF in cancer cells involved in the antiangiogenesis mechanism of TPT.

Key word: Topotecan; Antiangiogenesis; EA.hy926 cells; VEGF.

Acknowledgments We are grateful to Professor Edgell, Department of Pathology, University of North Carolina for presenting the EA.hy926 cells.

P030003

MZ3 Induces Apoptosis in Human Leukemia Cells through Mitochondrial Pathway

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MZ3 exhibited high anticancer activity in six leukemia cell lines ($IC50\ 1.2 \times 10^{-8.0}$ M), including two drug-resistant cell lines. MZ3-induced DNA fragmentation in HL60 cells was observed with a dose-dependent and time-dependent manner. An elevation of reactive oxygen species was also observed in HL60 cells treated with $10^{-8.0}$ M MZ3 at 2 h, and a loss of mitochondrial membrane potential was detected at 8 h. The protein changes related to mitochondrial dysfunction indicated that MZ3 induced the activation of caspase-3, influenced the expression of Bcl-2 family members, MAPKs and other proteins relative to apoptosis. Furthermore, the anticancer activity in vivo was evaluated on SCID mice model of human leukemia engrafts. A prolonged survival time of MZ3 group (MST 33.5 days) was observed after treatment with MZ3 compared with the MST (15 days) in the control group. Together, our data suggested that MZ3 is a potent compound against leukemia cell lines both in vitro and in vivo, and the mitochondrial pathway mediated by Bcl-2 protein family and MAPKs might be involved in signaling MZ3-induced apoptosis.

Key Words: leukemia, Bcl-2 protein family, MAPKs, caspases, mitochondria

P030004

Antiproliferative activity of Fenretinide in human heptoma cells in vitro and in vivo

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To evaluate the anticancer activity of fenretinide against hepatoma cells both in vitro and in vivo and the potential mechanisms. Fenretinide exhibited high efficiency on cell growth inhibition of Bel-7402, HepG2 and Smmc-7721 in vitro with IC_{50} values 12.5-13.9 mM, measured by MTT method. We used flow cytometry to analyze the ratio of apoptotic Bel-7402 cells induced by 15.0 mM fenretinide for 0-48 h, with results ranging from 3%-48% respectively. In a Bel-7402-xenografted athymic mice model, administrations i.p. once per three days with fenretinide (25.0-100.0 mg/kg) for 21 days significantly inhibited tumor growth and the inhibition rates ranged from 37.2% to 57.2%. By western blotting, downregulation of procaspase-3, XIAP and cleaved PARP were observed in Bel-7402 treated with 15.0 mM fenretinide for 48 h. In addition, overexpression of p53 was in a time-dependent manner, along with the decrease of the ratio of Bcl-2/Bax. Fenretinide effectively inhibited the proliferation of Bel-7402 both in vitro and in vivo, and p53 and procaspase-3 mediated apoptosis pathway was involved in its potent anticancer mechanisms.

Key words: Fenretinide; hepatoma cells; apoptosis; xenografted

P030005

HYPOXIA - MEDIATED FENRETINIDE (4 - HPR) RESISTANCE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Our purpose was to investigate whether hypoxia is able to inhibit the effect of 4-HPR for ALL cell lines and its mechanism. By MTT method, we found that hypoxia (2%O2) induced 4-HPR resistance in the tested Molt-4 and Molt-3 with at least 2.8-fold increase in IC_{50} values ($p < 0.01$) relative to these in normoxia (20%O2). Apoptotic detection by flow cytometry showed that 2%O2 significantly suppress 4-HPR-induced apoptosis and the percentages of apoptotic cells induced by 4-HPR for 12h and 24h were 1.2% and 11.0% respectively, compared with 12.6% and 76.3% in 20%O2. In addition, in 20%O2, but not in 2%O2, 4-HPR obviously downregulated protein expression of procaspase-3, ERK1/2 and XIAP, and increased cleavage of PARP expression. Significant DYm loss in response to 4-HPR was observed in normoxia, but not in hypoxia. In conclusion, hypoxia is able to induce 4-HPR resistance in Molt-4 cells and the mechanism may be associated with regulation of mitochondrial pathway-related protein expression and inhibition of the DYm depolarization and apoptosis caused by 4-HPR.

Key words: hypoxia, drug resistance, 4-HPR

P030006

Thalidomide Attenuates Chemotherapy-Induced Intestinal Lesions Via Down-regulation of TNF-alpha

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In the present study, we tested the hypothesis that the increased intestinal TNF-αexpression and intestinal epithelial apoptosis by chemotherapy could be suppressed by anti-TNF-αagent. Thalidomide was used in our study to antagonize CPT-11 induced intestinal lesion. Diarrhea, intestinal lesion, cytokines and epithelial cell apoptosis were monitored. Our results demonstrated that administration of CPT-11 resulted in severe diarrhea and histological damages, accompanied with increased TNF-αexpression and intestinal epithelial cell apoptosis in rats. Combination of thalidomide significantly attenuated diarrhea and histological lesion caused by CPT-11, accompanied by inhibition of TNF-αexpression and intestinal epithelial cell apoptosis. These findings suggest a potent inhibitory role of anti-TNF-αagent on chemotherapy-induced gastrointestinal toxicity via modulation of intestinal TNF-αproduction and intestinal epithelial cell apoptosis. This observation might be of therapeutic value for identifying new agents that alleviate chemotherapy-induced intestinal toxicity.

P030007

Anticancer effects and mechanism of inducing apoptosis by Pyrazolone-semicarbazide Complex in human epidermiod carcinoma cell lines

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OBJECTIVE To evaluate the effects of Lgf-YL-9(Pyrazolone-semicarbazide Complex) on KB and KBv200 cell growth inhibition and the signal transduction pathway in apoptosis. METHODS MTT assay and cell xenograft model were to investigate the effect in vitro and in vivo. Reactive oxygen species (ROS) and mitochondrial membrane potential(ψm) levels in cells were tested by flow cytometry. Symptoms of cell apoptosis were assessed by DNA ladder and Hoechst33258 staining, activation of caspase-3 was measured by Western blot. RESULTS Cytotoxic effect on the two cells was similar. Antitumour activity of in KB cell xenografts was insignificantly increased, but no difference in KBv200 cell xenografts. ψm were decreased and ROS weren't different distinctly after cells were treated with Lgf-YL-9 for 24h. DNA ladder appeared and apoptotic cells stained brightly and displayed condensed and fragmented nuclei. Cleavage of caspase-3 was detected by Western blot. CONCLUSSIONS Lgf-YL-9 plays an important role in the anticancer function and the apoptosis mechanism was associated with the decrease of ψm and the activation of caspases signal transduction pathway.

Key word: Pyrazolone; Semicarbazide; Apoptosis; Caspase

P030008

No Interaction Between P-gp and Survivin, XIAP in MDR Cancer Cells

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OBJECTIVE: To study the interaction between P-gp and Survivin, XIAP in MDR cancer cells. METHODS: Human epidermoid carcinoma cells KB, breast cancer cells MCF-7, their resistant cells KBv200 and MCF-7/Adr overexpressing P-gp were used. The mRNA and protein levels were examined with RT-PCR and western blot. Transfection was used to alter gene expression. The immunoprecipitation assay was used to examine the direct combination between proteins. RESULTS: Either KBv200 or MCF-7/Adr cells expressing the mRNA and protein levels of MDR1, Survivin and XIAP were higher than those of KB and MCF-7 cells. After transfected with the plasmid pECFPN1-Survivin coding Survivin cDNA or pCDNA3-6myc-XIAP coding XIAP cDNA, Survivin or XIAP protein expressions were increased but P-gp levels were unchangeable in four cells. Similarly, after transfected with the siRNAs against Survivin or XIAP, Survivin or XIAP protein expressions were downregulated but P-gp levels were still invariable in two resistant cells. After immunoprecipitation, P-gp didn?t directly combine Survivin or XIAP in two resistant cells. CONCLUSION: Neither Survivin nor XIAP interacted with Pgp in MDR cancer cells.

KEYWORDS: P-gp, Survivin, XIAP, MDR

P030009

ACTINOMYCIN D EFFECTS ON TYPE I COLLAGEN

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Changes in collagen metabolism and structure accompany most of tumor processes. Our aim was to investigate influence of antitumor antibiotic Actinomycin D on rat skin type I collagen amino acid composition and hydrocarbon component.

Study was conducted by means of amino acid analyzer T-339 (Czech Republic). Actinomycin D was introduced to male Wistar rats in dose 2 mg/kg b.w. intraperitoneally.

It was shown that Actinomycin D caused reliable changes in amino acid composition of type I collagen: contents of aspartic acid, proline and hydroxyproline increased simultaneously to decreasing of serine and alanine contents. Such changes could result in modification of collagen molecule surface charge and helix structure.

Collagen hydrocarbon component contents also reliably increased under the influence of Actinomycin D.

Conclusions: as a result of experiments in vivo Actinomycin D ability to cause qualitative changes in type I collagen was established.

Key words: collagen, Actinomycin D, amino acid composition

P030010

Study of effect and mechanism of Nd3 on Human Ovarian Carcinoma Cell in vitro

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To explore inhibitory effect of Nd3 compound, a derivative of norcantharidin, on the proliferation of Skov3 cells and compare it with norcantharidin. Cell proliferation inhibition was evaluated with SRB assay. Nd3-induced cell cycle block and apoptosis were investigated by using flow cytometry assay. The results proved that Nd3 showed the higher inhibition effect on proliferation of Skov3 cells than norcantharidin in a dose- and time-effective dependent manner. Flow cytometry analysis indicated that Nd3 induced cell accumulation in the G2/M phase and apoptosis. Following a treatment of 10, 20, 30 μmol·L-1 Nd3 for 48h, the percentage of G2/M phase cells was 0.12, 10.51 and 21.97% and the apoptosis rate was 8.61, 15.66, 33.35%, respectively. It is concluded that Nd3 exhibits higher ability to inhibit Skov3 cell proliferation than norcantharidin in a dose- and time-effective dependent manner, arresting the cell cycle progression and inducing programmed cell death.

Key words: Nd3; cell cycle; apoptosis; human ovarian carcinoma cell

P030011

HET0016, a Selective Inhibitor of CYP4A, Inhibits 9L Gliosarcoma Tumor Growth in vivo

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The present study examined the effects of N- hydroxy - N'- (4 - butyl - 2 methylphenyl) formamidine (HET0016), a selective inhibitor of 20 - hydroxyeicosatrienoic acid (20 - HETE) formation on the growth of 9L rat gliosarcoma in vivo. Chronic administration of HET0016 (10 mg/Kg/day, ip) for two weeks reduced 9L tumor volume by 80%. This was accompanied by a 4 - fold reduction in the mitotic index, a 3 - 4 folds increase in the apoptotic index, and a ~ 50% decrease in tumor vascularization. In addition, HET0016 treatment increased mean survival time of the animals from 17 to 22 days. LC/MS experiments indicated that neither 9L cells grown in vitro nor 9L tumors removed produce 20 - HETE when incubated with AA. However, the normal surrounding brain tissue avidly makes 20 - HETE, and this activity is selectively inhibited by HET0016. We also found that 20 - HETE stimulated proliferation of 9L cells in vitro. Taking together, these results suggest that HET0016 may act in part by inhibiting the formation of 20 - HETE by the peri - tumoral tissue. Thus, we concluded that HET0016 might be the prototype of a new class of anti - growth compounds in the treatment of malignant brain tumors.

P030012

Bcl - 2 and Bcl - XL siRNA induced hepg2 cells apoptosis and sensitized cells to 5 - FU or HCPT

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To investigate the drug sensitivity in Bcl - 2 and Bcl - XL siRNA transfected Hepg2 cells. Bcl - 2, Bcl - XL siRNA expression vector were constructed and stably transfected into Hepg2 cells. RT - PCR was used to detect mRNA level. Immunofluorescence and western blot was used to detect Bcl - 2, Bcl - XL, Bax and caspase - 3 protein expression. Drug sensitivity of the cells were analyzed with MTT and flow cytometry. The protein expression of Bax had no changed and caspase - 3 was up - regulated when Bcl - 2 and Bcl - XL protein were reduced. Bcl - 2 and Bcl - XL transfected had higher cell inhibitory after treated with 5 - FU or HCPT. siRNA targeting Bcl - 2 and Bcl - XL gene can specifically down - regulate Bcl - 2 and Bcl - XL expression in Hepg2 cells, no changed Bax expression and increase caspase - 3 activity which lead to increase cell spontaneous apoptosis and sensitize cells to 5 - FU or HCPT. Bcl - 2 and Bcl - XL siRNA may be a important agent against human hepatoblastoma.

Key words: Bcl - 2, Bcl - XL, RNA interference, Hepg2 cells

Acknowledgement: Project supported by the National Natural Science Foundation of China (No 30300426) and the Youth Foundation of Hunan province education department (No03B034).

P030013

Inhibitory effects of heparan sulfate proteoglycan on mice transplanted tumors

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To observe the anti - tumor activity and the mechanism of heparan sulfate proteoglycan (HSPG) on C3H mice transplanted tumors. The tumor model was established and randomly divided into five groups. HSPG groups (5, 10, 50 mg/kg), positive group and control group, intraperitoneal injection once a day for 20 days and measured the volume of tumors. Mice were treated at 24th day, then examined tumor weight, calculate thymus index, spleen index, determined the apoptosis by TdT - mediated Dutp nick end labeling (TUNEL) assay in situ, detected the expression of vascular endothelial growth factor (VEGF) by immunolistochemistry. The tumor volume in HSPG groups was reduced without the decrease of thymus index, spleen index. TUNEL assay in situ showed numerous heavy blue apoptosis cells in the HSPG groups significantly higher than control groups. The tumors in HSPG groups showed significantly lower VEGF expression than those in control group. The result showed HSPG has significantly anti - tumor effects on C3H mice transplantable breast cancer. It can induce tumor cell apoptosis and inhibit the VEGF expression, with on obvious influence on immune and hematopoietic system.

Key words: HSPG; MCF - 7; Anti - tumor.

P030014

Study on antitumor activity of isatin in vivo

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AIM: To study the effects of isatin on apoptosis and proliferation of human neuroblastoma cells in vitro and on the growth inhibition of implanted sarcoma S180, hepatoma H22, solid (EC) and ascitic form of Ehrlich ascites cancer (EAC) in mice. METHODS: The effect of isatin on apoptosis of human neuroblastoma cells in vitro was investigated by Hoechst 33258 staining method. The inhibitory rates and life prolonged rates against S180, H22, EC and EAC were observed in tumor transplant models in mice. The influence on the expression of B - cell lymphoma leukemia - 2 gene (Bcl - 2) and proliferating cell nuclear antigen (PCNA) proteins in S180 tumor tissues were also assayed. RESULTS: Isatin 200 μM (29.4 mg.ml - 1) showed apoptosis - reducing effect on neuroblastoma cells. Isatin (60, 180 mg kg - 1) inhibited the growth of the three implanted tumors, with no effect on white blood cells in S180 bearing mice. Isatin (60, 180 mg kg - 1) inhibited the expression of Bcl - 2 and PCNA proteins in S180. CONCLUSION: Isatin exerts antitumor activity by inhibiting tumor cell growth and inducing tumor cell apoptosis.

Key words: isatin; antitumor; tumor transplant; apoptosis

P030015

Mechanism of antitumor effects of pinellia tuber polysaccharides

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AIM To study the anti - tumor effect and mechanism of action of pinellia tuber polysaccharides (PTP). METHODS The anti - tumor effect of PTP was studied on mice sarcoma S180, hepatoma H22 and Ehrlich Ascites Cancer (EAC) and the effects on cell proliferation of human neuroblastoma (SHSY - 5Y) and mice adrenal pheochromocytes (PC12) were evaluated. RESULTS: ①Compared with the negative control group, PTP (300, 600 mg kg - 1) and cyclophosphamide (20 mg kg - 1) inhibited the growth of implanted tumor S180, H22 and EAC in mice. ② All of OD values of the three PTP groups were smaller than that of the control group ($P < 0.01$), indicating that PTP could dose - dependently inhibit the proliferation of PC12 cells. ③Compared with the control group, PTP could inhibit the growth of PC12 cells ($P < 0.001$). ④12h after PTP administration, DNA ladder could be seen and 72h later cell death was observed, indicating that PTP could induce apoptosis and cell death of PC12. Conclusion: PTP shows an anti - tumor effect and the mechanism of action is probably related to the inhibition of cell proliferation and induction of apoptosis.

Keywords: pinellia tuber polysaccharides; tumor; apoptosis

P030016

Subcapsule test application for human tumors sensitivity to anticancer agent prediction.

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Introduction One of the most perspective directions of cancer chemotherapy is extension of indications to application of main antitumor agents. Aim. Study of agents' efficiency.

Methods. Where used postoperative material (cancer of mammary gland (MGC), rectum (RC), uterus (UC), lungs (LC), stomach (SC), prostate, ovary (OC)) which were transplanted under kidney capsules of mice CBA. In three days intraperitoneal introduction of agents began (3 days) in doze LD10 of agent. On the seventh day efficiency was estimated according to inhibition level of xenografts growth. The significance criterion was equal to 25% of tumor growth inhibition. Research results. Inhibition of xenografts for bisphosphonate Mebiphone was for MGC - 62,9%; RC - 71,4%; UC - 62,4%; LC - 50,0%; SC - 55,8%; OC - 61,9% and for prostate cancer - 48,0%. For chloroethylamine Clophiden inhibition reached for RC - 64,3%, for UC - 70,9%; for SC - 55,0% and for LC - 44,4%. For antimetabolite Brotheoplin inhibition were for RC - 64,3%; UC - 76,1%; SC - 55,8%; LC - 37,9% and OC - 67,0%. Conclusion. Were shown definition of individual sensitivity and extension of indications to application of these medicines in clinic.

Key words: subcapsule test

P030017

Quinoline derivatives interact with G - quadruplex, induce senescence and apoptosis in human cancer cell lines

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Agents stabilizing G-quadruplexes have the potential to interfere with telomere replication and inhibit c-myc expression. In this study, we found that quindoline derivatives interacted preferentially with intramolecular G-quadruplex structures and were novel potent telomerase inhibitors. Treatment with quindoline derivatives reproducibly inhibited telomerase activity in human leukemia K562 cells, HL60 cells and colon cancer SW620 cells. SYUIQ-5, one of quindoline derivatives from Chinese herbal medicine, when added to K562 and SW620 cell culture at non-acute cytotoxic concentrations, increased time of population doublings of K562 and SW620 cells, induced a marked cessation in cell growth and cellular senescence phenotype after 35 d and 18 d respectively. Growth cessation was accompanied by a shortening of telomere length, and induction of p16, p21 and p27 protein expression. SYUIQ-5 also induced a delayed apoptosis and c-myc expression in HL-60 cells. These results indicate that quindoline derivatives as novel potent G-quadruplex interactive agents are promising agents for cancer treatment.

Key words: senescence, G-quadruplex, telomerase inhibition, apoptosis

P030018

Inhibition of PKB/AKT activity and tumor growth by SYUNZ-16, a derivative of shikonin from Chinese herbal medicine

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The phosphatidylinositol 3-Kinase-Akt survival signaling, which is deregulated and activated in a variety of cancers, is very important for cancer cell survival and growth. In this study, we found that SYUNZ-16, a derivative of Shikonin, which is used in traditional Chinese medicine, could inhibit Akt kinase activity in vitro obviously, reduced the phosphorylation of FKHRL1 and resulted in FKHRL1 translocation to nucleus in GLC-82 and Hep3B cells. With RT-PCR analysis, it also showed the upregulation of Bim and FADD expression.

However, the PI3K phosphorylation were unaffected under the same concertrations of SYUNZ-16. Furthermore, SYUNZ-16 also inhibited cell growth and induced apoptosis in GLC-82 and Hep3B cells. Silencing of FKHR resulted in significant reduction of apoptosis in GLC-82 cells. Systemic administration of SYUNZ-16 at nontoxic doses in nude mice resulted in inhibition of subcutaneous tumor growth of human cancer GLC-82 xenografts. These results indicated that SYUNZ-16 could be a promising anticancer drug targeting the constitutively active Akt/PKB signaling-dependent tumor cells.

Key words: PKB/AKT, Shikonin derivative, apoptosiss, anticancer drug

P030019

Dose heparan sulfate proteoglycan (HSPG) has antineoplastic action?

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Objective: To identify if HSPG has antineoplastic action. Methods: HSPG isolated from confluent human breast epithelial cells were purified by ion-exchange chromatography, enzymatic degradation and identified by heparintinases; The antineoplastic action of the HSPG were detected on target breast cancer cell MDA-MB-231 and transplanted breast cancer animal model (C3H mice). Results: The HSPG drives cultured MDA-MB-231 cell into detaching and death in a large scale and the attached cells exhibiting morphologic change. HSPG can distinctly block the growth of the transplanted breast tumor which expressed decreases of growth velocity and tumor weight. Flow cytometry and immunohistochemical analysis proved the antineoplastic action of HSPG derived from its direct killing and apoptosis induction on the target cells and tumor model. It should be indicated that the antineoplastic action has little impact on spleen and thymus index as well as haematopoietic system of the animal model. Conclusion: The HSPG from the confluent human breast cells has an indelible antineoplastic action and an unshakable benefit comparing with the current antitumor drugs.

Key words: HSPG, antineoplastic action, breast cancer model

P030020

The effect of CDK4 inhibitor to AML

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Mutationally activated tyrosine kinases provide a critical survival signal to cancer cells, thus, making such kinases and their downstream effectors attractive targets for cancer therapy. To study signaling of mutated kinases we have chosen the receptor tyrosine kinase Flt3 that harbors an activating internal tandem duplication (ITD) in about 25% of AML patients. The use of a Flt3 inhibitor (THRX-165724, Theravance, Inc.) in two Flt3 ITD AML cell lines (MOLM13 and MV4-11) led to the inhibition of the INK4/CDK4,6/Rb/E2F pathway within three hours as reflected by the downregulation of D-cyclin gene expression followed by a decrease in D-cyclin protein. As a result of reduced D-cyclin levels, CDK4,6 activity was downregulated as revealed by the hypophosphorylation of the main substrate of CDK4,6, the Rb protein. THRX-165724 had no effect on D-cyclin levels or Rb hyperphosphorylation in THP-1 and U937 cells, two AML cell lines that express wildtype Flt3. Furthermore, THRX-165724 did not affect the proliferation or survival of these two cell lines.

P030021

The effect of polymer beta peptide and pegylates on liver cancer recurrence and metastasis

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Objective To study the inhibition effect of polymer beta peptide and pegylates on adhesion and invasion ability of tumor cell line and the prevention of them on liver cancer recurrence and metastasis after hepatectomy in a nude mouse model. Methods We studied the influence on the adhesion and invasion ability of tumor cell by MTT method and cell migration experiment. LCI-D20 human liver cancer metastasis model was used to observe the effect on recurrence and metastasis in nude mice. Results 1. The polypeptides and pegylates could all inhibit the adhesion of tumor cells to FN specifically. The inhibition effect on the adhesion of pegylated polypeptides is stronger than that of polypeptides 2. The inhibitory rates of invasion were 36.8, 46.6%, 45.6% and 50.8% for HCCLM6 tumor cells, and 33.6%, 35.9%, 38.3% and 41.2% for SMMC-7721 tumor cells. 3. Polypeptide and pegylates can inhibit the weight of recurrence tumor at incisal margin, and also inhibit the distant metastasis obviously. Conclusions The polypeptides and pegylated can inhibit adhesion and invasion ability of tumor cells obviously, and could also prevent and inhibit liver cancer metastasis and recurrence.

P030022

The expression of CYP4X1 in the human breast carcinoma and its role in regulating breast carcinoma cell growth

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Objective: To investigate the relationship between cytochrome P450 4Z1 (CYP4Z1) and carcinogenesis in mammary gland. Methods: Expression of CYP4Z1 in 15 cases of non-cancerous mammary gland tissues and 64 cases of human breast carcinoma tissues was detected by using RT-PCR. After the human breast carcinoma cell lines being treated with progesterone (a CYP4Z1 inducer) and CYP4Z1 short interfering RNA (SiRNA), the effect of cell growth was evaluated by MTT. Apoptosis was detected by using flow cytometry, and meanwhile, the change in caspase-3 activity was detected. Results: CYP4Z1 was over-expressed in 57% of breast carcinomas with no significant difference in breast tumor type. The expression of CYP4Z1 was correlated with differentiation and postoperative TNM staging of breast carcinoma tissues, but not with lymph node metastasis. CYP4Z1 was expressed in the human breast carcinoma cell lines (T47-D and MCF-7). Treatment with progesterone could increase the expression of CYP4Z1 (10 fold), promote growth of carcinoma cells and decrease activity of Caspase-3. This effect could be prevented by co-treatment with progesterone and CYP4Z1 SiRNA. Conclusion: Overexpression of CYP4Z1 is correlated with carcinoma cell growth, which may be a new target for therapy of breast carcinoma in the future.

Key words: CYP4X1; progesterone; breast carcinoma; growth control

P030023

Inhibitory effects of Stellera chamejasme L. extracts (SCLE) on the metastatic melanoma B16F10

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Chinese plant Stellera chamejasme L. has been found to possess significant anti-tumor activity. Melanoma is one of the most frequently metastasizing malignant neoplasias. In present study, we evaluated the inhibitory effect of Stellera chamejasme L. extracts with water (SCLE) on the highly metastatic melanoma B16F10 in vivo and in vitro. C57BL/6J mice were implanted i.v. with B16F10 (2×10^6 cells) in the experimental model in vivo. SCLE (10.0 ml/Kg b.w, i.p.) was found to significantly reduce the frequency of pulmonary metastasis and to prolong the survival time of B16F10-implanted mice. As for in vitro assay, drug-serum was derived from mice 2hrs after orally administered with SCLE. According to the data from serum-pharmacological experiments, it showed that treatment with SCLE drug-serum strongly suppressed the proliferation of B16F10 cells and inhibited the invasion of B16F10 cells through the reconstituted basement membrane (matrigel) in vitro. Taken together, these results demonstrate that SCLE possesses a notable inhibitory effect on the metastasis of B16F10 melanoma and may be applied for cancer therapy in clinic.

Key words: Stellera chamejasme L.; melanoma B16F10; metastasis

P030024

Daunorubicin and daunorubicinol tissue concentrations in gastric cancer patients after local administration

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Background: In view to study a model to maximize gastric cancer tissues exposure to antineoplastic drugs and contemporarily to reduce their systemic bioavailability, we implemented a preliminary investigation on disposition of daunorubicin liposomal preparation (D) in gastric cancer patients after submucosa injection.

Methods: Twelve candidates to gastric resection, because of gastric cancer, were administered with 2 doses of 50 mg of liposomal daunorubicin 1 week before surgery. Results: Tissue (gastric mucosa and lymph-nodes) concentrations resulted higher than those in serum and urine, these last being present only in traces.

Conclusions: Local administration of anticancer drugs may allow to reach significant concentrations in gastric mucosa and lymph-nodes, and in the meantime to avoid significant systemic concentrations. This procedure could be useful against metastases diffusion through the lymphatic system.

P030025

Effects of SN-38, an active metabolite of irinotecan on p53 mediated apoptosis in human hepatocellular carcinoma cells

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Irinotecan, topoisomerase I inhibitor, was reported as to have an apoptotic effect, although its detailed mechanism is still unclear. We investigated the apoptotic mechanisms of SN-38 in a human hepatocellular carcinoma cell line (Huh7). The cells were cultured with SN-38, for 24 hours. Apoptotic cells were stained by TUNEL, and analyzed by Western blotting to investigate the expression of p53, phosphorylated p53 at Ser15, and apoptosis-related proteins. In addition, Huh7 cells were precultured with p53 antisense oligodeoxynucleotide (AS ODN), followed by treatment of SN-38 and analyzed for apoptosis-related proteins. SN-38 significant increased apoptosis in Huh7 cells. SN-38 increased expression of p53 phosphorylation at Ser15 and its protein in the nucleus. SN-38 also increased Bax, caspase-9, caspase-3 and decreased Bcl-xL. These changes were recovered by p53 AS ODN pretreatment. Furthermore, SN-38 has increased p53 DNA-binding activity in the nuclear of Huh7 cells. We found that SN-38 binding motifs were detected in the proximal promoter of p53. These results suggest that p53-mediated apoptosis is an important mechanism on anti-cancer effects of SN-38 in hepatocellular carcinoma.

P030026

Improved tolerability and antitumor efficacy of gemcitabine - cisplatin through circadian dosing in mice

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We studied the relevance of gemcitabine (GEM) timing for chronotherapeutic optimization. Mice received single or multiple GEM doses ± cisplatin (CDDP) at 3, 7, 11, 15, 19 or 23 h After Light Onset (hALO) for toxicity and efficacy studies. GEM produced least body weight loss and least neutropenia after dosing at 11 vs 23 hALO, whether it was given alone or with CDDP ($p \leq 0.003$). GEM-CDDP tolerability was improved by GEM at 11 hALO and CDDP at 15 hALO ($p < 0.001$). The delivery of this schedule to Glasgow osteosarcoma-bearing mice increased median survival 3-fold as compared to schedules where both drugs were given simultaneously at 11 or 23 hALO (Log rank $p = 0.02$). The circadian amplitudes of body temperature and activity in mice implanted with telemetry transmitter were significantly damped following GEM at 23 hALO, but were not modified after GEM at 11 hALO. In conclusion, tolerability and efficacy were simultaneously improved by GEM dosing in the late rest span. The optimal schedules in humans would correspond to GEM delivery upon awakening and CDDP near mid-activity.

Keywords: Circadian rhythm, Gemcitabine, Chronotherapeutics

Supported by A.R.T.B.C., Hôpital P-Brousse, Villejuif & Lilly, St. Cloud, France

P030027

Ammonium trichloro(dioxoethylene-o,o')tellurate (AS101) sensitizes tumors to chemotherapy by inhibiting the tumor interleukin 10 autocrine loop.

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The study shows that B16 melanoma, stomach adenocarcinoma and glioblastoma multiforme (GBM) constitutively secrete IL10 in an autocrine/paracrine manner. IL10 is essential for tumor cell proliferation because its neutralization decreases clonogenicity. Addition of recombinant IL10 increases cell proliferation. AS101 decreased cell proliferation by inhibiting IL10. This activity was abrogated by exogenous addition of recombinant IL10. IL10 inhibition by AS101 results in dephosphorylation of Stat3 and reduced expression of Bcl2. These results are associated with sensitization of tumor cells to chemotherapeutic drugs, resulting in their increased apoptosis. AS101 sensitizes human GBM tumor to taxol in vitro and in vivo by IL10 inhibition. This sensitization can be obtained by transfection of GBM cells with IL10 antisense. Sensitization of GBM tumors to taxol in vivo was obtained by AS101 or by implantation of antisense IL10-transfected cells. The results indcate that the IL10 autocrine/paracrine loop plays an important role in the resistance of tumors to chemotherapeutic drugs. Therefore, AS101 combined with chemotherapy, may be effective in the treatment of certain tumors.

Keywords: IL10, AS101, tumors, stat3

P030028

Effects of Rosa roxburghii Extract on Proliferation and Differentiation in Human Hepatoma SMMC-7721 Cells and CD34+ Haematopoietic Cells

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This study investigated the effects of ethanol extract and a triterpene of Rosa roxburghii on proliferation and differentiation in human hepatoma SMMC-7721 cells and in umbilical cord blood CD34+ haematopoietic stem progenitor cells. Both extracts inhibited the proliferation of hepatoma cells in a concentration- and timedependent manner, and decreased the release of alpha-fetoprotein from hepatoma cells. Apoptosis was increased only at the highest dose of the ethanol extract in hepatoma cells. Both extracts of Rosa roxburghii did not affect the differentiation of cord blood CD34+ cells to granulocyte and monocyte, as evidenced by flow cytometry analysis of CD11b and CD15. The ethanol extract slightly inhibited proliferation of cord blood CD34+ cells, but no the triterpene. Thus, the triterpene and ethanol extract of Rosa roxburghii are effective in the inhibition of human hepatoma SMMC-7721 cell growth, without affecting the differentiation of CD34+ cells.

The triterpene has less toxicity to human bone marrow depression than the ethanol extract of Rosa roxburghii, and it appear to be a better anticancer drug.

Key words: Rosa roxburghii extract; hepatoma SMMC-7721 cells haematopoietic cells

P030029

Immuno-monitoring for patients with acute leikemia

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The aim of this research was to study the cellular, humoral immunity and some serum cytokine levels of patients with acute leukemia (AL). The study group consisted of 48 children aged 2-18 years who were diagnosed with AL. We were study of peripheral blood T cell subsets by Flow cytometry. IgA, IgM and IgG were measured by Mancini and serum cytokine were determined by ELISA. The number of CD3+ and CD4+ T cells among patients aged 2-14 with AL was significantly decresed ($p < 0.01$). The numbers of CD8+ T cells did not change. IgM and IgG level in patients with AL has decreased ($p < 0.05$) with less decrease of IgA ($p < 0.01$). Level of IL-1, IL-2 in patients with AL were incresed ($p < 0.01$). TNFa increased but this was not significantly valid ($p > 0.05$). IFNg was measured lower ($p < 0.05$). In patients with AL is shown by decreased numbers of CD3+T, CD4+T cells, CD4+/CD8+ ratio and significant decrease in serum IgG and IgM levels ($p < 0,01$). Serum cytokine level was changed related with the tumor pathogenicy.

Key words: Acute leukemia, immunity, cytokine

P030030

Cytotoxic activities of Stephania venosa tuber extracts

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Stephania venosa tuber has been used for many medicinal purposes as well as cancer remedy. This study aimed to compare pharmacological activities of S. venosa tuber extracts on human peripheral blood mononuclear cells (PBMCs). Both water and ethanol extracts exhibited cytotoxicity in a dose-dependent manner, with 50% inhibitory concentration (IC_{50}) values of 40 and 200 μg/ml, respectively, by AlamarBlue reduction assay. This result was verified by trypan blue dye exclusion. The antiproliferative activities of the extracts on mitogen-stimulated PBMCs were determined by MTT assay. The ethanol extract demonstrated higher potency than the water extract on inhibiting phytohemagglutinin-, pokeweed mitogen-, and Staphylococcus protein A-stimulated PBMC proliferation. The apoptotic induction activities of the extracts were also elucidated by annexinV staining. The ethanol extract showed higher potency on apoptotic induction. These results suggested S. venosa tuber may possess cytotoxic, antiproliferative, and apoptotic activities for antitumor action. The ethanolic soaking solution seems to be more potent than the boiling water when it was used as anticancer remedy.

Keyword: Stephania venosa, cytotoxicity

P030031

Effect of Acetaminophen on Doxorubicin accumulation and toxicity in hepatoma-derived HepG2 cells.

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Acetaminophen (AAP) in high doses has been used for therapy of hepatocellular cancer in combination with anticancer drugs. This study was performed to determine if AAP can modulate the doxorubicin (DXR) intracellular concentration and DXR-induced damage to HepG2 cells. Cells were exposed to AAP (2 and 5 mM) with or w/o DXR (2, 5 and 10 micro M). Viability was studied by the Alamar Blue assay. Apoptosis was assessed by flow cytometry and electron microscopy. DXR-efflux assay and western blot analysis measured Pglycoprotein (P-gp) activity and content. We demonstrated that AAP increased viability of DXR-exposed cells, normalized cell cycle and decreased apoptosis. AAP induced P-gp efflux activity and decreased DXR cellular accumulation. In conclusion, AAP strongly reduced the DXR lethal effect on HepG2 cells. This phenomenon may be due to stimulation by AAP of P-gp transport activity and expression. Co-administration of DXR and AAP, intended to improve anticancer therapy, may have an opposite effect, resulting in cancer cell survival.

Key words: acetaminophen, doxorubicin, P-glycoprotein, HepG2 cells.

Acknowledgments: Supported in part by the Dan David Foundation and the Kamea Fund (Dr. I. Manov)

P030032

Anti-tumor action and mechanism of polycydic iminoquinonic analogues

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Purpose This study was designed to investigate anti-tumor action and mechanism of 12 polycydic iminoquinonic analogues. Methods Tetrazdium assay was used to determine cytotoxicity. KBv200 and KB cell xenograft model was established to investigate the in vivo anti-tumor activity. Cell apoptosis rate was measured by flow cytometry. Activation of caspase-3, and capase-9, and Parp was measured by Western Blot. Results AM10 showed the most potent cytotoxicity. IC_{50} of AM10 to MCF-7/Adr, MCF-7, KBv200 and KB cell lines was 0.27 ±0.07, 0.84 ±0.15, 0.19 ±0.02 and 0.08 ±0.00 μmol/L, respectively. In mice bearing KBv200 and KB cell xenografts, AM10 inhibited the growth of tumor in dose-dependent manner. The apoptosis rate of KBv200 cell induced by 2, 4, 8 μmol/L AM10 was 29.1 ±3.6%, 36.8 ±1.3% and 49.6 ±7.5% at 48h, respectively. 2, 4, 8 μmol/L AM10 induced the cleavage of caspase-3, 9 and Parp in KBv200 cell line. Conclusions In vitro antitumor activity of AM10 was strongest among the 12 screened polycyclic iminoquinonic analogues and it potently inhibited the growth of KBv200 and KB cell xenografts in nude mice.

Keywords: Polycydic iminoquinonic analogues; apoptosis; xenografts

P030033

Substituted Imidazole Analogue Inhibits Angiogenesis Induced by Vascular Endothelial Growth Factor

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Angiogenesis is a process that involves proliferation, migration, differentiation and tube formation of endothelial cells. Vascular endothelial growth factor (VEGF) plays an important role in mediating many diseases such as cancer and diabetes. Inhibition of these angiogenic steps is a therapeutic strategy for the diseases. Substituted imidazole analogue (SIA) inhibited human umbilical vascular endothelial cells (HUVEC) viability in a dosedependent manner and caused apoptosis examined by PI staining assay. SIA also inhibited HUVEC migration induced by VEGF and tube formation on Matrigel. In Vivo study showed that SIA reduced angiogenesis in Matrigel plug assay. These results indicate that SIA could be a candidate for the treatment of angiogenesis related diseases.

Key words: angiogenesis, endothelial cells, VEGF

P030034

Modulation of hematopoiesis in myelosuppressed mice by Ganoderma lucidum polysaccharides

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To determine effect of Ganoderma lucidum polysaccharides (GLPS) on hematopoiesis in myelosuppressed mice. Mice were injected intraperitoneally (i.p.) once daily with 2.5 mg/kg, 25 mg/kg, 250 mg/kg of GL-PS, and vehicle respectively for 7 days 24 hours after i.p. cyclophosphamide (Cy, 300 mg/kg). On day 1 after Cy treatment, Splenocyt-conditioned medium (SCM) was prepared in the culture without GLPS or with 50 μg/ml GL-PS (GLPS-SCM). HPP-CFC, CFU-Mix, BFU-E, CFU-MK, CFU-GM and CFU-F colony of bone marrow cells (BMC) was tested. GLPS-induced SCM enhanced HPP-CFC, CFU-Mix, BFU-E, CFU-MK, CFU-GM and CFU-F proliferation than non-stimulated SCM in vitro, but GLPS alone could not promote these colonies proliferation. Injection of low-dose GL-PS in vivo promoted recovery of BMC, red blood cells, white blood cells and CFU-GM, BFU-E and CFU-E colony formation. The results demonstrate that GLPS promotes myelopoiesis by effect on hematopoietic microenvironment to produce the colony-stimulating activity and provide a basis for using GLPS in lessening chemotherapy-induced myelosuppression.

Keywords: Ganoderma lucidum polysaccharides; Myelosuppression

P030035

Noscapine induces apoptosis via caspases activation in P53-independent pathway

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Noscapine, an alkaloid derived from opium, has been used as an oral antitussive agent with low toxicity. One study has shown that noscapine can reduce stroke-

induced mortality rate. Some more recent publications indicate that noscapine can arrest mitosis and induce apoptosis. Also, an anti-tumor effect has been considered for noscapine. This property might be due to the fact that noscapine induces apoptosis. The molecular mechanism responsible for induction of apoptosis is not fully understood. The present study is undertaken to show some mechanisms of apoptosis induced by noscapine treatment. We investigated apoptosis induction in P53-independent pathways in P53-null K562 cells. This was done by observation of DNA fragmentation, caspase assays, and PARP-1 cleavage. Noscapine (20 μM) treatment for 24-48 hours increased caspases 2, 3, 6, 8, 9 activity and caused PARP-1 cleavage followed by DNA fragmentation. Thus, our results indicate that noscapine has the potential to be an effective anti-apoptotic drug used in treatment of malignancies.

P030036

Down-regulation of annexin-1 expression in thyroid cancers is associated with tumour aggressiveness

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The correlation of aggressiveness of thyroid tumours and resistance to apoptosis with expression of annexin-1 (ANXA1) was examined by Western blotting analysis in thyroid carcinoma cell lines and by immunohistochemistry in thyroid cancers with a different degree of differentiation. The highest level of ANXA1 expression was detected in the papillary carcinoma cells (NPA) and in the follicular cells (WRO). The most undifferentiated thyroid carcinoma cells (ARO and FRO) presented the lowest level of ANXA1 expression. The ARO cells were resistant to TRAIL-induced apoptosis. In surgical tissue specimens from 32 patients with thyroid cancers, we found high immunoreactivity for annexin-1 in papillary (PTC) and follicular (FTC) thyroid cancers while in undifferentiated thyroid cancers (UTC) the expression of the protein was barely detectable. In summary, 70% of UTC examined weakly expressed annexin-1, whereas 65% of PTC or FTC specimens tested showed high expression of ANXA-1. Thus ANXA1 expression may correlate with tumorigenesis suggesting that ANXA1 may represent an effective differentiation marker. The down-regulation of ANXA-1 expression may have a role in cancer development.

P030037

Cytotoxic Activities of Constituents from Peucedanum japonicum on Human Promyelocytic Leukemia cells (HL-60)

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The CHCl(3)-soluble fraction obtained from the 80% MeOH of Peucedanum japonicum Thunb. showed an cytotoxic effect on human promyelocytic leukemia cells (HL-60). Among the tested compounds, hyuganin C, showed the most potent cytotoxic effect. Exposure of human promyelocytic leukemia cells to hyuganin C resulted in the induction of apoptotic cell death characterized by DNA fragmentation, chromatin condensation and increase of the proportion of sub-G1 hypodiploid cells were observed. The results suggest that the inhibitory effect of hyuganin C on the growth of HL-60 cells appears to arise from the induction of apoptosis. Further investigation into the in vivo anticancer activity as well as apoptosis induction against several human cancer lines is required for providing biological evidence of this compound as a potential anticancer agent.

Key words: Peucedanum japonicum Thunb, hyuganin C, HL-60, Apoptosis

P030038

Antiproliferative Effect of Amlodipine, a Dihydropyridine Ca^{2+} Channel Blocker, on Human Epidermoid Carcinoma A431 Cells.

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We have previously shown that amlodipine, an L-type voltage-dependent Ca^{2+} channel blocker, inhibited the cell growth of human epidermoid carcinoma A431 cells that lack relevant Ca^{2+} channels. In this study, we examined the effect of amlodipine on cell cycle distribution and cell cycle-specific protein expression in A431 cells by flow cytometric analysis and Western blotting, respectively. Treatment with amlodipine (20-30 uM for 24 hrs) induced G1 phase accumulation, which was associated with decreases in the phosphorylated form of retinoblastoma protein (pRB), a regulator of G1-S-phase transition and in protein levels of cyclin D1 and cyclin dependent kinase 4 (CDK4), G1-specific cell-cycle proteins. On the other hand, the expression of p21 Waf1/Cip1, an inhibitor protein of CDK/cyclin complexes, was increased by amlodipine. These data suggest that amlodipine induced the expression of p21 Waf1/Cip1 and concomitantly inhibited the CDK4 and CDK4-mediated phosphorylation of pRB, which resulted in G1 cell-cycle arrest and growth inhibition.

P030039

Cordycepin, an active ingredient of Cordyceps sinensis, **inhibits tumor growth by stimulating adenosine A3 receptor.**

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[Objective] We have previously reported that orally administered cordycepin (3'-deoxyadenosine), an active ingredient of Cordyceps sinensis, inhibits the growth of B16-BL6 melanoma cells inoculated into mice without causing adverse effects. In the present study, we investigated whether cordycepin affects the growth of other tumor cells, and investigated the molecular target of cordycepin. [Methods] Mouse Lewis lung carcinoma, B16-BL6 melanoma, human HT1080 fibrosarcoma, Caco-2 and CW-2 colon carcinoma cells were incubated for 24, 48 and 72 hours in the presence of diverse adenosine receptor agonists and antagonists or indirubin, a glycogen synthase kinase-3beta (GSK-3beta) inhibitor. The viable cells were enumerated with a Coulter counter. [Results] Cordycepin significantly inhibited cell growth of various tumors in a dose-dependent manner. MRS1191, a selective adenosine A3 receptor antagonist, and indirubin ameliorated the growth suppression induced by cordycepin. [Discussion] These findings suggest that cordycepin displays an inhibitory effect on cell growth in various tumors and its molecular target is adenosine A3 receptors on the tumor cells.

Key Words: cordycepin, adenosine A3 receptor, GSK-3beta

P030040

Induction of apoptosis by curcumin in K562 cells involves down-regulation of p210bcr/abl level and inhibition of its tyrosine kinase activity

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Curcumin (Cur) has been reported to be an inhibitor of the EGF-R tyrosine kinase. Here, we demonstrated that Cur also induces apoptosis in a dose and time dependent manner in a P210bcr/abl positive CML cell line K562. Several hallmarks of apoptosis including DNA laddering, chromatin condensation and fragmentation were observed after the cells were treated with Cur. In order to reveal the mechanism by which Cur induces apoptosis, the effects of Cur on the expression of bcr/abl gene, the content of p210bcr/abl protein and tyrosine kinase activity of p210bcr/abl were studied by using RT-PCR, flow cytometry and western blot analysis with monoclonal antibody against BCR protein. The non-radioactive tyrosine kinase assay was used to determine the activity of tyrosin kinase in different fractions of K562 cells. It has been found that Cur remarkably inhibited the expression of p210bcr/abl protein and its tyrosine kinase activity in a dose and time dependent manner. The results suggest that down-regulation of p210bcr/abl level and inhibitions of its tyrosine kinase activity are involved in Cur mediated apoptotic cell death.

P030041

Inactivation of NF-KB is involved in an anticancer effect of a new saponin component from Gymnocladus chinensis Baillon

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Gymnocladus chinensis Baillon is widely distributed in China, and its fruits have been used in treatment of rheumatism, furunculosis, soreness and swelling in traditional Chinese medicine for a long time. However, few biological components were isolated. In present study, a new triterpenoid saponin (GC-1) was extracted from the fruit of Gymnocladus chinensis Baillon, and its biological actions were investigated. The results showed that GC-1 inhibited growth of a panel of

human cancer cell lines in vitro, but relatively lower inhibitory effect on normal cell lines by MTT and SRB assays. Moreover, GC-1 was also demonstrated to induce HL60 cell apoptosis in a dose dependent way. By using a reporter gene assay, NF-kB activity induced by TNFα was decreased gradually by addition of an increasing concentration of GC-1. In parallel, the blockage of NF-kB translocation from cytoplasm to nuclei was determined by western blotting. It is the first time to investigate the link of antiproliferative action of the compound with the inhibition of NF-kB activation. The mechanism of the actions of GC-1 might be due to the interruption of NF-kB translocation in signal pathway, and contribute to the chemotherapy potential.

Key words: triterpenoid saponin, Gymnocladus chinensis Baillon, anticancer, NF-kB

P030042

Synergistic Effect of Combining Paeonol with Cisplatin on apoptotic induction of Human Hepatoma Cell Lines

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Paeonol (Pae), a naturally occurring agent extracted from Moutan cortex, has shown great promise in the antitumor activity. The aim of this study was to investigate the effect of Pae and its combination with cisplatin (CDDP) on cell growth and apoptosis of human hepatoma cell lines HepG2 and SMMC-7721. The cytotoxic effect of drugs on the two cell lines was measured by MTT assay. The interaction of Pae and CDDP was evaluated by coefficient of drug interaction. Morphologic changes were observed by acridine orange fluorescence staining. Cell cycle progression and apoptotic rate were detected by flow cytometry. The results indicated that Pae and CDDP had cytotoxic effect on the two cell lines in a dose-dependent manner. Pae combined with various concentrations of CDDP showed synergistically cytotoxic and apoptosis-inducing effect on the two cell lines. And the interaction between Pae and CDDP was specific to each cell line. Additionally, a combination of Pae with CDDP resulted in a stronger G2/M arrest, compared to these agents alone in the two cell lines. Pae may be effective and useful as a new biochemical modulator in chemotherapy.

Key words Paeonol; Cisplatin; Synergistic effect; Apoptosis

P030043

Studies of Preparation and Biologic Activity of the Production of Recombinant of HMGN2a and Modified Form of PE Domain III

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To construct a recombinant immunotoxin (RIT) composed of the high Mobility Group protein N2 (HMGN2) a-helical domain and the modified form of Pseudomonas exotoxin (PE38KDEL) domain III and evaluate their anti-cancer capacity in vitro and in vivo. The prokaryotic expression vector pET-32a(+)-HMGN2α-PEIII was constructed and expressed in the E. coli strain BL21 induced by IPTG. The product of fusion protein was purified with Ni-NTA cHeLate agarose, then the tag protein was cleaved by thrombin digestion and the RIT HMGN2α-PEIII was purified with RP-HPLC. Using fluorescence microscope, we found that the fluorescence labeling RIT distributed in the HeLa cell. MTT assay indicated that the RIT kept potent and specific cytotoxicity to HeLa cells. DNA binding assay showed that the RIT binded to HeLa cell DNA selectively. In the in vivo study, it was found that the RIT could inhibit the growth of tumor in nude mice bearing HeLa cancer at 12 mg/kg. The rate of inhibition was 75.4%. In micrograph, the tumor appeared obvious necrosis and apoptosis. All these results suggested that the RIT has potential therapeutic application in tumor.

KEY WORDS HMGN2α, PE, immunotoxin

P030044

Triphenyltin 2-phenyl-1,2,3-triazole-4-carboxylate, a Novel Antitumor Agent, Induces Mtochondrion-Dependent Apoptosis in Human Cervical Adenocarcinoma Cells

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Triphenyltin 2-phenyl-1,2,3-triazole-4-carboxylate (TPT-1) was synthesized as a potential antitumor agent. TPT-1 exhibited antiproliferative effect on different human cancer cell lines, and this effect is far more potent than cisplatin. TPT-1 arrests HeLa cells cycle at G0/G1 phase assessed by FCM analysis and at low concentration (25 nM) TPT-1 induces HeLa cells apoptosis rather than necrosis at high concentration (50 nM), as shown by morphologic observations, DNA fragmentation analysis and FCM. Moreover, treatment of HeLa cells with TPT-1 results in a dramatic up-regulation of Bax and down-regulation of Bcl-2 analyzed by immunohistochemistry, and significantly decreased mitochondral transmembrane potential. Furthermore caspase-3 activation was observed in HeLa cells treated with TPT-1, and z-VAD-fmk rescues apoptotic cells induced by TPT-1. These results suggest that the major pathway by which TPT-1 induced HeLa cell apoptosis is by a mitochondriondependent mechanism. Taken together, we propose that TPT-1 could has the potential to be developed into a new therapeutic agent for treating cervical cancer.

KEY WORDS: triphenyltin 2-phenyl-1,2,3-triazole-4-carboxylate; HeLa cells; apoptosis

P030045

Quantification of tamoxifen and metabolites by LC-MS-Identification of 4'-hydroxylated metabdites in patients? plasma

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Tamoxifen (Tam) is used against estrogen receptor positive breast cancer. One third of patients do not benefit from this therapy. This could be due to variability in metabolism. To investigate Tam metabolism under steady state a LC-MS method to quantify Tam and 5 metabolites was developed. The compounds were quantified following liquid-liquid extraction of plasma samples, separation on a C8 column, electrospray ionization, and detection of the respective protonated molecule ions in single ion monitoring mode using 2 deuterated internal standards. Lower limits of quantification were sufficient to quantify all metabolites investigated in clinical samples from 21 patients receiving 20 mg Tam daily. The following mean concentrations were found: N-desmethyl Tam: 219 ng/mL, Tam 124 ng/mL, N-dddesmethyl Tam 31.3 ng/mL, N-desmethyl-4-OHTam 8.4 ng/mL, 4-OHTam 1.6 ng/mL, and a-OHTam 0.4 ng/mL. In addition, 4'-OHTam, N-desmethyl-4'-OHTam, and N-desmethyl-a-OHTam were identified in patients' plasma for the first time. Variability of active metabolites may contribute to the variability in treatment response. However, this needs to be further investigated.

Supported by the Robert Bosch Stiftung

P030046

Siegesbeckia glabrescens induces apoptosis with different pathways in human breast carcinoma MCF-7 and MDA-MB-231 cells.

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Breast cancer is one of the most common malignancies diagnosed in women with an increasing incidence. Siegesbeckia glabrescence (SG) has been used in traditional oriental medicine to treat cardiovascular diseases. This study examined whether or not SG could induce apoptosis in human breast carcinoma cells. The treatment of MCF-7 and MDA-MB-231 cells with a variety of SG concentrations resulted in a dose-dependent sequence of events that were marked by apoptosis. Furthermore, this apoptosis was accompanied by the cleavage of procaspase-9, -3, and poly(ADP-ribose) polymerase (PARP) in the MCF-7 cells, and procaspase-8, -3 and PARP in the MDA-MB-231 cells. Although, the SG-induced apoptosis was associated with a decrease in the Bcl-2 mRNA expression level and an increase in the Bax mRNA expression level in MCF-7 cells, there was no detectable change in the MDA-MB-231 cells. This suggests that SG might exert antiproliferative action in human breast carcinoma cells via two different apoptotic pathways, namely an intrinsic signal in MCF-7 cells and an extrinsic signal in MDA-MB-231 cells. Therefore, regardless of the ER status, SG might be a promising proapoptotic agent for treating breast cancer.

P030047

Recent advances in anti-metastatic drug developments

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Tumor metastases cost for more than 70 % of cancer patient's death in clinics. There have been great heaps of advances in the foundational and clinical research in cancer metastases studies, including subjects of pathology, molecular biology and pharmacology. Tremendous relevant molecular targets, pathways and interrelations of pathology and a great deal of agents are put into researches. Laboratory models have been normand divided into in vitro and in vivo categories. And in vivo models are further divided into artificial and spontaneous ones. Genetic means also help us a lot—like finding metastatic genes, enhancing spontaneous metastatic rates of tumor models, early clinical diagnoses and drug targeting explorations etc. Pharmacology and clinical investigations for old and new compounds have been undergoing in larger scale than ever that results number of new drugs for tumor metastases to be licensed. This paper presents general ideas and personal discussions for these topics.

P030048

Effects of BclKex, cisplatin, and the combination of BclKex and cisplatin on tumor growth in a nude mouse model of lung cancer

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Overexpression of bcl-2 (B cell lymphoma/leukemia-2) protein inhibits apoptotic pathway in the tumor development process. BclKex, a 22-mer phosphorothioate oligodeoxynucleotide derived from Gene Meth technology, has been shown to prevent bcl-2 gene transcription, thereby blocking expression of bcl-2 mRNA and subsequent protein production. To explore whether BclKex has an anti-tumor effect and potentiates anti-tumor activities of chemotherapy agents, dose-response effects of BclKex, cisplatin, and the combination of BclKex and cisplatin on tumor growth were studied in a nude mouse lung cancer model. Multiple daily intraperitoneal injections of BclKex alone up to 15 mg/kg/day for 14 days did not produce any anti-tumor effects or toxic effects whereas cisplatin produced dose-dependent anti-tumor effects and toxic effects. Moreover, multiple injections of BclKex potentiated cisplatin's anti-tumor effects but not toxic effect. The results suggest that the bcl-2 transcription blocker BclKex does not have anti-tumor activity itself but potentiates anti-tumor effect of cisplatin.

Key words: DNA methylation, bcl-2, lung cancer, cisplatin

P030049

Bufalin enhanced differentiation of All-trans retinoic acid-induced in the patients with acute promyelocytic leukemia in vitro

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Objective To investigate the effect of bufalin combined with all-trans retinoic acid-induced (ATRA) differentiation of acute promyelocytic leukemia (APL) cells in primary culture. Methods Fresh leukemia cells were obtained from heparinized bone marrow aspirations of 12 APL cases in patients. Cell viability was determined by trypan blue dye exclusion. The apoptosis of APL cell was assessed by morphological analysis. Differentiation of APL cell was assessed by morphological analysis and the nitro blue tetrazolium (NBT) reduction test and expression of the granulocyte/macrophage-specific antigen CD11b. Results Bufalin combined with ATRA can induce differentiation of APL cells towards mature stages, NBT reduction was increased 15 % ~52 % and CD11b expression was also increased 16 % ~69 % in combination of bufalin and ATRA were higher than that of ATRA alone, while the concentration of ATRA needed in the combination was reduced to 30 % and the time of differentiation was reduced from 7 days to 4 days. Conclusion The combination of ATRA with bufalin can significantly enhance the differentiation of acute promyelocytic leukemia cells in primary culture by ATRA.

P030050

Induction of apoptosis and inhibition of telomerase activity by histone deacetylase inhibitors in human cancer cells

Yung Hyun Choi, Ched Park, Byung Tae Choia and Gi Young Kima Department of Biochemistry and aAnatomy, Dongeui University College of Oriental Medicine and Department of Biomaterial Control, Dongeui University Graduate School, Busan 614-052, Korea; bFaculty of Applied Marine Science, Cheju National University, Jeju 690-756, Korea, The objective of the present study was to investigate the effect of trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, on the cell growth and apoptosis, and its effect on the telomerase activity in human cancer cells. Exposure of human lung carcinoma A549 and leukemic U937 cells to TSA resulted in growth inhibition and induction of apoptosis in a dose-dependent manner. The increase in apoptosis was associated with the up-regulation in Bax expression, and down-regulation of Bcl-2 and Bcl-XL. TSA treatment inhibited the levels of IAP family members and induced the activation of caspase-3, which was associated with concomitant degradation of PARP and-catenin protein. TSA treatment markedly inhibited the activity of telomerase in a dose-dependent fashion. Additionally, the expression of hTERT, a main determinant of the telomerase enzymatic activity, was progressively down-regulated by TSA treatment. We therefore conclude that TSA demonstrated anti-proliferative and apoptosis-inducing effects on A549 and U937 cells in vitro, and that changes in Bcl-2 family protein levels as well as telomerase activity may play an important role in its mechanism of action.

Key words: TSA, apoptosis, Bcl-2, IAPs, caspase, telomerase

P030051

Antitumor and Calcium Mobilisation Activities of 1,4 bis-(heteroaryl substituted) benzene (BHB) Derivatives on U2OS Cells

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1,2(3)(4)(5)-BHB derivatives have several pharmacological effects such as antitumor, cytotoxic and Ca^{2+} channel inducer. We prepared title compounds in order to investigate cytotoxic activities and effects of intracellular Ca^{2+} mobilisation in vitro.

Cytotoxic effects of derivatives was measured by MTT assay. U2OS (human, Osteosarcoma) cells were incubated by four various derivatives at 24 or 48 h and IC_{50} values were found between 0.001 and 0.06 mg/ml for 24h. In this study benzimidazole substituted compound was the most toxic derivative on U2OS cells. However after 48 h, the higher cytotoxicity was obtained by benzothiazole substituted that showed the cytotoxic effects of these compounds seems to be time-dependent on this cell line.

Depending on IC_{50} values, effects of compounds on intracellular Ca^{2+} concentrations were measured by spectrofluorophotometry and 5-nitro benzimidazole substituted derivative was found to increase the calcium mobilisation through the cell membrane.

These results show that 1,4-BHB derivatives possesses antitumor activity and also results in decline the intracellular calcium on U2OS osteosarcoma cells.

Key words: Antitumor, 1,4-BHB, U2OS.

P030052

Circadian Profile Study of Dihydropyrimidine Dehydrogenase, Thymidylate Synthase, Glutathione and Hematologic Parameters in Healthy Chinese Volunteers

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To know the circadian expression profiles of dihydropyrimidine dehydrogenase (DPD), thymidylate synthase (TS), and reduced Glutathione (GSH) in peripheral veinal blood for healthy Chinese volunteers.

Methods: Peripheral veinal blood of total 14 healthy volunteers (8 males and 6 females) was collected at 6 time points (08:00, 12:00, 16:00, 20:00, 24:00, and 04:00) during a single 24 hours from all the participants.

Radioimmunity assay was used to measure plasma cortisol level. Dihydrouracil and uracil ratios (UH2/U ratios) and reduced GSH was measured with HPLC method. The real-time quantitative RT-PCR method was used for measuring the expression profiles of DPD gene and TS gene. Results: Obvious circadian rhythms were displayed in most of the hematologic parameters, plasma cortisol level, and in whole-blood reduced GSH level. There was no rhythm found for plasma UH2/U ratios, DPD gene and TS gene. Conclusion: Based on the results of interindividual variation in DPD activity in peripheral blood mononuclear cells, a fixed chrono-chemotherapy program using 5-FU for all different individuals should be considered again.

Key Words: circadian rhythm, DPD, TS, GSH

P030053

The research about the effect of abdominal infusion of DDP and 5-FU under thermotherapy via radiofrequency on malignant seroperitoneum

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OBJECTIVE: To investigate the effect of abdominal infusion of DDP and 5-FU under thermotherapy via radiofrequency on malignant seroperitoneum. METHODS: 60 patients were randomly divided in two groups. The patients of chemotherapy group (control group) were only infused DDP and 5-FU abdominally, and the patients in therapy group were treated with thermotherapy via radiofrequency besides the chemotherapy.
RESULT: The clinical effect, life quality, life span, and toxicity were differently improved in therapy group in respective period. CONCLUSION: The combination of thermotherapy via radiofrequency and chemotherapy by abdominal infusion of DDP and 5-Fu on malignant seroperitoneum may decrease peritoneal liquid and improve life quality without toxicities increased.
KEY WORDS: DDP; 5-Fu; Thermotherapy via radiofrequency; Malignant seroperitoneum

P030054

The antitumorigenic potential of total saponins from radix Astragalus membranaceus as chemotherapeutic adjuvant in treating colon cancer

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Discovery of novel chemotherapeutic agents with high remission rate and low toxicity is imperative. We have recently found that the total saponins extracted from Astragalus membranaceus (AST) can inhibit the growth of HT29 colon adenocarcinoma cells. AST resulted in a remarkable decrease in cell proliferation (determined by BrdU assay). Western blot analysis had shown that these effects were associated with a dose-dependent downregulation of the anti-apoptotic factor Bcl-xL and concurrent PARP cleavage. Besides, expression of the cyclin-dependent kinase inhibitor p21 as well as the novel pro-apoptotic protein NSAID-activated gene (NAG)-1 was also upregulated. Real-time PCR had demonstrated that NAG-1 mRNA level was also increased by AST.
In HT29-xenografted nude mice, AST treatment resulted in a 35% tumor regression, as compared to 37% growth suppression by the chemotherapeutic drug 5-fluorouracil, without causing significant body weight loss in the animals as in the case of the latter agent. These findings have implicated that total Astragali saponins possess antitumorigenic potential in treating colon cancer.
Keywords: colon cancer; Astragalus membranaceus; NSAID activated gene-1; nude mice

P030055

Gefitinib affects DNA topoisomerase I activity and alters the etoposide-induced G2/M arrest in PC3 cells.

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Gefitinib is an inhibitor of epidermal growth factor receptor tyrosine kinase (EGFR-TK). The response of cells to gefitinib treatment is not correlated with the level of EGFR, suggesting additional molecular targets for gefitinib. Certain tyrosine kinase antagonists, tyrphostins, are potent inhibitors of the cellular topoisomerase I (topo I). Here, the effect of treatment of prostate carcinomas cells (PC3) with Gefitinib alone or in combination with etoposide on the cellular topo I and on cell cycle parameters was examined. Cytotoxicity, additivity, cell cycle progression, topo I activity, topo I and other cellular proteins levels were determined in the various treatments. Gefitinib decreased the activity of topo I but not the level of topo I protein in PC3 cells. Treatment with gefinitib combined with etoposide, had additive inhibitory effect on cell proliferation. Gefitinib arrested cells at G1 and etoposide at G2/M while the combination of both drugs at a specific sequence and concentrations caused the accumulation of cells in S phase.
The reduction in topo I activity by gefitinib contribute to the anti cancer properties of this drug and to the design of an effective anti-cancer strategy.

P030056

The Effects of Fotemustine, Atorvastatin and Dexamethasone on the C6 Glioblastoma

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Aim: Fotemustine (FM) is an antineoplastic agent used in the systemic treatment of the glioblastoma. In this study beside fotemustine, dexamethasone (DM) was used as an antiangiogenic agent and atorvastatin (AV) which is antilipidemic but also putative apoptatic was used. Our aim was investigate the effects of the combination of three agents on the mice C6 glioblastoma (C6G) tumour mass, cell proliferation, angiogenesis and lipid profile.
Material and Metods: 5x106 C6G cell was inoculated to flank of the Bulb-C mice. 10 days after inoculation, FM10 mg/kg, DM3 mg/kg single dose(ip) and AV10 mg/kg/day(oral) for 8 days were applied. On 18th day, extracted C6G were weighted and their lipid profiles were detected by gas chromatography. Cell proliferation was measured by KI-67 antibody. Angiogenesis was detected by PTEN antibody.
Results: In all treatment groups, tumour mass was decreased by 48-68 % in comparison with control. DM augmented FM effects by 2.4 %. On the other hand AV had no effect. Amount of total fatty acid was increased by DM, reduced by FM, but not effected by AV. Lindeic acid/arachidonic acid ratio (18:2/20:4) was decreased with FM 60 % in comparison with control.

P030057

The Study of the Pharmacodynamic Properties of L-VCR and Its Mechanism

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The present study was conducted to analyze the pharmacodynamic properties of liposome Vincristine (L-VCR), its pharmacological effects against tumor. and the characteristics of L-VCR and the possible mechanism of its actions were discussed. Wistar rats were inoculated with Walker256 tumor. Blood drug level was analyzed by HPLC-UV and pharmacodynamic parameters were calculated. Mice were inoculated with transplantable tumors, such as S180 tumor carneus, B16 melanoma, Colon26 colon carcinoma and L-VCR was given i.v. Tumor inhibitory rate and non-tumor body weight was calculated. Compared with F-VCR, the plasma level of L-VCR was higher and sustained significantly longer. Its AUC0.25-t was increased about 121 times after treatment. The weight of the tumor mass in all three cases were reduced significantly, the tumor inhibitory rate of 2 mg/kg F-VCR was comparable with that of 1 mg/kg L-VCR, and non-tumor body weight was significantly higher in the L-VCR treated group. The effect of L-VCR was due to the coating of liposome and its level in tumor was increased.
Key Words: L-VCR, HPLC-UV, Pharmacodynamics

P030058

Anticarcinogenic Effects of Carvacrol and Thymol on C6 Glioblastoma multiforme Cell Line

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Glioma multiforme is one of very aggressive and expansive tumor types and it is almost incurable, since current therapies are largely unsatisfactory against this tumor. In the present study, two monoterpenes, carvacrol (CR) and thymol (TM), which are found in volatile oil of Oregano and Thymus, were used to test anticarcinogenic effect on C6 cell line. Test substances were applied at 1, 5, 10, 50 and 100 uM doses. MTT and neutral red tests were applied and results were obtained at the end of 24, 48, 72 and 96 hours. CR showed dose dependent effects on C6 cells and decreased C6 proliferation. While CR decreased mitochondrial activity (MA), it increased lysosomal activity (LA) at the first and second days. It also decreased either MA or LA at the third and fourth days. TM showed dose and time dependent antiproliferative activity on C6 cells. TM reduced MA and also LA. To best of our knowledge, this study is the first study about anticancer effects of CR and TM on brain tumor cells. However, it needs further studies to understand their mechanisms of action.
Keywords: carvacrol, thymol, glioma, cancer

P030059

Studies on the Photodynamic Effect and the Mechanism of CPD6 on Sarcoma 180 Transplanted in Mice[*]

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Objective: To observe the photodynamic effects and its mechanism of the chlorin photosensitizer derivative6 (CPD6) on sarcoma 180 transplanted in mice. Methods: Subcutaneous inoculation of cancer cells was made in mice. When it is 6-8 mm in diameter, the tumor were irradiated for 10 minutes by laser with 760 nm at 15 min after intravenous injection of CPD6, hematoporphyrin derivative (HpD) and normal saline, respectively. The tumors were taken to calculate the inhibitory rate at 30 day after therapy. DNA in cancer cells were purified and analyzed with Raman spectroscopy. Result: The tumor inhibitory rate (TIR) is 46.9% in CPD6 group, 55.8% in HpD group. The Raman spectra of DNA in cancer cells were different in the groups. The special bands of adenine, guanine and phosphodiester bond were shift or diminution. It shows that DNA in the cancer cell was damaged. Conclusion: CPD6 has the photodynamic effects on sarcoma 180 transplanted in mice. The effect of CPD6 is associated with the severe damage of DNA in cancer cells caused by it.

Key words: CPD6, sarcoma S180, Raman spectrum, damage of DNA,

* The research supported by Tianjin United Scientific Research Center on Opto-electronics (No. 003101511).

P030060

Comparison of Photodynamic Effects on Liver Cancer Cells in Vitro among Three Chlorin Photosensitizer Derivatives*

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Objective: To evaluate and compare the photodynamic effects of three chlorine photosensitizer derivatives (CPD) 2, 3 and 5 on liver cancer cells in vitro. Methods: Photodynamic effects of CPD2 CPD3 CPD5 on Liver cancer cells BEL-7402 were observed respectively. The phototoxic effects of the three CPD photosensitizers were compared and estimated by the experiment of elimination of red dye. Result: The results show that CPD3 was absorbed increase by liver cancer cells BEL-7402. The phototoxic effects of CPD2 and CPD5 were lower than that of CPD3 when the concentration is in 0.5 ~2.5 mg/ml. The mortality of liver cancer cells was increased going with pyramiding the dose of photosensitizers when the concentration is in 0.5 ~2.5 mg/ml. The relationship of close-effect of CPD is very marked. Conclusion: CPD5 has the best integrate capability.

Key words: Chlorin, photosensitizers, liver cancer cells BEL-7402, phototoxic effects,

* The source of research support is Tianjin United Scientific Research Center on Opto-electronics, the No. is 003101511.

P030062

Apoptotic activity of 23-hydroxybetulinic acid on Lovo cell line

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In order to investigate the apoptotic effects of 23-hydroxybetulinic acid on Lovo cell line and the mechanism of apoptosis. We used MTT-based cytotoxicity assay to test the Lovo cell proliferation. The apoptotic cells and the mitochondrial membrane potential were detected by fluorescence microscopy, flow-cytometric analysis. 23-Hydroxybetulinic acid inhibited Lovo cell proliferation in close and time-dependent manner. Apoptotic body—the characteristic morphology changes were noted after Lovo cells exposed to 23-hydroxybetulinic acid.

The apoptotic activity of 23-hydroxybetulinic acid enhanced as the close and time increased. Compared with control group, 23-hydroxybetulinic acid caused the Lovo cell mitochondrial membrane potential change obviously. 23-Hydroxybetulinic acid exerted apoptotic activity on Lovo cell line. The mitochondrion played acrucial role in the process of Lovo cell apoptosis induded by 23-hydroxybetulinic acid. The changes of mitochondrial membrane potential may result in the Lovo cell apoptosis.

P030063

The Effect and its Mechanism of Isorhamnetin on Lung Cancer

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To investigate the effect and its mechanism of isorhamnetin on lung cancers. A549 cells were treated with Iso. Their morphological and cellular characteristic were observed by light and electronic microscopy. Growth inhibition was analyzed with MTT assay, clonogenic assay and growth curve assay. Apoptotic characteristic of cells were determined by FCM, DNA fragmentation, comet assay, immunochemistry, westblot and TUNEL assay. Iso inhibited the growth of A549 cells which demonstrated apoptotic changes. Iso could up-regulate the expression of apoptosis genes Bax, Caspase-3 and P53, and down-regulate the expression of the anti-apoptotic gene Bcl-2 and PCNA protein. Tumor models were setup by transplanting Lewis lung carcinoma cells into C57BL/6 mice. The tumor weight and size treated with Iso were lowering compared to the control group. The results of apoptosis-related genes of transplanted Lewis cells were the same as those in vitro. Iso had antiproliferative activity against lung cancer in vitro and in vivo. Its mechanism may be involved in apoptosis of cells induced by down-regulation of oncogenes and up-regulation of apoptotic genes.

P030064

The anti-cancer effects and mechanism of artesunate on colorectal cancer

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The aim of our research is to study the anti-cancer effects and mechanism of artesunate on colorectal cancer.

The anti-cancer effects were examined by growth inhibition rate of colorectal cancer in nude mice. When dosage of intravenous injection was 200 mg/kg.d and 400 mg/kg.week, percentage of antitumor was 35% and 41%.

When artesunate was injected into tumors directly with 50 mg/kg.d, the inhibition rate was 51%.

The anti-cancer mechanism was studied in vitro. When colorectal cancer cells were exposured to different concentrations of artesunate, β-catenin translocation from nuclear to adherent junctions of membrane was detected by immunocytochemistry; RT-PCR results suggested the expression of c-myc and survivin, the target genes of β-catenin, was reduced; the apoptosis rate examined by flow cytomerty was increased and expression of Ki-67 tested by immunocytochemistry was decreased.

Artesunate can translocate β-catenin from nuclear to membrane, which inhibited the expression of c-myc and survivin. so the colorectal cancer cells were easy to apoptosis in vitro and the growth of colorectal cancer in nude mice was inhibited significantly.

Key words: artesunate; colorectal cancer; mechanism

P030065

Eeffect of scutellarria baicalensis stem-leaf total flavonid on human cervical carcinoma Hela-cell in vitro

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Objective: To study the effect of scutellarria baicalensis stem-leaf total flavonid (SSTF) on human cervical carcinoma cell in vitro. Methods: The human cervical carcinoma cell (4 ×105/mL, 100μL) was added to a well of 96 well plate. The negative group included 6 wells. The five concentration of positive drug 5-FU were 0.01, 0.1 1, 10, 100μg/mL respectively as well as the SSTF. There are three wells at each concentration. The A was assayed at 570nm wave on Enzyme-label instrument using MTT method and calculated the IC50 of SSTF inhibiting the growth of human cervical carcinoma cell in vitro. Results: SSTF could inhibit growth of the tumor cell, and its IC_{50} was 20.5μg/mL, that of 5-FU was 10.5μg/mL. Conclusion: SSTF has the effect of inhibiting on the growth of human cervical carcinoma cell in vitro, it has certain cell toxicity.

Key Words: SSTF; cervical carcinoma; Hela-cell

P030066

An imidazole derivative FG020326 reversing MDR in vitro and in vivo

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OBJECTIVE: To investigate FG020326 reversing multidrug resistance(MDR) in vitro and in vivo. METHODS: MTT, doxorubicin(Dox) accumulation and xenograft model were used to study FG020326 reversing MDR in two human MDR cancer cells MCF-7/adr and KBv200 and their parental sensitive cells MCF-7 and KB. The function, expression and [3H]azidopine labeling of P-gp were examined to explore the reversal mechanism. The CYP3A4 activity and FG020326 pharmacokinetic were examined by HPLC. RESULTS: FG020326 enhanced the cytotoxicity of Dox and vincristine(VCR) in two MDR cells, exhibited more 3-fold stronger reversal MDR activity than verapamil and increased the Dox intracellular accumulation in MCF-7/adr. In KBv200 cell xenografts mice, FG020326 enhanced the VCR antitumor activity without increasing the toxicity. FG020326 increased Rhodamine 123 accumulation, inhibited P-gp expression and [3H]azidopine labeling in KBv200. FG020326 didn't affect the CYP3A4 activity up to 50μmol/L and VCR pharmacokinetics with enough efficacious plasma concentration in mice. CONCLUSION: FG020326 is a potent MDR modulator in vitro and in vivo and may possess great promise to treat P-gp-mediated MDR cancers. KEYWORD: FG020326, P-gp, MDR

P030067

Research of the antitumor effect and mechanism of a Dithiocarbamic acid ester on hepatoma cells by cDNA microassay

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Hydrochloride 4-Methyl-Piperazine-1-Carbodithioc Acid 3-Cyano-3,3-Diphenyl-Propyl Ester is a novel structure compound of Dithiocarbamic acid esters with the symbol of 208, which could significantly inhibit the growth of human hepatoma implanted nude mice with lower cyto-toxicity. Two human hepatoma cells, originated from planted tumor and monolayer culturing separately, were used to compare the differences of gene expression profile between the control and experimental groups treated with compound 208 by cDNA microassay. Total RNA were extracted and cDNAs were labeled with Cy3 and Cy5, respectively. Then the cDNAs were hybridized on the gene chips containing 8000 kinds of human genes. The signals were examined by GenePix Pro 3.0 software. The two nucleic fluorescent dyes were exchanged each other, so that the experiments were repeated twice. The results showed that 237 kinds of genes were markedly different after treated by 208 compared with the control, in which 215 were down-regulated and 22 up-regulated. According to the reports 75 of 237 were involved with tumor. To some extent, compound 208 worked through the regulation of genes related with signal conduction and cell structure proteins.

Key words: gene chip, gene expression, human hepatoma cells

P030068

Study of the effects of mast cell mediators on SW756 cervical carcinoma cell migration.

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To investigate the effects of mast cell mediators on epithelial cell migration during carcinogenesis, an in vitro assay of scratch wound healing onto monolayers of SW756 cells (HPV18-positive cervical carcinoma cell line) was developed. SW756 cells migrate, but not proliferate in response to scratch wounding in serum-free medium. Migration rate of SW756 cells was significantly increased in the presence of serum-free medium supplemented with TNF-alpha (0.3-10.0 ng/mL), but no effect was observed after the medium was supplemented with histamine and serotonin (1.0-30.0 uM) as compared to serum-free media. 2-Arachidonylglycerol (0.1-3.0 uM) a mast cell endocanabinoid and full agonist of cannabinoid receptors, inhibited SW756 cell migration in a serumfree medium and in the medium supplemented with TNF-alpha. This effect was blocked by the addition of SR144528 0.5 uM (Sanofi Recherche, France), a CB2 antagonist of cannabincid receptors. These results suggest that mast cells may have both, stimulatory and inhibitory effects on SW756 cell migration, depending upon the type of mediator released.

migration, mast cell, cancer

Research supported by FONDECYT-Chile Grant 1020458

P030069

Inhibition of Functions of P-glycoprotein By HZ08 in Human Erthroleukemic Cell Line, K562/A02

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Objective: To assess a novel P-glycoprotein(P-gp) inhibitor, HZ08, on drug efflux of K562/A02, and to explore its mechanism. Methods: Cytotoxicity was assessed by MTT assay. Intracellular rhodamine123(Rh123) accumulation and apoptosis rate were measured with flow cytometry. The effect of HZ08 in vivo research was also appreciated with nude mice. P-gp expression, MDR1 expression, membrane lipid fluidity and ATPase activity were determined as well. Results: Rh123 accumulation was increased with HZ08 in K562/A02. 10μM HZ08 reversed the resistance of K562/A02 to Adriamycin(ADM) and Vincristine(VCR). HZ08 could increase apoptosis rate of VCR in K562/A02. Moreover, in vivo ADM co-administration with HZ08 inhibited the growth of K562/A02 in nude mice. HZ08 had no influence on expression of P-gp and MDR1, but reduced cell membrane fluid and enhanced ATPase activity in K562/A02. Conclusion: HZ08 was effective on inhibition of P-gp in K562/A02, which would impossibly be a new drug to reverse the resistant of tumor cells.

Key Words: P-glycoprotein; HZ08; multidrug resistance

Acknowledgement: We thank Dr. Wenlong Huang for her help for providing new chemical compound HZ08.

P030070

Antitumour action of 5,6-dimethylxanthenone-4-acetic acid (DMXAA) in rats bearing chemically-induced primary mammary tumours

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5,6-Dimethylxanthenone-4-acetic acid (DMXAA) is a vascular disrupting agent under phase II clinical trials. Its activity was evaluated in female Wistar rats bearing primary mammary tumours induced by s.c. injection of N-nitroso-N-methylurea (100 mg/kg). A single dose of DMXAA (1800 mg/m2) was given to animals when tumours were measurable. Tumour volumes, extent of necrosis and cytokine profiles were measured. Following DMXAA treatment, tumour growth was delayed and the overall survival of animals extended significantly, tumours showed an increase of comedo necrosis, occurrence of large areas of confluent necrosis of the epithelial and stromal components, vascular damages, including luminal thrombus, interstitial haemorrhage, loss of endothelium and impaired patency of small blood vessels, and increased levels of TNFα, IL-6, VEGF and IL-1α. The study shows for the first time that DMXAA has significant in vivo antitumour activity against non-transplanted autochthonous tumours in a host species other than the mouse.

Key words: DMXAA, antivascular therapy. New Zealand Cancer Society and Auckland Medical Research Foundation supported the work.

P030071

Lovastatin potentiates antitumor activity and improves Hemorheology in Lewis lung carcinoma in C57 mice

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The aim of the present studies was to investigate whether lovastatin has an antitumor activity on Lewis lung carcinoma and evaluate whether lovastatin has a direct effect on Hemorheology in tumor model. The C57 mice bearing with Lewis lung carcinoma were treated with lovastatin at concentrations of 10, 20, 40 mg/kg i.g. for 21days. The antitumor growth and metastasis of lovastatin on the Lewis lung carcinoma were examined. The blood viscosity and the electrophoresis on red blood cells were also observed. Treatment with lovastatin could significantly reduce the tumor formation and metastatic dissemination to the lungs from established oxter tumors ($p < 0.01$). Lovastatin-treated mice also exhibited decreased viscosity in blood ($p < 0.05$) and enriched the electric charge on red blood cells ($p < 0.05$). CONCLUSION: Lovastatin is effective in slowing the growth of tumor formation and metastasis, at the same time, lovastatin meliorate the hemorheology in tumor model. These in vivo results support further investigation of lovastatin as an antitumor agent in animal models.

Keyword: lovastatin, hemorheology, Lewis lung carcinoma.

Acknowledgement: 973 Program of the Ministry of Science and technology(No. 2004CB518902)

P030072

Modulation Mechanisms of Ganoderma Lucidum Polysaccharides (Gl-PS) on Human Multidug Resistance

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Multidrug resistance (MDR) is a problem in the treatment of cancer by chemotherapy. The ganoderma lucidum polysaccharides(Gl-PS), the main bioactive component, has been confirmed anti-tumor effects in our previous studies. In this study the effect of Gl-PS on MDR and its mechanisms were studied. Human K562 and K562/ADM cell lines were used. Measurement of cytotoxicity by MTT method; ADM concentration in cell was detected by FACS and Confocal; the expression of P-gp was assayed by FACS; the MDR-1 gene expression was detected by RT-PCR. The results showed that Gl-PS could transverse MDR in human K562/ADM cell lines. Gl-PS can obviously reverse the resistance of K562/ADM to ADM. The reverse factor were 6.97, 6.86 at the concentration of Gl-PS in 10, 20 mg/ml respectively. Pgp-170 expression and MDR-1 gene expression could be down regulated by Gl-PS at 10 mg/L and 50 mg/L. The results clue on us that Gl-PS inhibited the MDR by inhibiting of multidrug resistance proteins.

Key words: multidrug resistance; Ganoderma Lucidum polysaccharides; P-glycoprotein; MDR-associated protein1

P030073

B3, a novel modulator of P-glycoprotein mediated multidrug resistance in K562/A02 cells

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Objective: To research the effect of B3 on P-glycoprotein (P-gp) inhibition in K562/A02 cell line and its possible mechanism. Methods: Cytotoxicity was assessed by MTT assay. Intracellular rhodamine123 accumulation was measured with flow cytometry and mdr1 mRNA expression by reverse RT-PCR. Membrane lipid fluidity of K562 and K562/A02 were determined by fluorescence spectrophotometer and ATPase activity on cell membrane was measured after membrane protein preparation through differential centrifugation. Results: B3 conferred an increase on chemosensitivity of K562/A02 to Adriamycin and Vincristine. B3 could increase rhodamine123 retention in K562/A02 cells. 10 μmol/L B3 had no effect on the levels of mdr1 mRNA in K562/A02 cells($P > 0.05$). It is supposed that B3 can reverse multidrug resistance by decreasing cell membrane lipid fluidity. B3 with 3, 10 μmol/L can enhance ATPase activity significantly($P < 0.05$). Conclusion: B3 is a novel and potent MDR reversal agent and may be a potential adjunctive agent for tumor chemotherapy.

Key Words: P-glycoprotein; B3; multidrug resistance

Acknowledgement: We thank Dr. Wenlong Huang for her help for providing new chemical compound HZ08.

P030074

Anticancer activity of XY-8, a new water-solubility derivate from Camptotecin, on human carcinoma

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XY-8 is a new water-solubility derivate from Camptotecin, which, compared with Camptotecin, appears to be less toxicity and more cytotoxic against human tumor cells. In this study, we report the anticancer activity of XY-8 on 9 kinds of human tumor including lung carcinoma, stomach carcinoma(2 cell lines), hepatocarcinoma(2 cell lines), breast carcinoma, colon carcinoma, leukemia, cervix carcinoma, ovarian cancer and glioblastoma in vitro or in vivo. The average IC_{50} value of XY-8 1.22 μM. In contrast, the average IC_{50} values of Camptotecin were 10.54 μM. The LD50 was 280 mg/kg on mice by iv.. In nude mice, the T/C(%) were 44.2 at the dose of 2.5 mg/kg and 53.76 at the dose of 5 mg/kg on A-549 xenograft, the T/C(%) were 27.3 at the dose of 2.5 mg/kg and 36.6 at the dose of 5 mg/kg on BGC-823 xenograft, the T/C(%) were 43.3 at the dose of 2.5 mg/kg and 48.9 at the dose of 5 mg/kg on SGC-7901 xenograft,. The results showed that XY-8 had strong growth inhibition of human tumor in vitro and in vivo and may be useful in human tumor chemotherapy duo to its water-solubility and less toxicity.

Key words: anticancer activity, Camptotecin, derivate, in vivo

P030075

Multifactors are associated with Gleevec-acquired resistance in human leukemia cell line, K562/G02

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Objective Gleevec, a specific ABL tyrosine kinase inhibitor, was used for treatment of Ph+ CML, ALL and GIST. However drug resistance has been observed, especially in blast phase of CML, and has become a significant therapeutic problem in clinic. This work was to study the molecular mechanisms involved in the drug resistance. Methods A Gleevec-acquired resistant leukemia cell line K562/G02 was selected by exposure of K562 to increasing concentrations of Gleevec. RT-PCR, Western blotting, IP, FISH, and proteomics assay were used to study the resistance mechanisms. Results Not only were bcr-abl up regulated in gene level, protein level, and kinase activity but also showed mdr1/Pgp overexpression in K562/G02 cells. Additionally, 20 differentially expressed protein spots were identified using 2-DE followed by MALDI-TOF MS between K562 and K562/G02 cells. Conclusions A Gleevec-resistant leukemia cell line was established. The resistance mechanisms involved increased expression of bcr-abl and mdr1/Pgp, amplification of bcr-abl fusion gene, increased activity of BCR/ABL, and overexpression of Proteasome, Calmodulin, etc. The results provide clues to elucidate the mechanisms underlying Gleevec resistance in leukemia.

Key words: leukemia Gleevec resistance mechanism

Acknowledgement: This work was supported by a grant from The Nature Science foundation of Tianjin city government, China (Grant No.: 043610311).

P030076

Chelidonium majus L. derivate Ukrain inhibits metastasing

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Objectives. To clarify the potential and the mechanisms of the antimetastatic activity of the drug Ukrain.

Methods. Expression of genes and proteins involved into the tumor invasion associated extracellular matrix remodelling was studied in an ex vivo human glioblastoma panel as well as in the murine Lewis carcinoma model.

Results. Dose-related decrease of glioblastoma cell proliferation and a tendency to down-regulation of SPARC were found; in the C57BL/6 mice model, the tumor growth and metastases inhibition index were 71.5% and 73.1%, respectively. Additionally, an increase of the thymus endocrine activity, serum interferon, adhesion of peritoneal macrophages and formation of antibodies against thymus-dependent antigen by splenic plasma cells were observed.

Conclusions. The results obtained suggest that the antitumor and especially the antimetastatic effect of Ukrain is due to several distinct mechanisms, including previously described antiangiogenetic activity with stimulation of peritumoral fibrotic tissue development. Very promising is the ability of this drug to prevent the formation of new metastases and to inhibit the growth of existing ones.

P030077

The Anticancer Effects of Oridonin in Vitro

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Background: oridonin was one of active ingredients in Rabdosia rubescens (hamal.). The plant was mainly attributed a zone of Henna province of China, which was often used to prevent and treat esophageal cancers in local regions. Its anticancer activities were attracted since 1970's, but profound studies had been lacked. Aim: the inhibitory effects of oridonin on the growth of 15 human and murine cancer cell lines were investigated in this study, including those from esophageal (TE1 and Eca109), leukemia (HL60 and k562), hepatoma (HepG2 and Bel-7402), breast (MCF-7), lung adenocarcinoma(A549), gastric (BGC823 and SGC7901), cervical (Hela), colon (HCT and HT-29), pancreatic (PC3), and murine melanoma B16-BL6. Method: The ability of oridonin to inhibit the proliferation of cancer cells were examined by MTT assay. Results and conclusion: Oridonin effectively inhibited the proliferation of those cancer cells with IC_{50} ranging from 2.036 to 19.060 μg/ml. The anticancer activities in vivo and mechanisms were under investigation.

P030078

The effects of piroxicom, mefenamic acid and ibuprofen on oral bioavailability of paditaxel

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Paclitaxel an antineoplastic agent has a low oral bioavailability. The P-glycoprotein inhibitors can improve the oral bioavailability of it. we investigated the possibility of enhancing bioavailability of paclitaxel using NSAIDs.

Paclitaxel in cremophor EL: ethanol (50:50) in doses of 10-100 mg/kg was administered orally to cannulated jugular vein rats after pretreatment by piroxicam, mefenamic acid or ibuprofen (10 mg/kg) in individual groups (n = 5). Serum concentration of paditaxel was analyzed by HPLC method. In the control group, paditaxel was nŸ detectable, whereas in treatment groups (that received paclitaxel after NSAIDs), serum concentrations up to 0.72 µg/ml were achieved and piroxicam had the most effect. By decreasing the oral dose of paclitaxel from 100 to 25 mg/kg after pretreatment by ibuprofen, Cmax and AUC were decreased and dose-serum concentration relationship was nonlinear.

Key words: paditaxel, oral bioavailability, HPLC, NSAIDs

P030080

Effects of Brunellae cumfructu on human beast cancer cell lines

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Objective To investigate the effect of brunellae cumfructu on MCF-7/S and MCF-7/R cells in vitro. Methods the survival rates of cells were assayed by MTT assay. The protein expression of p53 and breast cancer resistant protein (BCRP) were measured by Western-blot. Results The inhibition of brunellae cumfructu on MCF-7/S and MCF-7/R cells was dose/time-dependent, IC_{50} were 81.12 ±5.12 and 77.73 ±5.2, respectively. Pretreated with 12.5 mg/ml brunellae cumfructu, the IC_{50} (ug/ml) of MCF-7/R cells to adriamycin was decreased from 52.81 to 28.78. The expression of p53 was up-regulated by 12.5 mg/ml brunellae cumfructu and down-regulated by 75 and 100 mg/ml brunellae cumfructu. BCRP was down-regulated in MCF-7/R cells pretreated by 12.5 mg/ml brunellae cumfructu. Conclusion Brunellae cumfructu can inhibit the proliferations of MCF-7/S and MCF-7/R cells significantly; and may reverse the multidrug resistance of MCF-7/R cells by down-regulating the expression of BCRP and p53.

Key word: breast cancer; p53; breast cancer resistant protein (BCRP); brunellae cumfructu; adriamycin

P030081

Reversal of Multidrug Resistance in Cancer Cells by dextroisomer R-Verapamil and the Related Midecular Mechanisms

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Objective To study the reversal of multidrug resistance (MDR) by R-verapamil (R-VPM) in vitro and the animal toxicity of R-VPM.

Methods: Cytotoxicity was determined by MTT assay. Cellular accumulation of Dox was measured by fluorescence spectrophotometry. Animal toxicity was tested by i.p drug administration in BALB/c mice.

Results: R-VPM 1.25umol/L increased the sensitivity of KBv200 cells to VCR and Dox ($P < 0.01$). This effect was dose-related. R-VPM reversed MDR and increased cellular Dox accumulation of KBv200 cells as effectively as VPM, but possessed lower acute toxicity in BALB/c ($P < 0.05$). Conlusions: R-VPM reversed the MDR to VCR and Dox at a clinically tolerable concentration, and is a good candidate as chemosensitizer in clinic.

Key words: Multidrug resistance R-Verapamil

P030082

Curcumin analogs-glutathione interactions and proposed redox-dependent mechanism of the anti-cancer effect of the novel analogs

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A series of novel curcumin analogs were synthesized and screened for anti-cancer activities both at Emory University and National Cancer Institute (NCI). A majority of the analogs demonstrate a significant degree of anti-tumor activity. EF24 is the most active compound within the series. Follow-up studies showed that EF24 induces cell cycle arrest and apoptosis by means of a redox-dependent mechanism in MDA-MB-231 human breast cancer cells and DU-145 human prostate cancer cells. EF24 can serve as a Michael acceptor and react with nucleophiles such as glutathione (GSH). EF24 was treated with GSH/H_2O to produce a colorless solution with cytotoxic properties comparable with EF24 alone. This suggested the in situ formation of an EF24-GSH conjugate which releases EF24 in a rapidly established equilibrium. Treatment of breast cancer cells separately with EF24 and EF24-GSH revealed that the two compounds are almost equally efficacious in their cell-kill capacity. The EF24-GSH and its congeners appear to represent a promising new series of stable and water-soluble anti-tumor pro-drugs.

Key words: Curcumin analogs, Anti-cancer, EF24-GSH conjugate.

Acknowledgement: We are grateful to Emory University for supporting the work

P04. Psychopharmacology

P040001

Melatonin reverses the oxidative stress but not cognitive impairment in intracortical ferric chloride model of posttraumatic epilepsy in rats

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In the present study the effect of melatonin a potent antioxidant was studied against intracortical ferric chloride ($FeCl_3$) induced cognitive impairment and oxidative stress.

Male Wistar rats were injected $FeCl_3$ (5ul, 100 mM) in cortex intracortically. Rats were assessed for cognitive impairment on day 1 and 2 and subsequently sacrificed for the estimation of oxidative stress markers i.e. malondialdehyde (MDA) and catalase in brain tissue. Melatonin was injected at a dose of 50 mg/kg, i.p, 10 min before $FeCl_3$ injection. Intracortical $FeCl_3$ caused cognitive impairment as evident by increase in retention latency in elevated plus maze (80 + 18s) and decrease in step through latency (35 + 4.6 s) on day 2 as compared to day 1 (55 + 8.3s and 600 + 3.2s respectively). A significant increase in levels of MDA and decrease in levels of catalase was seen in the vehicle treated $FeCl_3$ group. Pretreatment of melatonin (50 mg/kg, i.p.) significantly ($p < 0.05$) prevented the increase in MDA and prevented the decrease in catalse levelsas compared to the vehicle treated $FeCl_3$ rats. However, melatonin had did not prevent the cognitive impairment.

P040002

Effect of BCPT on AVP content of hypothalamus and pituitary, and AVP mRNA of hypothalamus in chronic stress rats

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To study the effect of bioactive compounds from paecilomyces tenuipes (BCPT) on AVP content in hypothalamus, pituitary and expression of AVP mRNA in hypothalamus and behaviour in chronic unpredictable stress model in rats. The depression animal model was induced by chronic unpredictable stress. The behaviour of rats was tested in the open field. The effect of BCPT on AVP content in hypothalamus and pituitary was tested by radioimmunoassay. RT-PCR was used to test the expression of AVP mRNA in hypothalamus. BCPT could decrease the expression of AVP mRNA of hypothalamus and decrease AVP content in hypothalamus and pituitary in chronic stressed rats obviously. BCPT could increase ambulation and rearing score of chronic stressed rats in the open-field test. BCPT exhibited an antidepressant-like effect may in part be associated with the decreasing AVP content of hypothalamus and pituitary, and the expression of AVP mRNA of hypothalamus in chronic unpredictable stress model of depression in rats.

Keywords paecilomyces tenuipes; AVP

Acknowledgements: Supported by Department of Education of Anhui Province (No. 2004KJ194ZD) and Department of Technology of Anhui Province (No. 04023048)

P040003

Correlation between testosterone, gonadotropins and prolactin and severity of negative symptoms in male patients with chronic schizophrenia

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The aim of this study was to evaluate the relationship between plasma levels of testosterone, FSH, LH and prolactin and the severity of negative symptoms in patients with chronic schizophrenia. Fifty four male inpatients with chronic schizophrenia participated in this cross sectional study. All patients were on risperidone 4 mg/day or haloperidol 10 mg. The patients were assigned to groups with predominant negative and non predominant negative symptoms on the basis of the Positive and Negative Syndrome Scale (PANSS). Plasma levels of testosterone and free testosterone in the patients with predominant and non predominant negative symptoms were significantly lower than those in the normal controls. Plasma level of prolactin in the predominant negative symptoms group was significantly higher than the aged matched normal males. Significant inverse correlation between negative subscale scores of PANSS and plasma levels of testosterone and free testosterone in the patients with predominant negative symptoms were detected. Our results indicate that assessment of sex hormones and function of hypothalamic-pituitary-gonadotropin axis could be an important biological marker for the severity of negative symptoms.

P040004

GABA-B receptor antagonist, CGP51176, produces antidepressant-like effects in rodents

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Antidepressant drugs (AD), which mostly affect uptake or metabolism of monoaminergic neurotransmitters, exert multiple adverse effects and their efficacies are unsatisfactory. One of the promising targets for a novel antidepressant therapy is modulation of GABA-ergic system. The aim of our study was to investigate potential antidepressant-like effects of GABA-B receptor antagonist, CGP 51176 in the forced swim test (FST) in C57Bl/6J mice as well as in the olfactory bulbectomy (OB) and the chronic mild stress (CMS) models of depression in Wistar rats. We found, that CGP51176 produced a significant, dose-dependent decrease in the immobility time of mice in the FST, without affecting the locomotor activity. Moreover, our results have shown that repeated administration of CGP51176 (3 mg/kg) attenuated the OB-related behavioural changes of rats and moreover it dose-dependently (0.3-30 mg/kg) reversed CMS-induced anhedonia in the manner similar to that seen following chronic (but not acute) treatment with ADs. These preclinical data suggest that selective GABA-B receptor antagonist may be useful in treatment of depression.

Acknowledgement: Supported by grant from Polpharma to A. Pilc.

P040005

Novel NMDA receptor antagonist neramexane, enhances antidepressant-like effects of imipramine but not imipramine-induced increase in BDNF expression

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An improvement in effectiveness of the treatment of depression is achieved by combined use of several antidepressants. In the present study, the novel NMDA receptor antagonist, neramexane (MED 5 mg/kg) as well as imipramine (MED 5 mg/kg) shortened immobility time in the tail-suspension test in mice. An ineffective dose of neramexane (2.5 mg/kg) potentiated the anti-immobility effects of 5 and 20 mg/kg of imipramine. This enhancement was not synergistic, because the mean (CL) theoretical and observed ED50 doses for imipramine plus neramexane were 13.8 (3.8-21) and 13.6 (0.9-23.3) mg/kg, respectively. In contrast, as assessed by Northern blot analysis, 14-days treatment with imipramine increased Brain Derived Neurotrophic Factor (BDNF) mRNA expression in the cortex while neramexane decreased it. Combined treatment produced no effect on BDNF expression. Present data support the view that NMDA receptor antagonists enhance the potency of antidepressants, but leave an open question as to whether enhanced BDNF expression is a necessary feature of antidepressant treatment.

P040006

Antidepressant-induced increase in extracellular zinc concentration in the rat cortex

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The mechanism of antidepressant drugs (AD) is still the mater of dispute. Recently antidepressant-like properties zinc has been demonstrated. To evaluate the role of zinc in the mechanism of AD we used in vivo microdialysis in conscious rats to measure the extracellular zinc concentration in rat frontal cortex.

The rats were anesthetized and microdialysis probes were implanted. On the next day, the microdialysis probes were perfused with artificial cerebrospinal fluid. Baseline samples were collected every 15 min for 60 min, next, the amitriptyline was injected and dialysate fractions collected for another 60 min. Samples were collected and zinc was determined by voltametric striping method.

Amitriptyline administration dose dependently increased (10 mg/kg by 46%; 20 mg/kg by 166%) extracellular zinc concentration in rat frontal cortex.

The results demonstrated involvement of zinc homeostasis in the pharmacological mechanism of amitriptyline, and further indicate the role of zinc in the mechanism of AD action.

Keywords: amitriptyline, microdialysis, zinc, rat.
MEiN grant 2 P05A 017829, Inst Pharmacol PAS, CM Jagiell Univ, Krakow

P040007

Possible involvement of group II/III metabotropic glutamate receptors in the mechanism of action of antidepressant drugs

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We investigated the effect of chronic (21 days) treatment with antidepressant drugs on group II/III mGlu receptors reactivity in rat brain. Chronic imipramine treatment reduced the ability of group II mGlu receptors agonist, 2R,4R-APDC, to inhibit forskolin-stimulated cAMP formation in slices of rat cerebral cortex. Moreover, we observed the attenuation of mGluR2/3 agonist (L-CCG-I) stimulated cAMP accumulation in the same preparation. Prolonged treatment with imipramine or citalopram did not changed the action of group III mGluR agonist ACPT-I on forskolin-stimulated cAMP accumulation. Binding studies have shown no influence of chronic treatment with antidepressants on the density (Bmax) or affinity (Kd) of [3H]-LY341495 binding to mGluR2/3 receptors in the rat cerebral cortex or hippocampus. In behavioral studies we also investigated potential antidepressant-like effect of group II mGluRs antagonist, LY341495 as well as ACPT-I. We have found that both compounds produced a significant, dose-dependent decrease in the immobility time in mice or rats, suggesting, that modulation of group II/III mGlu receptors may produce an antidepressant-like effect.

Supported by KBN grant no P05 0465/03/25 to A. Pilc

P040008

The effect of music on calcium-dependent dopamine synthesis in the brain

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The effect of music on brain function was investigated through animal experiments. Previous our studies indicated that calcium increases brain dopamine (DA) synthesis through a calmodulin-dependent system.

Increased DA levels reduce blood pressure and prolong ethanol-induced sleep time. In this study, the effect of music was examined on this pathway. Systolic blood pressure in spontaneously hypertensive rats (SHR) was reduced, and ethanol-induced sleep time in mice was prolonged by exposure to Mozart's music (K.205). These effects vanished when calcium-dependent DA synthesis in the brain was inhibited. Exposure to music also increased serum calcium and neostriatal DA levels. These results suggest that music leads to increased DA synthesis in the brain, thus causing reduction in blood pressure and enhancement in alcohol's effect. Music might regulate and/or affect various brain functions through dopaminergic neurotransmission, and might therefore be effective for rectification of symptoms in various diseases that involve DA dysfunction.

Key words: calcium/calmodulin; dopamine synthesis; music. This study was supported by a grant from the Yamaha Music Foundation.

P040009

The effect of exercise on dopaminergic brain functions

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The effect of exercise on brain function was investigated through animal experiments. Previous our studies indicated that calcium increases brain dopamine (DA) synthesis through a calmodulin-dependent system.

Exercise leads to increased serum calcium levels, and the calcium is transported to the brain. This in turn enhances brain DA synthesis, and increased DA levels regulate various brain functions. There are abnormally low levels of DA in the neostriatum and nucleus accumbens of epileptic mice (El mice) and spontaneously hypertensive rats (SHR). The low DA levels in those animals were improved following intracerebroventricular administration of calcium chloride. Blood pressure and DA levels in SHR were also normalized by exercise. In epileptic El mice, convulsions normalized DA levels and physiologic function. These findings suggest that exercise or convulsions affect various brain functions through calcium-dependent DA synthesis. This leads to the possibility that symptoms in various diseases that involve DA dysfunction such as Parkinson's disease or senile dementia might be improved by exercise.

Key words: calcium/calmodulin; dopamine synthesis; exercise.

P040010

CORRELATION BETWEEN VITAMIN E AND ZOCAR LEVELS AND THE DEVELOPMENT OF ALZHEIMER'S DISEASE IN A MOUSE MODEL OF AD.

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Alzheimer's disease(AD) is the most common type of dementia(75%), manifesting as a severe deterioration of mental functions. The brains of people with Alzheimer Disease exhibit deposition of amyloid-B and progressive degeneration of nerve cells.

Scientists are studing ways that may help decrease or prevent neurodegeneration and may help injured neurons to regrow. We determined whether the antioxidant vitamine E+ZOCARaffected the presence of amyloid plaques in brain of aged PDAPP transgenic mice. Beta-amyloid precursor protein (APP), is important for the pathogenesis of Alzheimer's disease (AD), which is characterized by progressive decline of cognitive functions, formation of A beta plaques, and neurofibrillary tangles, and loss of neurons.

In the present study we have examined that treatment by vitamin E + Folic acid in PDAPP aged mice compare with control groups is able to decrease 17% in AB plaques levels of cerebral amyloidosis in necortex. More over, our results suggest that treatment by vitamins is able to prevent the disruption of basal cholinergic forebrain system and prevent of loss of cholinergic basal forebrain neurons(Ms + HDB, VDB).

Key words: Alzheimer's disease, vitamin E + Zocar

P040011

Acute elevated platform stress decreases MEK/MAPK signaling in rat frontal cortex

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Acute elevated platform stress inhibits long-term potentiation at synapses from the hippocampus to the prefrontal cortex in the rat. This stress model has been suggested to recapitulate some deficits in the anterior cingulate/orbitofrontal cortices found in depressed patients. The present study examined the effects of acute elevated platform stress on the phosphorylation state of proteins in the MEK/MAPK signaling pathway. A time course experiment showed that acute stress reduced the levels of phospho-Ser217/221-mitogen-activated protein/ERK kinase (MEK) and, subsequently, phospho-Thr202/Tyr204-p42/44-mitogen-activated protein kinase (MAPK) in the frontal cortex after 15, 30 and 60 mins. Phosphorylated MEK/MAPK returned to baseline levels after 140 mins. Treatment with imipramine, a tricyclic antidepressant, increased the levels of phosphorylated MEK/MAPK and counteracted the effect of stress on these phosphorylation events. These data indicate that MEK/MAPK signaling is altered in a stress model known to regulate synaptic plasticity. The fact that these alterations are counteracted by imipramine supports the notion that this stress model recapitulates certain deficits of depression-like states.

P040012

Histaminergic neurons and cognition: A study of H1 receptor mutant mice

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The aim of this study was to investigate the role of histamine H1 receptor (H1R) in cognition in physiological and pathological conditions by using H1R mutant (H1-/-) mice. In normal condtion, several behavioral studies indicated H1-/- mice show impaired object recognition and spatial memory, improved conditioned fear memory. Moreover, hippocampal long-term potentiation was reduced in H1-/- mice. These results indicate that H1R is involved in memory process for which the cortex, amygdala and hippocampus interact. In pathological condition, both H1-/- and control mice were subjected to social isolation, an animal model of schizophrenia.

Four-week later, behavioral and neurochemical changes were evaluated. Social isolation impaired locomotion in home-cage, prepulse inhibition of startle response and water maze performance in control mice, but not in H1-/- mice. Mutation of H1 receptor decreases isolation-induced hyperactivity of cortical dopaminergic neurons.

These data indicate blockage of H1R attenuates social isolation-induced behavioral changes. In conclusion, blockage of H1R impairs cognition in normal condition, whereas H1R blocking inversely improves cognition in disease models of schizophrenia.

P040013

Positive modulation of NMDA receptors by pregnendone sulphate (PS) potentiates glutamate and taurine levels in the striatum of freely moving rats

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PS is a relatively abundant sulphated neurosteroid in brain, and is able to influence the activity of different ligandgated ion-channel receptors. In the present study, we investigated the effect of PS on glutamate and taurine release in the rat striatum. Male rats were anaesthetised and guide cannulae were stereotaxically inserted. Rats were allowed 7 days for recovery. A microdialysis probe was inserted into the striatum, and perfused with aCSF at 2 ul/min. Samples were collected every 20 min. Dialysate amino acid were analysed by HPLC. PS (0.1, 0.5 and 2.0 mM) enhanced glutamate levels by approximately 20, 50 and 100% respectively with no effect on taurine levels. Co-administration of PS (0.1 and 0.5 mM) with a sub-threshold concentration of NMDA (50 uM) significantly potentiated PS induced glutamate and taurine levels. The effect of PS on glutamate levels is likely to be the result of positive modulation of the NMDA receptor. The mechanism of enhancement of PS on NMDA induced taurine levels, however, is not understood, but may be due to the consequences of enhancement of glutamate release and/or potentiation of NMDA function.

P040014

The psychopharmacological analysis of Ladasten effects

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The goal of the study was to investigate the psychopharmacological properties of novel substance N-(2-adamanthyl)-N-(parabromphenyl) amine –Ladasten. Ladasten prevented the fear response in Balb/c mice and MR rats tested in open-field and elevated plus maze, without any effect in MNRA rats and C57Bl/6 mice. In C57Bl/6 mice and MNRA rats Ladasten caused the psychostimulating action measured by Optovarimex test. In former test Ladasten had no effect upon Balb/c mice and MR rats. Ladasten prevented stress induced by decrease in benzodiazepine binding in Balb/c mice and MR rats, and had no such an effect in MNRA rats and C57Bl/6 mice.

In MNRA rats Ladasten stimulated the rise of monoamine level and had no analogic effect in MR rats. Data obtained allow the conclusion about double mechanism of Ladasten action, dependent on emotional stress response phenotype and mediated psychostimulating and anxiolytic action.

P040015

Effects of Afobazol in a model of Haemorrhagic Stroke

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The present study reports the data on Afobazol effects examined under subchroni-

cal (14 days) administration in the experimental model of haemorrhagic stroke (HS) in rats produced by the destruction of brain tissuee in capsula interna area. All rats with haemorrhagic stroke were shown to have minor disturbances (flaccidity, slowed movements, weak extremities), and severe in 40% of animals (riding-arena movements, paresis, extremities paralysis) impairments of the neurological status. A significant reduction of locomotor activity was observed in HS rats when compared to that of sham-operated animals. Afobazol (5 mg/kg) significantly decreased ($P < 0.05$) the severe neurological impairments, raised the locomotor activity in the open field test and increased the survival of animals with HS. Drug was found to improve learning and memory processes in rats with HS tested in passive avoidance model. Thus, the results obtained from the reported research evidently showed that Afobazol is able to improve behavior and memory impairments, as well as they proved to augment the survival rate in animals with haemorrhagic stroke.

Key words: Afobazol, Haemorrhagic Stroke

P040016

Substances with analgesic activity among dermorphine analogues

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The synthesis and study of the structure-activity relationship were carried out in 43 fragments and analogues of dermorphine (aDM) by consecutive replacement individual amino acids residues. The optimal structures for analgesic, thermoregulatory and vasomotor activities were determined. Based on these findings new aDM were purposefully synthetised, including those stereochemically modified Pro in the 6-th position. The aDM demonstrated a high analgesic activity (used tests as follows: tail flick; acetic acid caused writhing, hot plate, mechanical pressure of the tail, formaline test) The aDM caused no negative influence upon breath, exhibited a low narcogenic potential, and wide safety range.

P040017

Psychotropic activity of Betulin-containing dry extract from Birch tree bark in mice.

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Dry extract from Birch tree bark (EBTB) contains 70% of betulin along with minor components (betulinic acid, luped, phytosterols, etc.). EBTB produces low toxicity and displays antiviral, hepatoprotective, antihypoxic and adaptogenic properties in animals and humans. The present work studied psychotropic action of EBBT in C57BL/6 mice. 2 and 6 hours after EBBT administration (50 and 25 mg/kg, p.o.) significant improvement in efficiency of exploratory behavior in closed exploratory cross-maze and decrease in scopolamine-induced amnesia in passive avoidance paradigm were observed. These outcomes indicate that EBTB produces nootropic (cognition enhancing) activity. The same doses of EBBT produced antidepressive and thymoleptic effects in the Porsolt swim test and slip funnel inescapable situation. Also EBTB elevated motor activity and diminished haloperidol-induced catalepsy suggesting involvement of brain dopamine-positive mechanisms in EBBT effects. The probable mechanisms of the effects described are discussed.

P040018

NMDA receptors and Ca^{2+}/Calmodulin-Dependent Protein kinase II in the Nucleus accumbens, discrete with each other, are both involved in Methamphetamine-induced Conditioned Place Preference expression

Pan Wynn H.T*, Wu Hsiao-Hua, Lin Ski-Kwang, Yeh Pen-Ho.

Neural adaptations in the nucleus accumbens (NAc) are thought to mediate several of the behavioral enhancements after chronic exposure to abused drugs. In present studies, we explored the role of NMDA receptors and their associated signal, Ca^{2+} Calmodulin-Dependent Protein kinase II (CaMKII)" in the Nc for the methamphetamine (MA)-induced conditioned place preference (CPP) expression. Our results showed that bilaterally intra-NAc infusion of KN-93 or L-AP5 both abolished MA-induced CPP expression. Besides, western bolt analysis showed that the activity of CaMKII (P-CaMKII/CaMKII) in the NAc was decreased, rather than increased, following the CPP test as compared to Pre-CPP state. In addition, intra-NAc infusion of KN-93 immediately abated the activity of CaMKII in the NAc, which was stationary even following the CPP test. However, intra-NAc infusion of L-AP5, unlike intra-NAc infusion of KN-93, hardly affected the activity of CaMKII in the NAc either before or after CPP test as compared to the control group. Taken together, our data demonstrated that the activation of NMDA receptors and the activity of CaMKII in the NAc, discrete with each other, are important in MA-induced CPP expression.

P040019

Oral anti-S100 protein antibodies - a novel anxiolytic with antidepressant and neuroprotective potential

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Despite recent advances in psychopharmacology, there is still a need in effective and well-tolerated anxiolytics. In animal models Tenoten (oral ultra-low doses of antibodies to S100) has shown anxiolytic and antidepressant effects. It also induced potent protective effects in rodent models of ischemic and hemorrhagic brain stoke, and of Alzheimer's disease.

To study clinical efficacy of Tenoten in anxiety disorders, we performed an open-label, flexible dose trial. A total of 247 patients (baseline HAMA 28.0+/-0.4) were assigned to receive Tenoten (6-12 tabl/day, n=127) or diazepam (15 mg/day, n=120) for 4 weeks. Tenoten was almost as effective as diazepam in reducing anxiety (assessed by Hamilton anxiety scale, HAMA): baseline-to-endpoint decrease in HAMA amounted to -15.3+/-0.6 and -17.6+/-0.6 respectively, the proportion of responders (with >=50% improvement) to 69.3% and 78.3% respectively, and almost half of patients in both groups achieved at least partial remission (HAMA <=10). However, the adverse events rate in Tenoten arm was 7 times lower than in diazepam arm.

Tenoten is a promising option in the treatment of anxiety. Its additional psychopharmacological potential may be of great benefit.

P040020

Effect of BCPT on CORT、ACTH in plasma and expression of CRH mRNA in hypothalamus in chronic unpredictable stress model in rats

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The aim of the present study was to explore the anti-depression effects of bioactive compounds from paecilomyces tenuipes (BCPT) on CORT and ACTH level in plasm, CRH mRNA expression in the hypothalamus in chronic unpredictable stress model (CUS) model in rats. CUS-induced preference behaviour change has been used as a model to predict the clinical efficacy of many types of antidepressant treatment. BCPT exhibited a significantly increased sucrose intake in the CUS model in rats, but there was no effect in unstressed animals. We used radioimmunoassay (RIA) to detected the CORT and ACTH content in the plasma, and used RT-PCR to test the expression of CRH mRNA in hypothalamus. BCPT at a dose of 40 and 80 mg/kg could decreasing the expression of CRH mRNA in hypothalamus and the plasma level of CORT、ACTH in CUS model in rats obviously. Our results suggested that BCPT exhibited antidepressant-like effect may in part be associated with regulating the hyperaction of the function of hypothalamic-pituitary-adrenal axis (HPAA).

Keywords: paecilomyces tenuipes; HPA

Acknowledgements: Supported by Department of Education of Anhui Province (No. 2004KJ194ZD) and Department of Technology of Anhui Province (No. 04023048)

P040021

The medial hypothalamic 5-HT_{1A} receptors are involved in the inhibition of stress-induced hyperactivity of HPA axis

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It is known that 5-HT_{1A} agonists inhibit stress-induced increases in ACTH release. The objective of this study is to test the hypothesis that the inhibitory effect of 5-HT_{1A} agonists is mediated by the 5-HT_{1A} receptors in the medial hypothalamus. 5-HT_{1A} receptors in the hypothalamus were selectively reduced by injection of an adenovirus with 5-HT_{1A} antisense sequence (1AP-AS-Ad). 8-OH-DPAT-induced inhibition of ACTH response to a stressor, elevated plus maze, was examined at 10 days later. The reduction in the hypothalamic 5-HT_{1A} receptors was determined by autoradiography of ^{125}I-MPPI binding. Although stress or systemic administration of 8-OH-DPAT increase ACTH secretion, 8-OH-DPAT significantly inhibits stress-induced increase in ACTH secretion in the control adenovirus injected mice. The inhibitory effect of 8-OH-DPAT was blunted in the mice received 1AP-AS-Ad. These data demonstrated that 5-HT_{1A} receptors in the medial hypothalamus inhibit stress-induced hyperactivity of HPA axis. Defensive behaviors and anxiety-like behaviors of these mice were also examined.

Key words: ACTH, defensive behavior, T maze, anxiety-like behavior
Supported by NARSAD and USPHS MH072938 for Qian Li

P040022

Evaluation of Antipsychotic Drugs as Inhibitors of Multidrug Resistance Transporter P-glycoprotein

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Previous studies have revealed that P-glycoprotein (P-gp) may be involved in efflux transport of several antipsychotics. In the present study, the artipsychotics, i.e., risperidone, olanzapine, quetiapine, clozapine, haloperidol, chlorpromazine, a major metabolite of risperidone, 9-OH-risperidone, and a positive control inhibitor, PSC833, were evaluated for their inhibitory effects on P-gp-mediated rhodamine 123 (5 microM) cellular uptake in LLC-PK1 and L-MDR1 cells using a flow cytometric method. All the antipsychotics showed various degrees of inhibitory effects on P-gp activity. The concentrations of the inhibitor to cause 50% of the maximal increment of intracellular Rhd 123 fluorescence (EC_{50}) were: PSC833 (0.5 microM) < olanzapine (3.9 microM) < chlorpromazine (5.8 microM) < risperidone (6.6 microM) < haloperidol (9.1 microM) < quetiapine (9.8 microM) < 9-OH-risperidone (12.5 microM) < clozapine (30 microM). These results suggest that pharmacokinetic interactions due to inhibition of P-gp activity by the antispychotics appear possible, and warrant further investigation.

P040023

Morphine-induced conditioned place preference in mice withdrawn from chronic oral nicotine treatment

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In previous studies cross-tolerance between nicotine and morphine has been found using conditioned place preference (CPP). The purpose of this experiment was to study whether nicotine-withdrawal sensitizes mice to the rewarding effects of morphine (MOR). The mice received increasing concentrations (50-500 microg/ml) of nicotine via drinking water for seven weeks. Control mice were drinking tap water. Nicotine solution was changed for water on the 50th day of the treatment. The mice were habituated to the CPP apparatus on three consecutive days before withdrawal. The mice were conditioned with saline or MOR (5 or 10 mg/kg s.c.) with a biased system on days 1, 2, 4 and 5 after withdrawal and conditioning was measured on days 3 and 6. The nicotine-withdrawn mice showed CPP after two-day administration of MOR 5 mg/kg, whereas control mice were first conditioned by the higher dose (10 mg/kg) of MOR. Thus, it seems that nicotine-withdrawal sensitizes mice to the rewarding effects of morphine.

Key words: Nicotine, morphine, conditioned place preference.
This work was supported by the Academy of Finland.

P040024

The antipsychotic effects of (-)-stepholidine on the animal models of schizophrenia symptoms

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AIM: To evaluate the antipsychotic effects of (-)-stepholidine (SPD) in animals. METHODS: The apomorpline (APO) and amphetamine (AMP) induced the disruption of swimming in mice swimming normalization test. The immobility in mice in the forced swimming test was enhanced by repeated treatment of phencyclidine (PCP). These tests were used as animal models for the positive and negative symptoms of schizophrenia. RESULTS: SPD could ameliorate the disorder induced by APO or AMP and significantly increase the swimming numbers in a dose-dependent manner with the lowest effective doses at 10 mg.kg^{-1}. Also SPD could significantly attenuate the immobility enhanced by PCP in the forced swimming test. CONCLUSION: SPD possesses the potential antipsychotic activity for schizophrenia.

KEY WORDS (-)-stepholidine; phencyclidine; atypical antipsychotics

P040025

The Role of Nitric Oxide in the Anxiolytic and Antidepressant Activities of Sertraline in Mice

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It is known that nitric oxide is a neurotransmitter in central nervous system and has a role in the regulation of some behaviours such as anxiety and depression. The therapeutic importance of selective serotonin reuptake inhibitors increase gradually in the treatment of behavioral disorders such as anxiety and depression. In the present study, the role of nitric oxide was evaluated in the antidepressant and anxiolytic activity of sertraline which is a selective serotonin reuptake inhibitor. Material and Methods: Forced swimming test was used for evaluating antidepressant activity and elevated plus maze test was used for determining anxiolytic activity. Sertraline (5 and 30 mg/kg) was injected intraperitoneally. L-arginine (10 mg/kg) or L-NAME (10 mg/kg) were given with 30 mg/kg sertraline. Results: No anxiolytic and antidepressant acivity was observed by using 5 mg/kg sertraline. While there were significant anxiolytic and antidepressant activities of sertraline at 30 mg/kg. These effects did not change when sertraline 30 mg/kg was used with L-arginine. L-NAME (10 mg/kg) increased the anxiolytic and antidepressant activity of sertraline (30 mg/kg). These results suggest that the inhibition of nitric oxide is improved the anxiolytic and antidepressant activities of sertraline.

Key Words: Anxiolytic, Antidepressant, Sertraline, Nitric Oxide

P040026

Memory enhancing properties of E-6801, a 5-HT_6 receptor ligand with agonist properties, in the novel object discrimination test

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Introduction. 5-HT_6 receptor antagonists improve retention of recognition memory in the novel object discrimination (NOD) paradigm in rats (Woolley et al. 2004). Herein, a comparative study between two 5-HT_6 receptor antagonists (SB-271046 and Ro 04-6790), a 5-HT_6 receptor ligand with agonist properties (E-6801), and donepezil, an AchE inhibitor, is reported. Methods. Lister Hooded rats, administered i.p., were used. NOD paradigm utilised was that described by Ennaceur et al. (1998) as modified by Woolley et al. (2003), with a 4 hours inter-trial interval (ITI). Results. Vehicle treated rats, after 4 h. ITI, spent an equivalent time exploring the novel and familiar objects. SB-271046 (10 mg/kg) and Ro 04-6790 (5 and 10 mg/kg) produced a significant increase in the time spent exploring the novel object. E-6801 was active at 2.5, 5 and 10 mg/kg, with a nonsignificant increase at 1.25 mg/kg. Donepezil was active between 0.1 and 3 mg/kg. Conclusion. E-6801 enhanced the performance in the NOD test in rats, being as efficacious as donepezil. Further investigation is going on to clarify whether a 5-HT_6 receptor agonist or antagonist represents a better approach for the treatment of memory deficits.

P040027

Alterations of phosphorylated microtubule associated protein 2 (MAP2) expression following chronic stress

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Objective: Stress causes morphological changes in brain. Microtubule-associated proteins (MAP2) is a maker of dendrites of neurons. The aim of this study is to investigate the expression of MAP2 following chronic stress.

Methods: Total and phosphorylated MAP2 protein expression in hippocampus was

analyzed by immunoprecipitation combined with western-blot.
Results: MAP-2 protein expression did not differ between control and stressed groups, but phosphorylated MAP2 decreased significantly in chronic stressed rats compared to control rats.
Conclusions: The phosphorylation of MAP2 are regulated by many signal transduction elements. Our results suggest that the changes in phosphorylated state of MAP2 in hippocampus in stressed rats may represent a condition of abnormal dendrites, likely representing structural or functional changes in the dendrites as well as the dysfunction in post-receptor signal transduction in response to stress.
Key words: chronic stress, microtubule-associated protein 2, immunoprecipitation
Acknowledgement: This work was supported by the National Natural Science Foundation of China (grant number: 30472018).

P040029

The activity of a Galanin-3 receptor antagonist HT-2157 and paroxetine on midbrain dopamine neurons

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We examined the antidepressant-like profile of a novel galanin-3 receptor antagonist (HT-2157) and the selective serotonin reuptake inhibitors (SSRI), paroxetine and on the activity of spontaneously active dopamine (DA) neurons in the substantia nigra pars compacta (SNC) and ventral tegmental area (VTA) in anesthetized adult male Sprague-Dawley rats. This was accomplished using the technique of in vivo extracellular recording. Our data clearly shows that repeated administration (1 mg/kg i.p., injection per day for 21 days) of paroxetine or HT-2157 (30 mg/kg i.p.) produced a significant increase in the number of spontaneously active VTA DA neurons, with no significant effects on the SCN DA neurons. This is congruent with the activity of other SSRIs in this test paradigm and suggests that the selective GALR-3 receptor antagonist (HT-2157) may possess SSRI-like antidepressant properties.

P040030

Diverse monoamine-HPA axis changes and anxiety after stress and re-stress in an animal model of posttraumatic stress disorder (PTSD)

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PTSD develops after repeated trauma and is characterized by monoaminergic and hypothalamic-pituitary-adrenal (HPA)-axis abnormalities. Understanding the diverse monoamine-HPA axis changes after stress and restress may provide a better understanding of the neurobiology and treatment of PTSD. Hippocampal and frontal cortex serotonin (5-HT), noradrenaline (NA) and dopamine (DA), plasma corticosterone (CORT) and anxiety were studied in rats on day 1/day 7 post acute stress (AS) and on day 1/day 7 post re-stress (RS). Immediately after AS, there was a significant increase in anxiety and CORT that normalised on day 7. RS evoked hypocortisolemia immediately after RS and a later increase in anxiety on day 7 post RS. Hippocampal 5-HT, NA and DA were unchanged immediately after AS, but significantly raised on day 7 post AS. RS reduced 5-HT and NA immediately and on day 7 post RS, respectively, while DA was unchanged. Frontal cortex DA was significantly elevated after AS and reduced on day 7 post RS, with no change in 5-HT and NA. These biobehavioral changes after AS and RS suggest treating acute and chronic PTSD by selectively targeting the HPA-axis and limbic monoamines using appropriate drug treatment.

P040031

Maternal deprivation and corticosterone administration as a neurodevelopmental model of schizophrenia: reduced effect of apomorphine on prepulse inhibition

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It is postulated that schizophrenia is caused by both early and late developmental disruptions. In a developmental animal model combining maternal deprivation (MD) and adolescent corticosterone (CORT) treatment, we found a reduced effect of a single dose of the dopamine receptor agonist, apomorphine (APO), on prepulse inhibition (PPI). In the current study, we extended these findings with a dose response experiment and dopamine D_2 receptor binding. Wistar rats were MD for 24 hrs or 20 sec on postnatal day (pnd) 9 and subcutaneously (s.c.) implanted with a 100 mg CORT or cholesterol pellet for 2 weeks starting at pnd 56. At pnd 84, the effect of 0.1, 0.3 and 1.0 mg/kg of APO on PPI was tested. APO induced a dose-dependent decrease of PPI in all groups except those treated with both MD and CORT. Autoradiography showed no changes in D_2 binding that could explain this dopaminergic insensitivity. Our results indicate impaired dopaminergic regulation of behaviour in this animal model of schizophrenia. We are currently assessing D_1 receptor density and possible second messenger coupling to elucidate the mechanism.
Keywords: Developmental, apomorphine, PPI, dopamine, schizophrenia.

P040032

NOREPINEPHRINE TRANSPORTER KNOCKOUT AFFECTS BRAIN EXPRESSION OF GALANIN AND ITS RECEPTORS

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Neuropeptides such as galanin (GAL) and/or their receptors have been proposed to be involved in antidepressant action. Since norepinephrine transporter knockout ($NET^{-/-}$) mice were shown to behave like antidepressant-treated mice, we examined in the CNS mRNA expression of galanin and its three receptors (GALR1-3) in $NET^{-/-}$ and $NET^{+/+}$ mice. The mRNA expression was determined in the whole brain and in certain brain regions (olfactory bulb, cortex, cerebellum, brainstem, striatum, hippocampus, hypothalamus) by quantitative real-time PCR (qPCR). In $NET^{+/+}$ mice the highest mRNA expression of GAL and its three receptors was observed in the hypothalamus. Knockout of the NET induced a decrease in mRNA expression of GAL and its receptors GALR-1 and GALR-3 in the cerebellum. In addition, GALR-1 mRNA was decreased in the brainstem whereas GALR-3 mRNA tended to be increased in the striatum. These results indicate that the NET knockout induces brain region-specific and differential mRNA regulation of GAL and its receptors. It remains to be shown whether similar results are obtained at the protein level.
Key words: galanin, norepinephrine transporter knockout, brain, qPCR

P040033

The effect of antipsychotic drugs on serotonin-1A (5-HT_{1A}) receptor mediated disruption of prepulse inhibition (PPI).

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An interaction with 5-HT_{1A} receptors has been suggested to explain the excellent clinical efficacy of some atypical antipsychotics. The aim of this study was to assess the effect of these drugs on 5-HT_{1A} receptor mediated disruption of PPI, a measure of sensorimotor gating which is deficient in schizophrenia. Treatment of male Sprague-Dawley rats (n = 8 per group) with the 5-HT_{1A} receptor agonist, 8-hydroxy-dipropyl-aminoetralin (8-OH-DPAT), caused a dose-dependent decrease of PPI, as measured with automated startle chambers. Haloperidol or raclopride at 0.25 mg/kg, but not 0.05 mg/kg, significantly blocked the action of 0.5 mg/kg of 8-OH-DPAT. A similar inhibition was seen with 5 mg/kg, but not 1 mg/kg of aripiprazole. On the other hand, clozapine (1 or 5 mg/kg), olanzapine (1 or 5 mg/kg), risperidone (0.2 or 1 mg/kg) or amisulpride (10 or 50 mg/kg) had little or partial effects. None of the antipsychotic drugs disrupted PPI. These data confirm that part of the action of some antipsychotic drugs may be by interacting with 5-HT_{1A} mediated disruption of PPI, either directly or via dopamine D2 receptor blockade
Keywords: prepulse inhibition, serotonin-1A receptors, antipsychotics, dopamine.

P040034

Effectiveness of Risperidone Oral Solution for Psychotic Agitated Patients

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[Objective] In overseas practices, combination therapy using Haloperidol (HPD) and lorazepam for intramuscular injection has been adopted against psychotic agitated patient. Combination use of risperidone oral solution (RIS-OS) and lorazepam liquid for oral administration was recently reported to be equally effective. Using cases where domestically permitted lorazepam tablets (LOR) was used for the combination therapy.
[Method] Patients of psychotic raptus were treated by either of oral administration

of RIS-OS 2 ml + LOR 1 mg or intramuscular injection of HPD 5 mg + oral administration of LOR 1 mg. The psychotic agitated state was assessed under CGI and five items of BPRS, after 30 minutes, 60 minutes and 120 minutes.
[Results] 52 patents were treated using RIS-OS and 17 patients using HPD. No statistically significant difference was found in CGI and 5-items of BPRS.
[Conclusions] Because no randomization was effected, the result may be biased; however, CGI and 5 items in BPRS indicated no statistically significant difference in the initial treatment period. No difference of therapeutic effect was apparent between the RIS-OS treatment and HPD intramuscular injection method.

P040035

METABOLISM AND CNS DISTRIBUTION OF THE ANTIDEPRESSANT, DESIPRAMINE, IN YOUNG COMPARED TO ADULT RATS

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The tricyclic antidepressants, including desipramine (DMI), are no better than placebo in treating juvenile depression in humans. As part of our animal studies related to this observation, we have compared the half-life of DMI and the brain concentrations of DMI and its active metabolite desmethyldesipramine (DDMI) in young and adult rats following 4 days of twice daily injections or chronic infusion for 2 weeks. These studies indicate that postnatal day (PND) 9-13 rats metabolize DMI at a slower rate than adult rats. The ratio of DDMI/DMI is much lower in the young rats compared to adult rats and is dependent on dose and age. PND 28 rats metabolized the DMI at a faster rate than adult rats. DMI and DDMI are distributed evenly throughout the brain of PND 21-35 and adult rats following two weeks of chronic infusion. In addition, DDMI has a higher affinity for the serotonin transporter than the norepinephrine transporter. These results suggest that PND 9-11 rats metabolize DMI much slower than PND 28 and adult rats, and that DDMI may contribute to the action of DMI in adult but not juvenile rats.

Key words: antidepressant, juvenile, psychopharmacology, pharmacokinetic
Research supported by MH64772

P040036

EARLY LIFE STRESS ALTERS THE OPEN FIELD BEHAVIOR OF FEMALE ADULT RATS

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Early maternal deprivation is considered an animal model of early life stress that causes long-term alteration in later life behaviors. Objective: The aim of this study was to explore the long-term effect of early life stress on locomotion and exploration activity in female adult rats. Methods: Female Wistra and their female pups were reared under 3 conditions: 1) 360 min daily maternal separation (MS), 2) handling by man for 5 min daily (H) both conditions were done on the first 10 days after birth and 3) no handling or separation (NH). At 21 days of age rats, were housed in each group for 4 weeks. Subsequently, rats were tested individually for 5 min in a circular open field arena. Results: The results showed that both stress conditions, handling and maternal separation, produced hyperlocomotion (increased total zone transition) and exploration activity (increased number of rears). Both effects were significantly increased in H group when compared with NH as control. Conclusion: These finding suggested that early life stress condtion alters long-term effect on locomotion and exploration behaviors of female adult rats.

P040037

Mechanism of SSRI-induced sexual dysfunctions: Preliminary data using male Wistar rats

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The exact cause of sexual dysfunction side effects of selective serotonin reuptake inhibitors (SSRI's) is not known and tests in rats may tell us more. Testing in male rats involves observations of mounts, intromissions, and ejaculations. When given chronically, paroxetine (an SSRI), inhibits sexual behavior. With 14 distinct serotonin receptors, the objective of our studes is to determine which ones are involved. Here we studied the role of the 5-HT_{1A} and 5-HT_{2C}. The 5-HT_{1A} agonist ±8-OH-DPAT strongly facilitates sexual behavior. The 5-HT_{1A} antagonist WAY100,635 when administered alone has no effect. However, when co-administered with an SSRI, we see a strong inhibition on sexual behavior. This may mimic the chronic effects of SSRI in humans where increased levels of serotonin may desensitize the 5-HT_{1A} receptor. The 5-HT_{2C} agonist RO60-0175 inhibited sexual behavior. The 5-HT_{2C} antagonist SB243,213 alone may facilitate sexual behavior. When administered with paroxetine, SB243,213 has no effects on sexual behavior and may even recover the sexual dysfunction side effects of the SSRI. The SSRI-induced reduction of sexual behavior seems to involve at least a 5-HT_{1A} receptor mediated effect.

P040038

Cannabidiol reverses MK-801-induced social withdrawal in rats

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The cannabis constituent cannabidiol has been suggested to have the properties of an atypical antipsychotic. Previous work in our laboratory has demonstrated that cannabidiol reverses deficits in sensorimotor gating induced by the NMDA receptor antagonist MK-801. The present study aimed to investigate the effect of cannabidiol on MK-801-induced social withdrawal in rats, an animal model of negative symptoms associated with schizophrenia. Separate groups of rats were treated with MK-801 (0.3 mg/kg) following pre-treatment with cannabidiol (5 mg/kg) or clozapine (3 mg/kg). Rats were placed in an open arena and videotaped for 10 min and social and locomotor behaviour was assessed. MK-801 produced a decrease in social behaviours such as investigation, following and climbing over. Cannabidiol reversed this decrease and reinstated social interaction to a similar level to control. Clozapine decreased locomotor activity and did not reverse social withdrawal, perhaps due to the hypomotility. These results support the evidence for the potential antipsychotic properties of cannabidiol.

Key words: Cannabidiol, clozapine, schizophrenia, social withdrawal

P040039

The efficacy of diazepam and esomeprazole in prevention of stress ulcer lesions development in rats

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Stress ulcer presents acute lesion of gastric mucosa. Pathogenesis of stress ulcer is not clarified, yet. Our aim was to test effects of diazepam and esomeprazole, given as a pretreatment, on progression of stress ulcer lesions induced with cold-restraint stress. Experiments were conducted in adult Wistar rats weight 200-250 gr. Cold restrained stress was induced in rats individually restrained in plastic cages at 4°C for 3h. Cold-restrained stress produced petechiae and erosions in the glandular part of the stomach. Macroscopic lesions were histologicaly verified. Diazepam at a dose of 5 mg/kg, given intraperitonealy 30 minutes before cold-restraint stress, increased both the number and the size of petechiae and erosions, but it was not statistically significant. Intragastric administration of esomeprazole at a dose of 20 mg/kg 30 minutes before cold-restraint stress did not decrease either petechial or erosions' number or size. On the basis of the obtained results it was concluded that neither diazepam or esomeprazole were efficient in these experimental models of stress ulcer in rats.

P040040

PSYCHOLOGICAL STRESS INCREASES THE ANXIOLYTIC-LIKE EFFECT OF NITRIC OXIDE SYNTHASE INHIBITOR, L-NAME

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Psychological stress in the early stages of life such as social isolation rearing from weaning has been reported to alter the behaviors of the adult animals and modify the effects of many psychotropic agents. Objective: To investigate the effects of social isolation rearing from weaning on the anxiolytic-like effect of the nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME). Methods: At day 21 postnatal, male Wistar rats were reared either in social groups of five rats/cage or in isolation (one rat/cage). After five weeks, these rats were placed individually onto the elevated plus-maze following intraperitonean administration with either saline or L-NAME 30 min before a 5 min test. Results: Pretreatment of L-NAME (5, 10 and 50 mg/kg i.p.) produced a dose-related anxiolytic-like profile in isolation reared rats. This effect was indicated by increase in the percentage of open:total arm entries and time spent on the elevated plus-maze. However, the anxiolytic-like effect of L-NAME

was not observed in socially reared rats . Conclusion : These results show that psychological stress in the early stages of life enhances the anxiolytic - like effect of L - NAME in the adult rats .

P040041

Atractylodis rhizoma extract attenuates methamphetamine and nicotine induced conditioned place preference by CB1 antagonism

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It has been reported that cannabinoid CB1 receptor is involved in the reward effect of various kinds of abused drugs . Recent evidences further indicate that CB1 receptor antagonists can reduce the rewarding properties of abuse drugs including methamphetamine (MAP) , cocaine , heroin , nicotine and alcohol . In present study , we design to evaluate whether Atractylodes rhizome extract (ARE) has the property of CB1 receptor antagonist and modulates MAP or nicotine induced drug addiction using conditioned place preference (CPP) test . In [^{3}H] CP55 ,940 binding assay using rat cerebral cortex membranes , ARE had a higher affinity for CB1 receptor (K_i = 161 .9 nM) . Furthermore , ARE (30 nM) significantly reduced CP 55 ,940 (100 nM) stimulated [^{35}S] GTPgammaS binding in rat cerebellum membranes . These results clearly demonstrate that ARE acts as CB1 receptor antagonist . Repeated administration of ARE (0 .1 mg/ kg) before the MAP (1 mg/ kg) or nicotine (0 .5 mg/ kg) treatment significantly inhibited MAP or nicotine induced CPP in mice . Therefore , these results suggest that ARE reduces MAP or nicotine induced dependence by the antagonism of CB1 receptors .

P040042

The effect of bee venom acupuncture on acute methamphetamine induced hyperactivity , hyperthermia and Fos expression in mice

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Acupuncture is a commonly used treatment option for the treatment of addiction including nicotine , cocaine and morphine . The present study was designed to investigate the effect of bee venom (BV) acupuncture on methamphetamine (MAP, 1 mg/ kg , s .c .) induced acute toxicity (behavioral hyperactivity and hyperthermia) in mice . BV was dissolved in saline at three doses (0 .1 , 1 and 10 mg/ ml) . Diluted BV (20 ul , s .c .) bilaterally administrated into acupoint (Zusanli , ST36) or control point (TE8 and tail base) . BV into acupoint injection dose dependantly reduced MAP induced toxicity , while BV injection into control points did not produce any effect . On the other hand , we observed that MAP injection signifcantly increased Fos expression in the several brain regions including nucleus accumebns (NAc) , caudate putamen (CPU) , ventral tegmental area (VTA) and substantia nigra (SN) . Notably , BV acupuncture further increased MAP induced Fos expression in the NAc , CPU, SN except VTA . These findings suggest that BV acupuncture (ST36) has a therapeutic effect on acute MAP toxicity , possibly by elevating neuronal activity in the specific brain regions .

P040043

Cyperi rhizoma extract has therapeutical potentials in several psychiatric disorders through CB1 receptor and sigma1 receptor

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It has been reported that CB1 receptor and sigma1 receptor are involved any therapeutical effect on the psychiatric disorder . We purified three substances [i .e . alpha cyperone(AC) , beta selinene(BS) and cyperotundone(CY)] from Cyperi rhizoma (CR) and evaluated these receptor specific anti - psychiatric effect . In radioligand binding study , AC and BS had an affinity CB1 cannabinoid receptor (ki = 109 .3 uM and 80 .57 uM, respectively) and sigma1 receptor (ki = 119 .8 uM and 12 .18 uM, respectively) , whereas CY had sigma1 receptor affinity (ki = 179 .1 uM) . In further [^{35}S] GTPgS binding assay , AC and BS act as CB1 receptor antagonist/ sigma1 partial agonist and CY has sigma1 partial agonistc profile . AC and BS produced antidepressant - like effect (forced swimming and tail suspension test) and anxiolytic effect (elevated plus - maze test) . On the other hand , CY significantly reduced methamphetamine or nicotine induced condtioned place preference . Therefore these results suggest that Cyperi rhizoma is a potential pharmacological plant for the treatment of psychiatric disorders through CB1 receptor and sigma1 receptor .

P040044

Influence of galantamine on acetylcholinesterase activity in rat brain evaluated in vitro and in behavioral tests .

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We have attempted to enhance the parasympatomimetic activity of galantamine (GAL) in the key areas of rat brain using L - carnitine (CAR) . Following the administration of the highest of the GAL doses used (2 .5 ; 5 ; 10 mg/ kg i .m .) , acetylcholinesterase (AChE) activity decreased mainly in the frontal cortex and hippocampus . In the interaction of GAL and CAR , AChE inhibition was stronger . A low GAL dose (2 .5 mg/ kg i .m .) did not induce a statistically significant change in AChE activity , but clinical symptoms of an increased activity of the cholinergic system were observed (tremor , convulsions , salivation) . For this reason , we made another attempt to evaluate the efficacy of GAL and its interaction with CAR using behavioral tests . The purpose of the experiment was to assess the effect of GAL and its combination with CAR on the activity of AChE that may not have been apparent in the previous in vitro experiments . We found that GAL in the dose of 2 .5 mg/ kg gives rise to statistically significant changes , predominantly those of the peripheral cholinergic transfer . Premedication by CAR did not lead to a change in the values of the parameters monitored .

Supported by grant IGA MZ CR NR7935 - 3/ 2004 .

P040045

EFFECTS OF CARBAMAZEPINE AND LITHIUM ON THE OPEN FIELD BEHAVIOR OF ISOLATION STRESS RATS

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We investigated the effects of social isolation on the open field behaviours and compared the effects of carbamazepine with lithium on these behaviours in socially and isolation reared rats . Male Wistar rats were reared from weaning in either alone or groups of five rats/ cage . After 5 weeks , these rats were tested individually for 5 min in a circular open field arena . The results demonstrated that isolation reared rats exhibited locomotor hyperactivity , had significantly more number of rears , entried more frequent and spent longer time in the inner zone of an open field arena than the socially reared rats . Pretreatment with carbamazepine (10 , 20 and 40 mg/ kg i .p .) did not alter locomotor activity in neither socially nor isolation reared rats . However , pretreatment with lithium chloride (50 , 100 and 150 mg/ kg i .p .) produced a dose - related hypolocomotion effect in both socially and isolation reared rats . These effects of lithium were more pronounced in socially than isolation reared rats . The results indicate that rearing rats in social isolation from weaning causes stress in the early stage of life which produces behavioural disturbances in the adult rats and alters the responsitivity to lithium .

P040046

Stress - alleviating action of GBE50 on rat physical - emotional stress model

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The effects of GBE50 (a new kind of Ginkgo Biloba extract) on the rat model of physical - emotional stress were evaluated from the levels of the behavior , hypothalamic - pituitary - adrenal axis (HPA) and hippocampal 5 - HT_{1A} receptor . Male Wistar rats were randomly divided into 5 groups : physical stress (PS) , emotional stress (ES) , PS or ES with GBE50 and control . The repeated foot shock was used as the chronic physical stress meanwhile witness as the emotional stress . The rats were tested for saccharine preference and locomotor activity . The plasma corticosterone was measured by protein binding assay . The change of 5 - HT_{1A} receptor in hippocampus was checked by radioligand binding analysis . The results showed that GBE50 could relieve the inhibition in rat behavior caused by PS and improve the agitation in the ES group . GBE50 produced regulations on the plasma corticosterone levels and the 5 - HT_{1A} receptor in PS and ES rats . So GBE50 could produce stress - alleviating action on both rat models of PS and ES .

Key Words : GBE50 , physical stress (PS) , emotional stress (ES) , hypothalam-

oic - pituitary - adrenal axis (HPA).

Acknowledgement: This research was funded by a Key Project of Shanghai Municipality (No.04DZ19855).

P040047

ACP-103, a potent 5-HT$_{2A}$ Receptor Inverse Agonist, as an Adjunctive Therapy in Schizophrenia

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ACP-103 is a potent and selective 5-HT$_{2A}$ receptor inverse agonist, with nanomolar potency at 5-HT$_{2A}$ receptors and 7-50 fold lower potency at 5-HT$_{2C}$ receptors in a variety of in vitro assays. ACP-103 lacks significant activity at D2 and over 50 other human receptors at concentrations up to 10 uM. In mice, ACP-103 was a potent inhibitor of head-twitch behaviors induced by 5-HT$_2$ agonist DOI, and enhanced haloperidol- or risperidone-mediated decreases in dizocilpine- or amphetamine-induced hyperactivity. ACP-103 also reduced haloperidol- or risperidone-induced catalepsy and hyperprolactinemia in rats. The ability of ACP-103 to reduce neuroleptic-induced side effects in animals was extended into the clinic. Initial clinical studies indicate that ACP-103 was generally safe and well tolerated, had a long plasma half-life, and occupied 5-HT$_{2A}$ receptors in human brain after oral administration. Moreover, ACP-103 administration reduced haloperidol-induced hyperprolactinemia in healthy volunteers, and haloperidol-induced akathisia in both healthy volunteers and schizophrenic subjects. Based on these observations, ACP-103 may have potential as an adjunctive therapy in schizophrenia with a wide therapeutic index.

P040048

Differential in vitro pharmacology of ACP-104 (N-desmethylclozapine) and clozapine

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N-desmethylclozapine (ACP-104), a metabolite of clozapine, has pharmacological properties predictive of antipsychotic activity but distinct from clozapine. While both ACP-104 and clozapine are potent 5-HT$_{2A}$ inverse agonists, in contrast to clozapine, ACP-104 is a dopamine D$_2$ partial agonist and a much more efficacious M1 muscarinic agonist. These properties suggest that ACP-104 may not only contribute to the antipsychotic properties of clozapine but may be responsible for clozapine's superior profile as an atypical antipsychotic agent (Sur et al. 2003, Weiner et al. 2004, Burstein et al. 2005). Ongoing studies have further compared the receptor pharmacology of ACP-104 and clozapine. We found that ACP-104 has markedly lower activity than clozapine at histamine H$_1$ and alpha 1 adrenoceptors. The lower affinity of ACP-104 for histamine H$_1$ receptors and alpha 1 adrenoceptors suggests that ACP-104 may have a lower propensity to cause sedation and a lower risk for producing adverse cardiovascular events than clozapine. These data suggest that ACP-104 may be as an efficacious antipsychotic agent with cognitive effect and tolerability superior to that of clozapine.

P040049

SUPERSENSITIVITY TO AMPHETAMINE IN PROTEIN KINASE-C INTERACTING PROTEIN (PKCI) / HINT$_1$ KNOCKOUT MICE

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PKCI/HINT$_1$ belongs to the histidine triad protein family that conserved in a broad range of organisms. However its physiological function remains unknown. Microarray studies reported decreased mRNA expression of PKCI/HINT$_1$ in the patients with schizophrenia. In view of the link between DA transmission and schizophrenia, the present study used behavioral and neurochemical approaches to examine the influence of PKCI/HINT$_1$ deletion upon: (i) basal and amphetamine-evoked locomotor activity; (ii) DA dynamics in the dorsal striatum and (iii) post-synaptic DA receptor function. PKCI$^{-/-}$ (KO) mice displayed low levels of spontaneous locomotion relative to wildtype littermates. Acute amphetamine administration significantly increased locomotor activity in WT mice; an effect that was enhanced in the KO mice. Quantitative microdialysis studies revealed no alteration in basal DA dynamics in the striatum and nucleus accumbens of the KO mice. In contrast, systemic administration of the direct-acting DA receptor agonist apomorphine significantly increased locomotor activity in KO mice. These results demonstrate that lack of PKCI/HINT$_1$ is associated with dysregulation of post-synaptic DA transmission.

P040050

The Role of Locus Coeruleus Alpha-2 Adrenoceptors in Learned Helplessness

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We examined the role of alpha-2 adrenoceptors (AR) in an animal model of Major Depressive Disorder. In learned helplessness (LH) animals exposed to inescapable stress later fail to escape an avoidable stress test. Unstressed controls and antidepressant treated animals receiving inescapable stress exhibit normal escape behaviors. Norepinephrine (NE) is important in stress response and autoinhibitory presynaptic alpha-2 AR in the locus coeruleus (LC) regulate NE release and neuronal firing rate. We found high affinity alpha-2 AR, measured by [^{125}I]-para-iodoclondine autoradiography, were reduced by 15-20% in the LC of LH Sprague-Dawley Holtzman rats as compared to controls. The role of alpha-2 AR in development of LH behavior was further examined by injection of alpha-2 AR agonist and antagonist drugs via cannula stereotaxically placed in the LC. The agonists clonidine and UK-14,304 dose dependently prevented LH behavior when administered 70 min following inescapable stress. Antagonist drugs yohimbine and RX821002 had no effect on LH behavior at any dose tested. We conclude that in LH animals LC alpha-2 AR are not tonically active and reduced receptor function contributes to development of LH behavior.

P040051

Modulation of serotonergic neuronal firing activity by cannabinoid CB1 agonists

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CB_1 receptor agonists are known for their capacity to affect mood regulation. However, their effects on serotonergic/5-HT (major monoamine involved in mood control) neurotransmission is poorly understood. Using in vivo electrophysiology, we showed that low doses of the CB_1 agonist WIN55,212-2 (0.05-0.2 mg/kg, i.v.) dose-dependently increased 5-HT firing activity of dorsal raphe neurons (control: 0.93 ±0.08 Hz; WIN 0.2 mg/kg: 1.76 ±0.24 Hz, $p < 0.01$), an effect reversed by the CB_1 antagonist Rimonabant (1 mg/kg, i.v.), but not by the vanilloid receptor antagonist Capsazepine (20 microgram/kg, i.v.). On the other hand, higher doses of WIN55,212-2 (0.3-0.6 mg/kg, i.v.) yielded no significant difference from controls. Following transection of the medial prefrontal cortex, WIN55,212-2 failed to increase 5-HT firing activity, while transection of the lateral prefrontal cortex did not perturb WIN55,212-2's modulatory effect. These results indicate that the modulation of 5-HT neurotransmission by CB_1 agonists is controlled by the medial prefrontal cortex.

Key words: CB_1 agonist, medial prefrontal cortex, 5-HT, mood.

Funding: Canadian Psychiatric Research Foundation, McGill University Health Center

P040052

Effect of N-methyl-D-aspartate receptor antagonists on hydroxyl radical generation in the posterior cingulate and retrosplenial cortex of free-moving mice on line with stereotyped behavior

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This study examined the effect of MK-801 and ketamine, N-methyl-D-aspartate (NMDA) receptor antagonists, on hydroxyl radical (·OH) generation in the posterior cingulate and retrosplenial (PC/RS) cortex of free-moving mice using microdialysis on line with stereotyped behavior. MK-801 (0.6 mg/kg) or ketamine (50 mg/kg) acute administration significantly increased ·OH levels in mouse PC/RS cortex. The basal OH levels after MK-801 and ketamine administrations for 7 consecutive days were significantly increased compared with the naive group. MK-801 (0.6 mg/kg) or ketamine (50 mg/kg) challenge after chronic administration further significantly increased dialysate ·OH levels. Our study also found that both acute and chronic administration of MK-801 or ketamine induced stereotyped behavior in mice, but the intensity of stereotyped behavior induced by MK-801 was more than that induced by ketamine. The results suggested that NMDA receptor antagonists participate to the generation of ·OH in the PC/RS cortex of mouse, and oxidative stress, derived from the formation of

free radicals, might play an important role in the pathophysiology of schizophrenia.

Keywords: MK-801; Ketamine; Hydroxyl radical; Microdialysis

P040053

Up-regulation of hippocampal neurogenesis is required for the chronic antidepressant efficacy of agmatine

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To explore the mechanism of antidepressant efficacy of agmatine (AGM), hippocampal neurogenesis was measured by Bromodeoxyuridine (BrdU) labeled immunohistochemistry, cell proliferation by colorimetric and 3H-thymidine assays in vitro, and chronic efficacy of AGM by sucrose consumption test and novelty suppressed feeding test. AGM 10 mg/kg (p.o) normalized the decrease of the open-field behavior, and the number of BrdU-labeled cells in hippocampal dentate gyrus in the stressed mice. Four weeks later, part of the new-born cells differentiated into neurons. Interestingly, 5-fluorouracil (5-FU, 15 mg/kg) deleted the chronic antidepressant efficacy of AGM in mice. In vitro, AGM 0.1-10 μM increased the proliferation of cultured hippocampal stem cells from neonatal rats, which were abolished by 5-FU (5 μM), MEK inhibitor PD98059 or PKA inhibitor H89 20 μM. It is concluded that up-regulation of hippocampal neurogenesis is required for agmatine's chronic antidepressant efficacy, which may be closely related to the activation of neurotrophic pathway and cAMP-PKA pathway.

KEY WORDS Agmatine; antidepressant; neurogenesis; chronic stress;
Acknowledgement National Natural Science Foundation of China (No 30300419)

P040054

Behavioral effects of five antidepressants on a poststroke rat model of emotional disturbances

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In human, ischemic stroke often induce mood disorders like depression and anxiety, but it is not known if any particular class of antidepressants has a clear therapeutical advantage. We studied the effects of several doses of five antidepressants: imipramine (7.5, 15, 30 mg/kg) minalcipran (30, 45, 60 mg/kg), desipramine (5, 10, 15 mg/kg), fluvoxamine (30, 45, 60 mg/kg) and fluoxetine (10 mg/kg), on a rat model of cerebral, global, transient ischemia. Ischemia was induced by the four-vessel occlusion technique. This model was validated to study emotional disturbances. Behavioral tests performed were: spontaneous motor activity, neurological scores, forced swimming test (FST) and elevated plus-maze (EPM). Antidepressants were administered intraperitoneally 23.5, 5 and 1 hour before the second session of the FST and 0.5 hour before the EPM. Brains were histologically controlled at the end of the experiments. Main results were that dual serotonergic and noradrenergic recapture inhibitors (SNRI), but not specific serotonergic recapture inhibitors (SSRI), demonstrated antidepressant properties and evidenced anxiolytic activities in postischemic animals.

P040055

Extinction training in conjunction with a NMDA receptor partial agonist erases memory trace

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Much evidence indicates that extinction training does not erase memory traces but forms an inhibitory learning that prevents the expression of original memory. Fear conditioning induces long-term potentiation (LTP) and drives synaptic insertion of AMPA receptors in the amygdala. Here we show that extinction training applied at 24hr after training attenuated startle potentiation but failed to affect the level of GluR1. Infusion of a partial agonist of NMDA receptors D-cycloserine (DCS) bilaterally into the amygdala 30 min before extinction training reversed the increase in GluR1. The effect was blocked by proteasome inhibitors suggesting the facilitation of NMDA-induced endocytosis of GluR by DCS. Furthermore, 10 μM NMDA which normally had no long-lasting effect on synaptic responses in the amygdala slices was able to induce long-term depression (LTD) in the presence of DCS. Surface GluR1 level was similarly decreased by the same treatment. These results provide the first evidence implicating the erasure of fear memory likely via facilitating endocytosis of AMPA receptors.

P040056

Effects of intracerebroventricular injection NMDA on the hypnotic effects of inhalation anesthetics

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Objective: To investigate the interaction between cerebral NMDA(N-methyl-D-aspartate) receptors and the hypnotic effects of enflurane, isoflurane and sevoflurane. Methods: 120 kunming mice (male or female) were divided randomly into 3 groups: enflurane, isoflurane, sevoflurane group. Each group was further divided into 4 subgroups: aCSF(artificial cerebral spinal fluid) group, NMDA25ng group, NMDA50ng group, NMDA75ng group. Intraperitoneally (ip) injected enflurane ($2.0\ ml \cdot kg^{-1}$), isoflurane ($1.2\ ml \cdot kg^{-1}$), sevoflurane ($5.0\ ml \cdot kg^{-1}$) to establish the mice model of hypnosis. Each animal intraperitoneally injected hypnotic doses of inhalation anesthetics. One minute after the mice losing of righting reflex, the treated groups intracerebroventricular administrated different doses of NMDA, and the control group intracerebroventricular administrated artificial cerebralspinal fluid. The recovery time of righting reflex (RT) was recorded. Results: There was no significant difference in the RT between NMDA (25、50、75ng) groups and aCSF group. Conclusions: Cerebral NMDA receptors may not play an important role in the hypnotic effects of inhalation anesthetics.

Key Words: NMDA receptors; inhalation anesthetics; hypnosis; mechanism

P040057

Antiepileptic effect of Phencynonate hydrochdride and its possibly antiepileptic mechanism

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Aim Antiepileptic effect of phencynonate hydrochloride (PCH) was tested and its antiepileptic mechanism was further investigated. Methods Through establishment of different epilepsy models, antiepileptic effects of PCH and other drugs were examined. Besides, the effects of phencynonate hydrochloride and other compounds against NMDA-induced lethality in mice, and NMDA-induced current in rat primary hippocampal neuronal cultures were also observed. Results: PCH produced a significant anticonvulsant effects on different epilepsy models in mice, its anticonvulsant potency was even more potent than the clinically used antiepileptics sodiumphenytoin. Furthermore, PCH could also dose-dependently execute its obvious protection against the lethal effects of N-methyl-D-aspartate(NMDA) in mice and selectively, uncompetitively block the current induced by 20 $mmol \cdot L^{-1}$ NMDA in a dose-dependent and voltage-independant manner while had no effect on the current induced by 2 $mmol \cdot L^{-1}$ GABA. Conclusion PCH had a notable anticonvulsant effects on typical epilepsy models, its antiepileptic mechanism might relate to its antagonism against NMDA receptor.

Key words: phencynonate hydrochloride; epilepsy; NMDA

P040058

ACUTE AND CHRONIC BUPRENORPHINE AND/OR CLORAZEPATE-USED IN SUBSTITUTION-CHANGE DIFFERENTIALLY BEHAVIOR AND OPIATE BINDING IN RODENTS.

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Buprenorphine (BPN) is very abused with benzodiazepines (BZD), especially Clorazepate (CRZ) during heroin substitution. Are Pharmacodynamic or behavioral explanations for BPN+CRZ craving BPN is a high affinity partial μ agonist and a delta + kappa antagonist for opioid receptors (OR). We tested acute (1 injection) or chronic (21 days) administration of BPN or CRZ or their association: (i) behaviors in mice with 1, 4 or 16 mg/kg of CRZ ± BPN, (ii) OR binding in rats. METHODS: (i) anxiety (black and white box) and memory (alternation in the Y-maze + passive avoidance tests). (ii) binding using a β-imager with 3 specific ^{3}H-Radioligands for μ(MOR), δ(DOR) and κ(KOR). RESULTS: (i) high doses of clorazepate totally reversed BPN hyperactivity and anxiogenic effects, and increased the BPN-induced spontaneous alternation impairment whereas it displays no effect on long-term memory processes. (ii) [a] CRZ alone diminished the down regulation of MOR [b] BPN induced changes are regionally modified when CRZ was added. [c] In most regions Kd increases were additive [d] surprisingly MOR Bmax and Kd simultaneously increased in thalamus CONCLUSION: changes induced on the OR with BPN could explain a persisting demand of BZD and the risks of overdose with IV route. In mice, the behavioural interactions between the OR and GABA/BZD complex are mainly im-

plicated in anxiety behaviour but not in memory functions.

Key words: buprenorphine, anxiety, memory, β-imager.

P040059

Effects of A Galanin-3 Receptor Antagonist on Neurite Outgrowth

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The neuropeptide Galanin mediates its effects through three known G-protein coupled receptor subtypes GalR1, GalR2 and GalR3, and has been implicated in many physiological processes including feeding behavior, pain and depression. Several studies have demonstrated the ability of Galanin to modulate the central 5-hydroxytryptamine (5-HT) function. HT-2157, a selective GalR3 antagonist, has been shown to increase extracellular levels of 5-HT in various brain regions. 5-HT-elicitated 5-HT_{1A} receptor activation increases neurogenesis and promotes neurite outgrowth which may contribute to its antidepressant effects. In this study, the effects of HT-2157 on enhancing neurite outgrowth were examined and the mechanisms underlying the modulation of neurite outgrowth were explored in both a PC12 sub-clone and primary mouse neuronal cultures.

The results demonstrated that HT2157 significantly enhanced neurite outgrowth of PC12 cells and primary mouse neurons. In addition, HT-2157 down regulated a transcription repressor of the 5-HT_{1A} receptor gene, indicating that the enhancement of neurite outgrowth by HT-2157 is mediated via derepression of 5-HT_{1A} receptor gene expression.

P040060

INVESTIGATION ON THE ANTIDEPRESSANT EFFECTS AND REGULATION OF ADULT HIPPOCAMPAL NEUROGENESIS OF SINISAN EFFECTIVE COMPONENTS

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OBJECTIVE: In the present study, the effects of Sinisan effective components (SNEC) on depression and hippocampal neurogenesis were investigated. METHODS: The animal model of lesions of olfactory bulb were used. The Brdu positive cells were marked by using the thymidine analog bromodeoxyuridine (BrdU), a maker for dividing cells, then showed and counted by immunohistochemistry. CREB gene was quantitively tested on basis of Taqman™ technology using MGB probe. RESULTS: Chronic SNEC treatment significantly increases the number of BrdU-labeled cells in the dentate gyrus and hilus of the hippocampus and CREB copy number. CONCLUSION: These results suggest that SNEC influences neurogenesis in the hippocampus by increasing CREB gene expressions. The reversal of reduced neurogenesis may be one target the antidepressant drugs exert their effects.

Key words: Sinisan effective components, antidepressant effects, hippocampal neurogenesis

P040061

Anxiolytic-like effect of oleamide in group housed and social isolated mice

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Social isolation has been suggested as an animal model of anxiety. In the present study, the putative anxiolytic activity of oleamide was examined in both group housed and social isolated male mice by using several experimental paradigms of anxiety. Use of the elevated plus-maze test revealed that oleamide (20 mg/kg, i.p.) increased the percentage of open-arm time in social isolated mice and oleamide (5, 10 and 20 mg/kg, i.p.) increased the percentage of open-arm time in group housed mice. In the light/dark test, oleamide (10 mg/kg, i.p. or 20 mg/kg, i.p.) prolonged the time spent in the light box in group housed and social isolated mice respectively without altering the locomotor activity of the animals. In the hole-board test, oleamide (10 and 20 mg/kg, i.p.) or oleamide (20 mg/kg, i.p.) increased head-dip counts and duration in group housed and social isolated mice respectively. Thus, these findings indicate that oleamide exhibits a fine anxiolytic-like effect in both group housed and social isolated mice.

Key words: Anxiety; Oleamide; Elevated plus-maze test; Light/dark transition test

P040062

Effects of Stepholidine Derivatives on Dopamine D1 and D2 Receptor[1]

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The pathogenesis of schizophrenia is suggested to involve dysfunction of dopamine D1 receptor in the medial prefrontal cortex accompanied by dopamine D2 receptor hyperactivity in subcortical regions. Stepholidine (SPD) has been demonstrated to have both D1 receptor agonism and D2 receptor antagonism effects. SPD derivatives was made for drug discovery. We got D1 and D2 receptors using baculovirus-Sf9 cell system. Then receptor binding assay of SPD vs D1R was performed by [^{3}H] SCH23390 and D2R was performed by [^{3}H] Spiprone. Receptor binding assays show 107、107-1、307、407、B3 have high affinities for both D1 and D2 dopamine receptor. HEK293 cells expressing D1 receptors and CHO cells expressing D2 receptors were prepared for [^{125}I] cAMP assays. Results show these SPD derivatives are able to stimulate cAMP production in HEK293-D1R cells, and inhibit Forskolin-stimulated cAMP accumulation in CHO-D2R cells. So SPD derivatives with some structures may have high affinities for both D1 and D2 receptor. These derivatives also show the similar effects of dual D1 agonist and D2 antagonist actions compared with SPD.

Key words: Stepholidine; Schizophrenia; derivatives; Receptor binding; cAMP

Project supperted by the Ministry of Science and Technology (973-2003CB515401) and the National Natural Science Foundation of China (No 30271495, No 30472009).

P040063

Oscillatory properties of dopamine neurons: Differences between the ventral tegmental area and the substantia nigra

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Dopamine (DA) neurons in both the substantia nigra (SN) and the ventral tegmental area (VTA) are thought to fire in two modes: single spiking and bursting. Alteration between the two has been suggested to be a key mechanism for DA neurons to regulate DA release. Using spectral analysis, we have recently shown that some DA neurons in the VTA also fire in a slow oscillatory mode. The main goal of the present study was to determine whether the SO is also present in SN DA neurons, which have been previously shown to exhibit more regular firing patterns and less bursting than VTA DA neurons. This study shows that the SO is present in both VTA and SN DA neurons. Compared to the VTA, however, there are fewer SO cells in the SN. The amplitude of the SO in individual SO DA cells is also smaller in the SN than in the VTA. In both areas, SO cells exhibit higher degrees of bursting and CV than non-SO cells. The two populations of cells, however, show similar firing rates. When compared between areas, both SO and non-SO cells show higher degrees of bursting and CV in the VTA than their counterparts in the SN.

Key Words: substantia nigra (SN), ventral tegmental area (VTA), Oscillation

Project supported by the Ministry of Science and Technology (973-2003CB515401)

P040064

l-Stepholidine, Adenosine A2A Receptor and Dopamine D_3 Receptor Interactions

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Aim: l-Stephdidine (SPD) is a lead compound of tetrahydroprotoberberines (THPBs). This work investigated the influence of SPD on Dopamine D_3 receptor (D_3R) and adenosine A_{2a} receptor ($A_{2a}R$). Methods: PC12 cells with endogenous $A_{2a}R$ were transfected with pIRES-D_3R plasmids by Lipofectamine™2000 transfection kit. Protein expression was assessed by Western blot. mRNA was detected by Real-time polymerase chain reaction. The accumulation of cyclic AMP from cells was measured by HitHunter cAMP II Assay kit. Results: Incubation of PC12 cells expressed D_3R with SPD resulted in a little decrease of D_3 receptors in protein expression. SPD can decrease of D_3R and $A_{2a}R$ mRNA level in PC12 cells which stably expressed D_3R. SPD or D_3 receptor agonist counteracted the $A_{2a}R$ receptor agonist-mediated increase in cAMP levels. Blockade of D_3R with the D_3R antagonist increased both basal and $A_{2a}R$ receptor agonist-stimulated cAMP levels. Conclusion: These results suggest SPD is a agonist for D_3R stably expressed in PC12 cells. The D_3R can modulate A_{2a} receptors at the level of gene transcription and the generation of second messengers.

Key words: l-Stepholidine; dopamine D_3 receptor; adenosine A_{2a} receptor

Project supperted by the Ministry of Science and Technology (973-2003CB515401) and the National Natural Science Foundation of China (No 30271495, No 30472009).

P040065

Antidepressant-like effects of icariin in animal models of depression

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Icariin is a major constituent of flavonoids isolated from Epimedium brevicornum. The present study was designed to examine whether icariin exert antidepressant-like activity of icariin in two behavioural procedures: the forced swim test (FST) in mice and the chronic mild stress (CMS) mode of rats. Icariin was orally administered for 21 days in the FST and for 5 weeks during the CMS models. Fluoxetine at 10 mg/kg was orally administered as a positive control. The duration of immobility time in FST was reduced by icariin exhibiting a typical inverted U-shaped dose-response curve. Icariin was unable to affect ambulatory or rearing behavior of mice in the open-field test. In CMS, the stress-induced decrease in the consumption of 1% sucrose solution was gradually reversed by chronic treatment with icariin. In addition, the present study demonstrated that rats subjected to CMS showed the elevated interleukin-1bata (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6) and tumor-necrosis-factor alpha (TNF-α) in serum. The cytokine impairments improved after icariin treatment, in parallel with decreases in anhedonic-like state. In conclusion, these results suggested that icariin exerted antidepressant-like effects in experimental animal models. The modulation of immunological response to the CMS exposure may contribute to antidepressant action of icariin treatment.

Key words: Icariin, Forced swimming test, Chronic mild stress, Cytokine

Acknowledgements: The work was co-financed by grants from NSFC (No. 30371755) and JSNSF (BK2003070).

P040066

The antidepressant effect of total flavone of A in mice exposed to cerebral ischemia

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objective: Post-stroke depression (PSD) has negative impacton rehabilitation following stroke. The effectiveness of antidepressant drugs in the management of PSD has been widely investigated. The aim of the present study was to confirm the putative antidepressant effect of total flavone of A (TFA, abelmoschus manihot extract) Methods: The antidepressant effect of TFA in mice exposed to cerebral ischemia, its antidepressant activity was compared with the selective serotonin reuptake inhibitors (SSRIs) fluoxetine in mouse with treatment of cerebral ischemia. The ischemia was induced by a right common carotid artery occlusion (CCAO). CCA-occluded and sham-operated mouse (surgery on day 0) were subjected to 'pre-test' swim, a forced swimming test (FST). on day 5, 24h later, each mouse was re-exposed to the 'test session'. each mouse received once daily administration of TFA 80, 160, and 320 mg/kg p.o. or vehicle from day 0 to day 6. Results: TFA (80, 160 mg/kg) markedly shortened the increased immobility time induced in FST. The depressive-like behaviors of mice exposed to cerebral ischemia were observed and the antidepressant effects of TFA in CCAO mice were assessed. Conclusions: TFA have the antidepressant effect on PSD, which was speculated that the neuronal damage caused by CCAO played an important role in the development of PSD. Further studies are needed to fully characterize the mechanisms of the antidepressant effect of TFA.

Key words: Post-stroke depression; CCAO; TFA; forced swimming test

P040067

Inhibition of Multidrug Resistance Transporter P-glycoprotein by Antipsychotics: Risperidone, Olanzapine, Quetiapine, Clozapine, Haloperidol, and Chlorpromazine

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Previous studies have revealed that P-glycoprotein (P-gp) may be involved in efflux transport of several antipsychotics. In the present study, the antipsychotics, i.e., risperidone, olanzapine, quetiapine, clozapine, haloperidol, chlorpromazine, a major metabolite of risperidone, 9-OH-risperidone, and a positive control inhibitor, PSC833, were evaluated for their inhibitory effects on P-gp-mediated rhodamine 123 (5μM) cellular uptake in LLC-PK1 and L-MDR1 cells using a flow cytometric method. All the antipsychotics showed various degrees of inhibitory effects on P-gp activity. The concentrations of the inhibitor to cause 50% of the maximal increment of intracellular Rhd 123 fluorescence (EC_{50}) were: PSC833 (0.5μM) < olanzapine (3.9μM) < chlorpromazine (5.8μM) < risperidone (6.6μM) < haloperidol (9.1μM) < quetiapine (9.8μM) < 9-OH-risperidone (12.5μM) < clozapine (30μM). These results suggest that pharmacokinetic interactions due to inhibition of P-gp activity by the antispychotics appear possible, and warrant further investigation.

Key words: P-glycoprotein, antipsychotics

Acknowledgement: This work was supported by NIH grant MH071811-01A1.

P05. Behavioral Pharmacology

P050001

Antagonistic activity of Ascorbic acid (Vitamin C) on dopaminergic modulation: apomorphine-induced stereotypic behavior in mice

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Interaction of antioxidant ascorbic acid with dopaminergic, nitrergic system and antipsychotic agents was investigated in mice against apomorphine-induced stereotypy. Ascorbic acid dose dependently inhibited stereotypic behavior produced by apomorphine. It potentiated the antipsychotic activity of haloperidol (0.1mg/kg i.p.), a typical antipsychotic agent. When administered along with clozapine (1-2 mg/kg i.p), sulpiride (10-20 mg/kg. i.p.) and risperidone (0.0025 mg/kg i.p.), ascorbic acid also potentiated their activity. L-NAME (30 mg/kg i.p.) inhibited stereotypic response, which was potentiated by ascorbic acid (800 mg/kg i.p.). When given along with SCH23390, additive effect was observed. Ascorbic acid also inhibited supersensitization response of apomorphine on reserpinization (2 mg/kg i.p.). Interestingly, at lower dose (100 mg/kg i.p.) ascorbic acid potentiated the dopaminergic activity of apomorphine (0.5 mg/kg) and 5-HTP (0.25 mg/kg i.p.). However, when given concomitantly it failed to alter the response of SKF38393. The study demonstrated that ascorbic acid potentiated the activity of antipsychotics and activity of nitric oxide synthase inhibitor

P050002

Effect of Oxytocin on Methamphetamine-Induced Behavioral Sensitization in Mice

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Oxytocin (OT), a neurohypophyseal neuropeptide synthesized in the brain and released at the posterior pituitary, affect adaptive central nervous system processes related to opiate, ethanol and cocaine addiction. Effect of OT on behavioral sensitization to methamphetamine (MAP) in mice has been investigated in this paper. Firstly, mice were acutely administered OT prior to the challenge with MAP, then the locomotor activity levels of the mice were recorded in the ambulometer. On the other hand, the effect of OT on the development, transfer and expression of the behavioral sensitization (BS) induced by MAP in mice was investigated with the locomotion monitored. It is found that OT (0.1, 0.5, 2.5 nmol, i.c.v.) dose-dependently inhibited the hyperactivity induced by acute treatment of MAP in mice, while it had no effect on the locotmotor activity when administrated alone. Meanwhile, chronic treatment with OT had no significant difference on the locomotion of the mice. There was no significant effect of OT on the development of BS induced by MAP. After BS has been established, OT (0.5, 2.5 nmol) inhibited the expression of MAP sensitization significantly. However, OT (0.1 nmol) markedly restrained the transfer of MAP sensitization. The data of the present study suggest that OT may influence the process of BS induced by MAP.

Keywords: oxytocin; methamphetamine; locomotion; behavioral sensitization

P050003

Tiagabine and its interactions with conventional antiepileptic drugs in amygdala-kindled rats.

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The aim of the study was the exact evaluation of interactions between tiagabine (TGB) and three conventional antiepileptic drugs (AEDs): valproate (VPA), carbamazepine (CBZ), or phenobarbital (PB) in amygdala-kindled rats, the established model of complex partial seizures in humans. The 50% effective doses of tested antiepileptic drugs causing 50% reduction of the afterdischarge duration made a base for further calculations. Isobolographic analysis of obtained data revealed that TGB interacts additively with all tested conventional AEDs for all fixed

ratios of mixture components (1:3, 1:1, and 3:1). Bidirectional analysis of pharmacokinetic interactions confirmed pharmacodynamic character of determined additivity. TGB. As regards undesired effects, TGB, VPA, CBZ, and PB (applied at their ED50 values) and their combinations in proportion of 1:1 did not impair motor performance evaluated in the chimney test. In conclusion, obtained results confirm that TGB may be a valuable drug candidate for add-on therapy of refractory complex partial seizures in humans.

P050004

Isobolographic characterization of interaction between levetiracetam and felbamate in the mouse maximal electroshock-induced seizure model.

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Despite the advanced knowledge of pathophysiological processes related with seizure initiation and propagation, there are still approx. 30% of epileptic patients inadequately treated with antiepileptic drugs (AEDs) used in monotherapy. For these patients, the combined therapy with two AEDs may be an efficacious treatment regimen. The aim of this study was to determine the exact type of interaction between two newer AEDs: levetiracetam (LEV) and felbamate (FBM) in the mouse maximal electroshock (MES)-induced seizure model using isobolographic analysis. The experiments were performed on male Albino Swiss mice in the MES test, being considered as an experimental model of tonic-clonic seizures in humans. Results indicated that LEV combined with FBM at the fixed drug dose ratio of 1:2, 1:1, 2:1, and 4:1 produced synergistic interaction in the MES model in mice. Pharmacokinetic evaluation of total brain concentrations of AEDs revealed that FBM increased significantly the total brain LEV concentrations in mice. Based on this preclinical study, one can conclude that despite pharmacokinetic interaction between FBM and LEV, their synergistic combination is worthy of consideration in further clinical practice.

P050005

Lesions of the medial prefrontal cortex block the devdopment but not expression of morphine induced behavioral sensitization in mice

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Considerable evidence suggests that the glutamatergic input from the medial prefrontal cortex (mPFC) to the VTA and the NAc appears to be involved in behavioral sensitization processes. However, dissociations regarding the role of the mPFC with respect to the development and expression of sensitization induced by morphine have not been fully studied. So the present study examined the role of the mPFC in the development and expression of morphine-induced sensitization. Bilateral kainic acid lesions of the mPFC were performed before the sensitization induced by morphine (10 mg/kg i.p.) for 7 days. On the day 1 and 7, the locomotor activities were measured. In the expression test, mice were trained by morphine for 7 days to induce sensitization, and then challenged with morphine after 5 days of withdrawal. On the day 1, 7 and13, the locomotor activities were measured. Kainic acid lesions prevented the development, but not expression of morphine sensitization. These data reinforce the view that the mPFC is involved in morphine sensitization and more specifically in the development of sensitization.

Keywords: Morphine; Behavioral sensitization; Kainic acid; Medial prefrontal cortex

P050006

INFLUENCE OF GAMMA-MSH PEPTIDES ON BEHAVIOURAL RESPONSES INDUCED BY FORCED ALCOHOLIZATION IN MICE

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Previously we have found that gamma2-MSH (g2) was capable to prevent gamma1-MSH (g1)-induced behavioural and dopaminergic activity, indicating that g1 and g2 may be involved in drug dependence processes. The forced alcoholization in mice was carried out: ethanol, 2 mg/kg i.p. daily for 10 days. Peptides were injected intracisternally on the day 1 (d1) and day 10 (d10) separately and in combinations (e.g. g1 on d1, and g2 on the d10). Other experiment: alcohol withdrawal after the d10, and peptide injections on d1 and d13. Control experiment: peptide injections without alcoholization. Behavioural responses were observed in elevated X-maze. G1 per se induced anxious that was prevented by prior administration of g2. The g2 (but not g1) reduced anxiolytic effects caused by alcohol injections for 10 days, however both peptides completely reduced excitement (increase in locomotion) caused by alcohol withdrawal. The data obtained indicate the importance of g1 and g2 in the development of alcohol dependence and their regulatory role in withdrawal-induced behavioural events.

Key words: g1, g2, behaviour, alcoholization.
Acknowledgements: ESF grant W-ESS3-007, Latvian Council of Science Grant Nr.01-0037.

P050007

The effect of non specific HCN blocker CsCl on learning and memory in mouse

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It has been suggested that HCN is involved in learning and memory. In the present study, we investigated the effects of HCN1 nonspecific-blocker CsCl on spatial learning and memory using Morris water maze and in situ hybridization methods in mice. The results suggested that CsCl (168 mg/kg i.p) 5-day later, the mean escape latency was 78.23 + 11.21s, but that of normal control group was 18.54 + 2.1s, (compared with CsCl group $p < 0.01$); In hippocampal tissue of HCN1 mRNA staining showed in the dentate gyrus (DG), CA1 and CA3 was weaker in the positive cell, compared with normal tissue ($p < 0.01$ in CA3, CA1; $p < 0.05$ in DG,) and average gray scale was increased. Our results showed that CsCl could affect significantly spatial learning and memory in mice, and the function changes of HCN1 channel is involved.

Key words: CsCl; HCN1 mRNA; mice
Acknowledgement: This work was supported by Natural Science Foundation of China (No.30371639).

P050008

Lumiracoxib: More than just a selective COX-2 inhibitor

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Clinical studies show that lumiracoxib exhibits differences, particularly in safety, compared other COX-2 inhibitors. We therefore examined the antinociceptive effect of lumiracoxib in the formalin test, a pain assay in which the role of peripheral COX-2 is limited. Lumiracoxib, but not celecoxib, produced peripheral antinociception when injected locally in the injured tissue. Further experiments showed that lumiracoxib peripheral effect was blocked by L-NAME, an inhibitor of nitric oxide synthesis, ODQ, an inhibitor of guanylyl cyclase, and by the potassium channel blockers glibenclamide, apamin and charybdotoxin. These results strongly suggest that the local antinociceptive effect of lumiracoxib is due, at least partially, to the activation of the nitric oxide-cyclic GMP-potassium channel pathway. Lumiracoxib then appears to be not only a selective COX-2 inhibitor, but a drug with a unique profile endowed of multiple mechanisms of action. Hence, analgesia can be achieved without excessive COX-2 inhibition. This, together with its pharmacokinetic properties, likely plays a role in the increased safety profile exhibited by lumiracoxib compared to other available selective COX-2 inhibitors.

P050009

Influence of Diazepam in behavior and electroencephalogram of rat poisoned by fipronil

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Objective To investigate the influence of Diazepam in the behavior and electroencephalogram of rat poisoned by fipronil. Methods Fifteen rats were randomly divided into fipronil group, fipronil + Diazepam group, and normal group. To observe the change in behavior and electroencephalogram (EEG). Results The rats in fipronil group showed the excited symptoms in 0.20 ±0.01h, and these symptoms got worse, the rats died in 30.80 ±19.25h. The rats in fipronil + Diazepam group appeared the excited symptoms in 3.34 ±0.32h, however, these symptoms did not aggravate, the rats died in 61.40 ±10.45h. Compared with fipronil group, the convulsion time and death time of rats were extended significantly in fipronil + Diazepam group ($P < 0.05$). EEG of the rats in fipronil group displayed α wave and β wave before ig fipronil, EEG showed the eleptiform wave 10 min after ig fipronil, later, there were vertex sharp transient wave and slow wave. EEG of the rats in fipronil + Diazepam group appeared the eleptiform wave which

was vertex sharp transient wave from 3h to 8h.. All EEG prior to death showed sharp wave. Conclusion Central nervous system of the rats poisoned acutly by fipronil is excited, however, the excited symptoms turn to the inhibited symptoms later, the rats show paroxysmal hyperspasmia. Diazepam can prolong the convulsion time and death time of the rats poisoned acutly by fipronil, and reduce the hyperspasmia. Besides, Diazepam also show the antagonistic action for the change of behavior and EEG.

P050011

Cydophosphamide induced inhibition of immunomodulation on herbal formulation

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A new frontier in pharmacology is yet to explore and develop agents that modulate immune response. The ayurvedic literature reveals that many herbs have proves immunomodulatory effect. The major uses of immunomodulators are generalized immuno deficiency disorders. The present study has been designed to evaluate the immunomodulation by inhibiting the immune response by using cyclophosphamide. The methods used are immobility test and tail suspension test in albino swiss mice. The method of carbon clearance and suspension of neurophils have been used to authenticate the immunomodulatory effect cannot be revealed as the patent is pending. The formulations A, B and C have antagonized the cyclophosphamide inhibition indcating the immunomodulation. The results indicate that the formulation have statistical significant immunomodulatory effect in the doses used. The mechanism of immunomodulatory effect has been substantiated by determining the influence on cyto cell lines such as IL-6, IL-12. The therapeutic and pharmacological aspects will be discussed with respect to the immunomodulation.

Key Words: Immunomodulation, Cyclophosphamide, Herbal formulation, Mice.

P050012

Antagonistic effect of melatonin on experimental models of Alzheimer's disease induced by okadaic acid in rat

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In an attempt to investigate the effect of melatonin on Alzheimer's disease, an animal model of Alzheimer's type was produced by microinjecting into the dorsal hippocampus of adult rat with okadaic acid (a phosphatase inhibitor). After injection of okadaic acid more than one time (0.5 microliter, once every 48 hours for three times), rats failed to perform the tasks in Morris water maze test, and neurofibrillary tangles and senile plaque were found in the hippocampus of rats by Bielshowsky stain. Melatonin (0.5-5.0 mg/kg daily for 14 days) reversed the effects of okadaic acid. These results suggest that melatonin can enhance the cognition of dement rats induced by okadaic acid.

Key words: melatonin; Alzheimer's disease; okadaic acid; dement rat

P050013

Interaction Between Dexmedetomidine and Ephedrine on Antinociception in Mice

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The clinical use of alpha2-adrenoceptor agonist dexmedetomidine (dex) for pain relief is restricted by the sedation and hypotension. This study was conducted to see whether the psychostimulant drug ephedrine (eph) has any effect on dex induced antinociception and locomotor inhibitory activity in mice. In experiments both sexes of Swiss albino mice (25-35 g) were tested with hot plate analgesia meter and holed open field test. The mice (n = 8) were injected (i.p) with saline + saline, dex (15 µg/kg) + saline, saline + eph (10 mg/kg) and dex (15 µg/kg) + eph (10 mg/kg). Dex produced significant antinociception at 30 min and the effect was decreased and abolished at 60 and 90 min, respectively. Eph showed very little antinociception at 30. and 60. min, it may depend on increase in locomotor activity. Coadministration of eph not only enhanced but also prolonged the duration of antinociception induced by dex for 90 min. At the same time the motor inhibitory effect of dex was counteracted by eph. We concluded that combining dex with eph may have beneficial effects in the treatment of pain without any sedation.

Key words: Dexmedetomidine, Ephedrine, Antinociception, Locomotor Activity

P050014

Enhancement of Antinociceptive Effect of Morphine by GB-115, a Novel Short Peptide Antagonist of CCK_2 Receptor.

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Stimulation of the brain cholecystokinin-2 (CCK_2) receptor by the octapeptide CCK-8 negatively modulates opioid responses (B.Pommier et al., 2002). In view of existence of physiologically relevant interactions between endogenous CCK and opioids, the effects of treatment with GB-115 ($Ph(CH_2)_5CO$-Gly-Trp-NH_2), a recently developed dipeptide CCK_2 receptor antagonist, and morphine in "hot-plate" and "tail-flick" tests were examined in mice. Given alone GB-115 at low doses 0.0125-0.5 mg/kg had no effect, whereas the large dose 4.0 mg/kg produced a weak, but significant, analgesic effect modified by naloxone (1.0 mg/kg) administration. In the "tail-flick" test the magnitude and duration of GB-115 antinociceptive effect was about 2 times less effective than in the "hot-plate" assay. However, GB-115 in a dose-dependent fashion naloxone-reversibly potentiated the morphine-induced analgesia in both tests. The present data demonstrate a crucial role of endogenous CCK, acting on CCK_2 receptors, in the control of pain perception at both spinal and supraspinal levels. These findings may have important implications for development of CCK_2 antagonists as analgesic adjuncts to the therapeutic use of morphine.

P050015

EXPERIMENTAL STUDY OF THE EFFECT OF METHYL PHENIDATE (RITALIN) ON MEMORY RETENTION AND RETRIEVAL IN MICE

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A review of effect of literature indicates that Methylphenidate is capable of affecting memory, and the degree is dose dependent. In this study, through use of passive avoidance apparatus, effect of different doses of Methylphenidate, on retention and retrieval of memory were investigated. mice were randomly allocated to groups consisting of 10 mice, then weight and numbered for future studies. The study was carried out on four successive days. After animal became familiar with the apparatus at first day, the complete stopped down times were measured on the second day, in memory retention testing after complete stepped down, animals received an electric shock and an IP injection of Methylphenidate, while in memory retrieval testing, after complete stepped down, animals received only an electric shock. On the fourth day, in memory retention testing animals complete stepped down times were evaluated, while in memory retrieval testing, after IP injection of methylphenidate, animals complete stepped down times were measured. The experimental finding indicates that methylphenidate (10 mg/kg) improved retention of memory, while 10 mg/kg dose, causes impairment of memory retrieval.

P050016

Addescent rats have higher levels of nAChR proteins in dopaminergic brain regions and self-administer more nicotine than adults

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Since human tobacco use usually begins during adolescence, we modified our established model to study the acquisition of nicotine self-administration (SA) in adolescents (PN43-45). Lewis rats had prolonged access (23h/d) to nicotine but received no prior shaping, conditioning, or food deprivation. Adolescent rats of both sexes showed similar dose-dependent (15-60 ug/kg/inj.) nicotine SA. Main effects (ANOVA) were shown for day and lever ($p < 0.001$). In comparison to adult females self-administering nicotine 30 ug/kg/inj., adolescents acquired nicotine SA at an accelerated rate ($p < 0.05$) and received a greater number of injections ($p < 0.05$) by d10. In addition, adolescents had greater Bmax values of 125I-epibatidine binding to nAChR in the ventral tegmental area, substantial nigra, and nucleus accumbens ($p < 0.05$). Thus, adolescent rats rapidly acquire nicotine SA within the dosage range previously observed in adult Lewis rats. However, adolescents have more nAChRs in mesolimbic reward areas, and females acquired SA behavior more rapidly, attaining higher levels of stable nicotine SA than adults.

Keywords: adolescent, nicotine, self-administration, nAChR
Acknowledgement: supported by DA-03977, DA-015525.

P050018

Orexin as a master switch to elicit multiple components of the defense response against stressor

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Defense response against stressor is characterized by simultaneous elicitation of multiple components of the autonomic, somatosensory, and behavioral responses such as a rise in blood pressure, heart rate, respiration, skeletal muscle vasodilation, analgesia, cortical arousal, and fight or flight. It has been long unknown how such a set of multiple systems is activated simultaneously. We have hypothesized that orexinergic neurons may be the key, since their cell bodies are located in the so-called defense area in the hypothalamus and their axons widely spread throughout the brain. To test our hypothesis, we used prepro-orexin knockout mice and orexin neuronablated mice. All the components of the defense response so far tested were attenuated in these mice. Moreover, basal blood pressure in these mice were lower by ~20 mmHg than the wild-type controls probably through lower sympathetic vasoconstrictor tone. We conclude that orexin plays as a master switch to elicit multiple efferent pathways of the defense response and as a critical determinant of the sympathetic outflow.

P050019

Attenuated defense response induced by stimulation of amygdala and bed nucleus of stria terminalis in orexin neuron-ablated mice

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We have previously shown that the defense response against stressor was attenuated in prepro-orexin knockout mice and orexin neuron-ablated mice and proposed that orexin plays as a master switch to elicit multiple efferent pathways of the defense response. It is still open question, however, how information of stressor activates the orexinergic neurons. In this study, we examined possible contribution of the amygdala and bed nucleus of stria terminalis (BNST) as one of the afferent nuclei to activate orexinergic neurons. In urethane-anesthetized mice, a GABA-A receptor antagonist, bicuculline, was microinjected into the amygdala or BNST, of which electrical stimulation induced simultaneous increases in blood pressure, heart rate, and respiratory frequency. Bicuculline dose-dependently induced cardiorespiratory excitation in both orexin neuron-ablated mice and wild-type controls. However, dose-response curve was rightward shifted in the orexin neuron-ablated mice. We conclude that the amygdala and BNST constitute one of the afferent pathways to the orexinergic neurons that involved in the defense response against stressor.

P050020

Improved Learning and Memory of Contextual Fear Conditioning and Hippocampal Synaptic Plasticity in Histidine Decarboxylase Knock-out Mice

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To explore the exact role of histamine in learning and memory and related mechanisms, synaptic plasticity at CA1 pyramidal neurons of the hippocampus was assessed in wild-type (WT) and knock-out mice lacking histidine decarboxylase gene ($HDC^{-/-}$) by extracellular recording. And contextual fear memory was tested in WT mice and $HDC^{-/-}$ mice 24 hours after foot shock. Glutamate and GABA in the cortex were measured by HPLC. We found that hipppocampal long-term potentiation (LTP) significantly increased in $HDC^{-/-}$ mice compared with that in WT mice. And the percent of time in freezing increased both in WT mice and in $HDC^{-/-}$ mice 24 hours after training. More importantly, $HDC^{-/-}$ mice froze significantly more than WT mice. Glutamate in the cortex of $HDC^{-/-}$ mice also increased significantly more than that of WT mice, while GABA exhibited no difference between the two genotypes. These data indicate that long-term histamine deficiency causes improved contextual fear memory, which may be partly due to the improvement of the hippocampal LTP and glutamate content in the cortex.

Key words: histamine; contextual fear memory; $HDC^{-/-}$ mice.

Supported by the National Natural Science Foundation of China (30000019).

P050023

Neuroprotective effects of Echinacoside in cellular and animal models of Parkinson's disease

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The present study investigated whether echinacoside (ECH), a major component of phenylethanoid glycosides from the Chinese herbal medicine Cistanches salsa, has neuroprotective effects in vitro, in vivo model of Parkinson's disease (PD) or not. In MPTP mouse model, ECH (5, 20 mg/kg) significantly attenuated behavioral disorders and cell death and led to a marked increase in the DA levels and tyrosine hydroxylase expression. In 6-OHDA rat model of PD, ECH (10, 20 mg/kg) notably decreased the asymmetric rotational behavior of rats induced by apomorphine and markedly increased the DA levels in the lesioned striata. Pre-treatment with ECH (10-40 μg/ml) significantly reduced activation of caspase-3 and caspase-8 and poly (ADP-Ribose) polymer cleavage in MPP+-induced apoptosis of neurons. The findings clearly indicate that ECH exerts neuroprotective effects through its potent inhibitory action on caspase-3 and caspase-8, suggesting that the compound may be an attractive candidate for several neurodegenerative disorders, including PD.

Key words: echinacoside; MPTP; 6-OHDA; Parkinson's disease

Acknowledgment: This study was supported by the National Program for Key Basic Research Projects (No. 2004CB519802)

P050024

Involvement of alpha-2-adrenoceptors in the local peripheral anti-hyperalgesic effect of oxcarbazepine

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We examined the effects of clonidine (CLON), alpha-2-adrenoceptor agonist and yohimbine (YOH), alpha-2-adrenoceptor antagonist, on the effects of oxcarbazepine (OXC) against concanavalin A-induced inflammatory hyperalgesia in a paw pressure test in rats. All substances were administrated intraplantarly (i.pl.) into the rat hind paw. OXC (1000-3000 nmol/paw; i.pl.) and CLON (1.9-7.5 nmol; i.pl.) caused a significant dose and time dependent reduction of the paw hyperalgesia. Isobolographic analysis of co-administration of OXC and CLON in a fixed dose ratio (1/4+1/4, 1/2+1/2 and 3/4+3/4 of ED50 of each drug) revealed an additive anti-hyperalgesic effect. Coadministration of YOH (260 and 520 nmol; i.pl.) with OXC (2000 nmol/paw; i.pl.) significantly decreased the anti-hyperalgesic effect of OXC in a dose and time dependent manner. These results indicate that the peripheral alpha-2 adrenoceptors are involved in the peripheral anti-hyperalgesic effects of OXC in a rat model of inflammatory hyperalgesia.

Key words: oxcarbazepine; anti-hyperalgesia; alpha-2-adrenoceptors.

We thank Novartis Pharma AD, Basel, Switzerland for supplying oxcarbazepine.

P050025

The examination of antinociceptive and toxic effects of oxcarbazepine and carbamazepine in mice

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The effects of oxcarbazepine (OXC) in acetic acid writhing test and rotarod test in mice were examined and compared with the same effect of carbamazepine (CBZ). All substances were applied intraperitoneally (i.p.). OXC (10-40 mg/kg; i.p.) and CBZ (5-25 mg/kg; i.p.) significantly and dose dependently reduced the number of acetic acid induced writhes. The corresponding ED50 (CL) were 14.8 (9.3-23.7) mg/kg and 7.6 (4.7-12.1) mg/kg for OXC and CBZ, respectively, indicating that OXC is about two times less potent than CBZ in inducing antinociception. In a rotarod test, OXC (80-200 mg/kg; i.p.) and CBZ (30-70 mg/kg; i.p.) caused significant dose and time dependent reduction of the time spent on rotarod. The corresponding TD50 (CL) were

150.7 (122.8 - 184.8) mg/kg and 40.4 (31.3 - 52.0) mg/kg for OXC and CBZ, respectively, indicating that OXC is about four times less potent than CBZ in impairing motor ability. The therapeutic index (TD50/ED50) of OXC was about twice greater than that of CBZ. Results indicate that OXC is less potent but potentially safer analgesic drug.

Key words: oxcarbazepine; carbamazepine; antinociception; toxicity.

We thank Novartis Pharma AD, Switzerland for supplying oxcarbazepine.

P050026

Effects of insulin in animal models of antidepressant, Anxiety, and learning and memory tests in mice

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Insulin is a polypeptide hormone that is present in mammals and its main function is the maintenance of blood sugar. Insulin receptors are widely but unevenly distributed in the brain. Insulin has been reported to be involved in the regulation of neurotransmitter release and the dysregulation of insulin signaling in the central nervous system has also been linked to the pathogenesis of neurodegenerative disorders. However, there has been no information on direct relationship of insulin with anxiety and depression among other CNS effects. This study therefore investigated the anxiolytic, antidepressant effects of insulin in addition to its influence on learning and memory and other various neurobehavioral animal models in mice. This experiment was carried out in mice administered intraperitoneally with Insulin at different doses of 0.5, 1.0 and 2.0 IU/kg. The results obtained showed that insulin increased grooming and decreased rearing in Novelty-induced behavior. Insulin has anxiogenic effects, induced a decrease in locomotor activity and impaired learning and memory. These results showed the neurobehavioral effects of insulin.

Keywords: Insulin, anxiolytic, antidepressant, learning and memory

P050027

Administration of oleamide induces antidepressant-like and decreased novelty-induced behaviours in mice

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Oleamide; a fatty acid amide accumulates selectively in the cerebrospinal fluid of sleep deprived cats and rats. Oleamide has been reported to have effects on a wide range of receptors and neurotransmitter systems such as Dopamine, Acetylcholine, Serotonin among others suggesting a wide range of its CNS effects. We investigated the effects of intraperitoneally administered Oleamide (5 and 10 mg/kg) on Novelty-induced behaviours, learning and memory and on forced swimming-induced depression in mice. Oleamide dose-dependently reduced ($p < 0.05$) rearing, grooming and locomotion activities. Spatial working memory was only significantly ($p < 0.05$) affected by the lower dose of 5 mg/kg while the dose of 10 mg/kg had no effect. In the forced swimming test, acute triple intraperitoneal administration of Oleamide (5 and 10 mg/kg) induced a dose dependent reduction in immobility with significant effect at the dose of 10 mg/kg suggesting its antidepressant-like property. In conclusion, these results confirm the multiplicity of CNS receptors and neurotransmitters that Oleamide interacts with hence its numerous and diverse neuropharmacological effects.

Keywords: Oleamide, learning and memory, antidepressant, behavior

P050028

Anxiety-like behavior in mice deficient in the phosphodiesterase 4B (PDE4B) enzyme

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Cyclic AMP (cAMP)-specific phophodiesterase 4 (PDE4), an enzyme catalyzing cAMP breakdown, plays a critical role in controlling intracellular cAMP, which is implicated in various CNS disorders. Using mice deficient in PDE4B ($PDE4B^{-/-}$) or PDE4D ($PDE4D^{-/-}$), two important isoenzymes of PDE4, we examined the function of PDE4 in anxiety-like behavior. The PDE4 inhibitor rolipram (0.1-1 mg/kg) dose-dependently decreased head-dips and the head-dipping time in the mouse hdeboard test (HBT). It also decreased transitions and the time spent on the light side in the mouse light-dark transition test (LDT). Interestingly, only $PDE4B^{-/-}$ mice displayed anxiety-like behavior, as evidenced by inhibited open-arm activity in the elevated-plus maze, decreased head-dips and the head-dipping time in the HBT, reduced transitions and the time on the light side in the LDT, and decreased ambulation and rears in the open-field test. Consistent with this, $PDE4B^{-/-}$ mice displayed increased levels of plasma corticosterone. These results suggest that PDE4, in particular the PDE4B subtype, is important for maintaining a normal mood status (Supported by research grants from NIMH and NICHHD).

P050029

Impaired memory retention in mice lacking pituitary adenylate cyclase-activating polypeptide (PACAP) in an object recognition test

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Neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) has been conserved remarkably during evolution and is widely expressed in the mammalian brain. In Drosophila, mutation of the PACAP homologue results in impairment of memory performance, suggesting the prominent role of PACAP in the learning and memory. Here we studied the function of endogenous and exogenous PACAP in vertebrate memory performance by using mice lacking PACAP ($PACAP^{-/-}$). Memory performance was evaluated in an object recognition test (ORT), based on the differential exploration of familiar and new objects. $PACAP^{-/-}$ and wild-type littermate exhibited a similar memory performance 1 h after the exploration training. In contrast, the memory performance of $PACAP^{-/-}$ was significantly impaired compared with wild-type mice when the test was performed 6 h after the training session. When PACAP (10-20 pmol/mice) was intracerebrally administrated 30 min before training, the deficit in memory performance of $PACAP^{-/-}$ was dose-dependently ameliorated without significant effects on that of wild-type mice. These results suggest that acute defect of PACAP signaling in brain results in impaired memory retention in vertebrates.

P050030

The involvement of Dopamine and Nitric Oxide (NO) on Morphine induced Straub Tail (STR) in mice

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Many central neurotransmitter systems are reported to be involved in the morphine-induced STR in mice. The object of this study was to clarify the role of dopaminergic and nitrergic in this phenomenon. We used 5 different groups of Male albino mice (20-25 g). There were 5 mice in each group. Mice were first given intraperitoneal (I.P) injection of different doses of morphine (2.5-100 mg/kg) 30 min before observation period. In other part of experiment, they were first injected with morphine and L-nitro-arginine-methyl-ester (L-NAME), a nonspecific NO inhibitor (10 mg/kg) and then were injected with morphine and Sulpiride, D2 antagonist (3.125-100 mg/kg). Finally Mice were given all these drugs. Results: L-NAME and Sulpiride decreased the morphine-induced STR when used alone and co-administration of L-NAME and Sulpiride decreased the morphine-induced STR but this decrease was less than L-NAME and more than Sulpiride. Statistical analysis was performed using ANOVA and scoring. In conclusion, probably this was the neuroprotective effect of NO inhibitor on dopamine receptor in presence of morphine. The results may suggest that morphine-induced STR is mediated through dopaminergic and nitrargic systems.

P050031

Effects of 4-Aminopyridine on classical conditioning of the rabbit (Oryctolagus cuniculus) nictitating membrane response

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A large body of data suggests that potassium channels may play an important role in learning and memory. Previous in vitro research in a number of species includ-

ing Hermissenda and the rablit suggests a 4 - aminopyridine (4 - AP) sensitive transient potassium channel may be involved in classical conditioning. We investigated the effects of in vivo 4 - AP administration (0.5 mg/kg) on classical conditioning of the rabbit nictitating membrane response using a battery of tests designed to assess the associative, sensory and motor contributors of 4 - AP to responding. 4 - AP enhanced both classical conditioning and conditioning - specific reflex modification compared to a saline vehicle control and these effects had several nonassociative components including an increase in the frequency of responding to the conditioned and unconditioned stimulus suggesting a sensitizing effect of the drug. Although 4 - AP can have peripheral effects, it may also modify cerebellar excitability or hippocampal neurotransmitter balance resulting in heightening responsiveness to stimulation.

P050032

Experimental studies on the modulatory role of nitric oxide in stress susceptibility and adaptation

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Emotionality is related to stress susceptibility and the present experimental study evaluated the modulatory role of nitric oxide (NO) in the regulation of stress susceptibility and adaptation. Albino rats were screened as "high" and "low" emotional and assay of brain homogenates showed that brain NO metabolites (NOx) levels were lower in "high", as compared to "low" emotional rats. Further, restraint stress (RS) suppressed (a) the number of entries/time spent in the open arms of the elevated plus maze (EPM) and (b) NOx levels in brain homogenates. The NO precursor, L - arginine reversed both RS - induced behavioral and biochemical changes, whereas, the NOS inhibitor, L - NAME, produced opposite effects. Chronic RS attenuated the observed acute RS - induced behavioral and biochemical changes and these were predictably modulated by NO - ergic agents. Cold restraint stress (CRS) consistently induced gastric lesions which were attenuated by L - arginine and aggravated by L - NAME. Exposure to chronic RS reduced the severity of CRS induced gastric lesions, and this adaptive response was facilitated by L - arginine and blocked by L - NAME. The results indicate the involvement of NO as a modulator of stress susceptibility and adaptation.

Key Words: Nitric Oxide, NO modulators, Stress Adaptation

The authors thank the Dept. of Science and Technology, New Delhi, for financial support

P050033

The pharmacokinetic - pharmacodynamic (PK/PD) relationship of diazepam in rats with anxiety after exposure to repeated stress.

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The study investigated the PK/PD relationship of diazepam after acute and chronic treatment in male Sprague - Dawley rats by using the elevated plus maze (EPM) as a pharmacodynamic endpoint of anxiety. Rats were exposed to a stress - restress paradigm, having received either diazepam or vehicle for 14 days (chronic) or at the end of the stress procedure (acute). Each group was divided into 2 groups of 12 rats each receiving either 3 mg/kg diazepam or vehicle. EPM assessments were conducted on day 14 at 6 peak and 6 trough diazepam levels in each group. The diazepam drug concentration in the plasma was determined simultaneously. A statistical significant decrease in aversive behavior was observed at peak diazepam concentration after acute treatment and at trough concentration after chronic treatment with 3 mg/kg respectively. A PK/PD relationship between plasma drug level and stress - induced aversive behavior could therefore be established for the 3 mg/kg dosing after both acute and chronic treatment. The difference between diazepam's anxiolytic effect at peak and trough concentrations was more profound after acute treatment.

Key words: PK/PD relationship, diazepam, rats, anxiety

P050034

GABA levels in the hippocampus and frontal cortex of rats following stress and - re - stress in an animal model of PTSD

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The precise role of GABA in post - traumatic stress disorder (PTSD) and the influence on GABA levels exerted by the stress that results in PTSD is still unclear. The current study investigated the effects of a stress - retress procedure on hippocampal and frontal cortex GABA levels at two different time intervals following re - stress. Male Sprague - Dawley rats were exposed to a time dependent sensitization (TDS) stress paradigm where after GABA levels were determined in the above two brain regions 1 and 7 days post re - stress using high performance liquid chromatography (HPLC) with electrochemical (EC) detection. Unstressed rats were used as controls. No difference in the concentration of GABA was found in the hippocampus at either of the time intervals. In the frontal cortex, however, an increase in GABA concentrations was evident both at day 1 and day 7 post re - stress. We conclude that frontal cortical, but not hippocampal GABA levels are more affected by stress, with these changes possibly underscoring the role of the cortex to exert control over the behavioral fear response after repeated trauma.

Key words: GABA, HPLC, stress, rats

P050035

ANALGESIC ACTIVITY OF HAWTHORN SEED EXTRACT

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Hawthorn (Crataegus monogyna, Rosaceae) extracts are among the most popular herbal medicinal products in the U.S.A. and various European countries. Hawthorn has been used for its several pharmacological effects more than 700 years as a folk medicine. Although various pharmacological activities of Hawthorn have been reported previously, to the best of our knowledge, there have been no reports related to its analgesic action. Here we aimed to investigate the analgesic activity of plant seed extract. Analgesic activity of morphine (2 mg/kg) and the extract (1 - 1000 mg/kg) were measured by tail - clip and tail - immersion tests. Naloxone (5 mg/kg) was used as opioid antagonist. In tail clip tests, 10 - 1000 mg/kg doses of the extract showed analgesic activity, whereas its 1 mg/kg dose did not. Naloxone antagonized its analgesic effect. No analgesic activity was observed in tail immersion test. LD_{50} value of the extract was estimated higher than 1000 mg/kg. Being important for the development of new analgesic drugs, this is the first report for the analgesic activity of Hawthorn seeds possibly due to endogenous opioid system. However, further studies are necessary.

Key words: Hawthorn, Crataegus, analgesia

P050036

Analgesic and Sedative Activity of 2 - (2 - Hydroxynaphthalen - 1 - yl) - 5,6 - dichloro - (1H) - benzimidazole (HNDCB)

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Benzimidazole derivatives are compounds having potential pharmacological effects such as analgesic, antiinflammatory, sedative, selective 5 - HT_4 receptor antagonists. As a benzimidazole derivative, we have synthesized HNDCB for screening its analgesic and sedative activities. In this study, HNDCB (500 mg/kg) was investigated for possible analgesic, skeletal muscle relaxant and sedative effects. Tail - clip and tail - immersion tests, activity cage and rota rod measurements were applied for these purposes. Morphine (1 mg/kg) and diazepam (2 mg/kg) were used as standards. Analgesic effect was observed both in tail - clip and tail - immersion tests. Significant decreases in the horizontal and vertical spontaneous activities were observed at applied dose. Skeletal muscle relaxation was more than control, but less than diazepam. In conclusion, HNDCB shows analgesic activity. Reduction in the locomotor activity and grip strength also shows the central nervous system depressant effect. Further studies are in progress to examine the pharmacological activities of other compounds in the same chemical group.

Keywords: benzimidazole derivatives, central nervous system, animal behavior

P050037

Central Nervous System Activity of 2 - (Naphthalen - 1 - yl) - 4,5 - dimethyl - (1H) - imidazole(NDI)

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There are several studies on the imidazole ring system and its pharmacological activities on central nervous system (CNS) such as antidepressant, anxiolytic, serotonergic, sedative, anaesthetic, etc . After preparing NDI by synthesis, we aimed to screen its pharmacological activity on CNS . A dose of 500 mg/ kg (i . p) test compound was used for the present study . Tail clip and tail immersion tests for analgesia, elevated plus maze and hole board tests for anxiety, activity cage measurements for spontaneous motor activity and hexobarbitone - induced sleeping time for sedative activity were studied . Analgesic effect was observed neither in tail - clip nor in tail - immersion tests . Test compound decreased the number of head - dips and mice spent more time in closed arm of maze . Significant decreases in the horizontal and vertical locomotor activities were observed at the applied dose . During sleeping test, the onset of sleeping time was decreased and the total sleeping time was increased . Our findings indicate that NDI possesses skeletal muscle relaxant and sedative activities . Other compounds having similar structure will be studied in the same pharmacological assays .

Key words : CNS, imidazole ring, animal behaviour

P050039

The spatio - temporal property instead of activity changes after focal cerebral ischemia in mice

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Cerebral ischemia induces sensorimotor and cognitive dysfunctions in rodents ; however, little is known about the changes in the spatio - temporal organization of locomotor activity after ischemia . We continuously (22 h) assessed these changes in an enclosure after focal cerebral ischemia in mice . The total traveled distances from 3rd to 24th were similar between the two groups . The control mice moved, stayed and stopped primarily in feeding and drinking zones, frequently in peripheral but rarely in central zones ; whereas the ischemic mice almost evenly in each zone . Mice were more active shortly after entered the enclosure and during night ; whereas ischemic mice recovered slower and was not more active in night . Most spatial parameters were closely correlated with the ischemic infarction, neuron densities and typical behavioral assessments . We conclude that focal cerebral ischemia alters the spatio - temporal properties, but not the activity amount, and that the spatial parameters may be useful indicators to evaluate the dysfunctions after focal cerebral ischemia .

P050040

THE INFLUENCE OF ADRENERGIC SYSTEM ON STRESS - INDUCED ANALGESIA

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Analgesic reaction is specific phenomenon that occurs during stressful event and there is a possibility of modifying it by different agents . It was examined the effect of adrenergic system through the beta receptor agonists and antagonists on stress - induced analgesia in rats . The electric steam applied during 2 minutes was used as stressful agent and the radiant heat method was used for testing central analgesic activity . Propranolol, metoprolol, atenolol, hexoprenaline, carvedilol and sotalol were applied i .p . 30 - 40 min . before the stress (according to drug pharmacokinetic parameters) . Any of the tested drugs didn't show analgesic effect . All drugs abolished analgesic effect caused by stress, but in various degree . While atenolol showed the slight prolongation of reaction time only 10th min . after stressful event, propranolol and carvedilol exhibited it at 10th and 30th min . Hyperanalgesic reaction was noted after 30th, 50th and 70th min . in hexoprenaline, atenolol and metoprolol treated groups, respectively . Based on these results it can be concluded that central and peripheral beta receptors modulate stress - induced analgesia .

Key words : stress - induced analgesia, adrenergic agonists and antagonists

P050041

The effect of addlescent carbohydrate bingeing on alcohol consumption and responsivity to amphetamine in adulthood alcohol - preferring rats

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Aims : The present study investigated whether adolescent sucrose bingeing affects adulthood alcohol consumption in alcohol - preferring rats, namely Fawn - Hooded (FH) and iP rats . In addition, the effect of adolescent sucrose bingeing on responsiveness to acute amphetamine was studied . Method : Rats (4 - week old) had food and water ad libitum and were allowed restricted access to 10 % w/ v sucrose solution for 21 days . Ten days later, all rats were allowed to choose between 5 % v/ v ethanol and water . Fluid consumption was monitored for 4 weeks . Separate cohorts had the same sucrose exposure protocol, but were then administered with amphetamine (1 .5 mg/ kg, i .p .) and their locomotor activity analyzed . Results : In iP rats consumption of sucrose did not impact upon alcohol consumption, while FH rats exposed to sucrose showed higher initial alcohol consumption and preference than control FH rats . In contrast, sucrose - exposure enhanced the locomotor response of iP rats to amphetamine compared to controls P rats, an effect not observed in FH rats . Conclusion : Sucrose bingeing during adolescence can alter behaviour patterns related to substance abuse in adulthood .

P050042

Long - Lasting Impairment of Behavioral Performance of Wistar Rats in the Open Field Test after Repeated Immobilization Stress ; Effects of Amphetamine

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The aim of this study was to determine whether repeated restraint stress to Wistar rats would produce longlasting behavioral impairment in the open field device and how small doses of amphetamine (AMPH) would modify the persisting behavioral changes . Rats were exposed for three consecutive days to 60 min lasting immobilization alone (IMO) or IMO combined with water immersion at 21 °C (IMO + C) , and the open field test was performed repeatedly for 5 weeks . All behavioral parameters after both stressors were reduced (total movement distance as an indicator of overall activity, rearing as vertical exploratory activity and time spent in the center of arena as an indicator of anxiety) . AMPH (0 .3 and 1 .0 mg/ kg i . p .) was given 60 min before the open field test that was performed 2 - 3 weeks after the application of stressors . AMPH given on days 23 and 30 increased behavioral parameters proportionally but its effect did not persist to the next testing . In summary, the interference of AMPH treatment with long - lasting changes in rat 's behavior following stress treatment was not persistent .

Amphetamine ; Open - field ; Restraint ; Wistar rats

Supported by grants MSM 0021620806, GACR 305/ 03/ H148 and GACR 309/ 06/ 0121 .

P050043

Effect of agmatine on the working memory in three - panel runway apparatus in rats .

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Effect of agmatine (endogenous imidazoline receptor ligand) was studied on the working memory deficits induced by scopolamine, a muscarinic receptor antagonist in rats using a three - panel runway apparatus . Scopolamine (1 mg/ kg, ip) was administered alone or in combination with agmatine (20 - 80 mg/ kg, ip) and memory errors and latency period of the session were recorded on a three - panel runway apparatus . Besides locomotor activity and passive avoidance tests were applied . Treatment with scopolamine produced significant working memory and locomotor activity deficits in rats . Treatment with agmatine significantly and dose dependently reduced the scopolamine - induced working memory deficits . These results suggest an important role of imidazoline receptors on working memory .

Key words : Agmatine, three panel runway, passive avoidance, locomotor activity

P050044

ANTIDRESSANT-LIKE EFFECT OF BARAKOL IN STRESS RATS

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Barakol was isolated from the fresh young leaves of Cassia siamea, a plant used in Thai traditional medicine. The present study investigated the antidepressant-like effect of barakol in socially and isolation reared rats. Male Wistar rats were obtained from weaning, and housed either alone (isolation rearing) or in groups of five-six rats/cage (social rearing). Six weeks later, these rats were tested for their sensitivity to barakol using the forced swimming test (Porsolt et al., 1978, Eur J Pharmacol 47, 379-391). The results demonstrated that the forced swimming behavior of the saline-treated isolation reared rats was not significantly difference from the socially reared controls. Sub-chronic administration of barakol (5 and 10 mg/kg i.p.) 24, 5 and 1 h to both isolation and socially reared rats, significantly reduced the immobility time (antidepressant-like effect) and increased struggling ($P < 0.05$) compare with the saline treated isolation reared rats. However, the antidepressant-like effect of barakol (5 and 10 mg/kg i.p.) was not observed in the socially reared rats. These results indicate that barakol has antidepressant-like effect (or antiimmobility effect) in social isolation stress rats.

P050045

THE EFFECT OF AZITROMYCIN ON GASTRIC STRESS-ULCER ULCER IN RATS INDUCED BY COLD RESTRAINT STRESS

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The present study examines the effect of azitromycin on gastric strees-ulcer in rats induced by cold restraint stress. Azitromycin was given to rats at 5 days before experiments, once daily in oral doses of 250 mg/BM. The protocols of the following experiments were supported by International declaration for care and use animals (Guide for Care and Use of Laboratory Animals, NIH Publication N0 85-23). For cold restraint stress, the rats were stained in individual close-fitting cages at 4 degrees C. The end of 3 hours, all the rats were sacrificed in ether anesthesia. The gastric erosions were manifested as focal erosions and petechiae of the mucosal fold, localized in glandular portion of stomach of rats. Azitromycin markedly reduced lesion area (from UI = 4.98 ±6.17 to UI = 0.36 ±0.83 mm2), but did not change number of petechiae. The results of this study suggest that antibiotic effect on stress-ulcer formation might be responsible for prevention of gastric lesion, modulated through mechanism that involves local inflammatory factors.

Key words: stress-ulcer, azitromycin, rat

P050046

Research of binding assay with 5-HT_1 and 5-HT_2 receptors by SCP-1 and SCP-2

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AIM. To study the combined effect of SCP-1 and SCP-2 with 5-HT_1 and 5-HT_2 receptors. METHODS. Using the radio ligand-receptor binding assay, choosing ^{3}H-5-HT for 5-HT_1 and ^{3}H-Spiperone for 5-HT_2 receptors as the specific ligand, we studied the competitive binding ability of SCP-1 and SCP-2 with 5-HT_1 and 5-HT_2 receptors. RESULTS. 1. In the ligand receptor saturation test of 5-HT_1, Bmax was 28.8 fmol/mg protein, Kd was 7.66 nmol/L. In the ligand receptor competition test, IC_{50} for SCP-1 and SCP-2 were 1.584 μM and 5.495 μM respectively, and nH were 0.96 and 1.05 respectively. 2. In the ligand receptor saturation test of 5-HT_2, Bmax was 121 fmol/mg protein and Kd was 5.91 nmol/L for 5-HT_2. In the ligand receptor competition test, IC_{50} for SCP-1 and SCP-2 were 1.0 μM and 2.512 μM respectively, nH were 0.86 and 0.88 respectively. CONCLUSION. SCP-1 and SCP-2 combined with single binding site of 5-HT_1 receptor and their binding with 5-HT_2 receptor is irregular, there are maybe negative interactions and several binding sites.

KEY WORS: SCP-1 and SCP-2, binding, 5-HT_1 and 5-HT_2 receptor, ^{3}H-Spiperone, ^{3}H-5-HT

Acknowledgement: This research are supported by government of china for 863.

P050047

EFFECTS OF THE 5-HT_{1A} RECEPTOR AGONIST 8-HYDROXY-2-(DI-N-PROPYLAMINO)-TETRALINE (8-OH-DPAT) ON FOOD INTAKE IN THE MOUSE

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The aim of the study was to investigate the effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT on food intake in mice. Male C57BC/6 mice (n = 16; b.wt. 28-32 g) were divided into two equal groups. Mice in Group 1 were injected s.c. with saline (control) and those in Group 2 with 8-OH-DPAT (25-200 mg/kg) and placed in experimental cages with free access to food and water. Food intake was measured over 120 min. Six days separated successive saline or drug trials. The results showed that 8-OH-DPAT (25-200 mg/kg) produced a dose-related increase in food intake in non-deprived mice, with doses of 100 mg/kg and above producing significant increases. For example, the 100 mg/kg dose increased cumulative food intake from a control value ±s.e.mean of 0.2 ±0.1g to 0.6 ±0.1g at 60 min ($p < 0.01$) and 0.7 ±0.2g to 1.7 ±0.2g at 120 min ($p < 0.01$). In further experiments, the hyperphagic effect of 8-OH-DPAT (200 mg/kg) was abolished by pre-treatment with the selective 5-HT_{1A} receptor antagonist WAY 100635. The results show that, in agreement with previous results obtained in rat and pig, 8-OH-DPAT also produces hyperphagia in the mouse by a 5-HT_{1A} receptor mediated mechanism of action.

Key words: Mouse, Food, 5-HT_{1A}, 8-OH-DPAT

P050048

Effects of Scutellaria flavonoid on memory deficits in aluminum toxic mice

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AIM To study of flavonoids from stems and leaves of Scutellaria baicalensis Georgi (SSF) on learning and memory deficits, automatic dyskinesia, neural and hepatic pathological changes and free radicals abnormal alterations. METHODS Aluminum toxic model of mice was produced by introperitoneal injection (ip) of $AlCl_3$ for 50 d. Behavioral test of mice was used to examine the learning and memory ability; the number of automatic action determined the automatic dyskinesia; the neural and hepatic pathological changes were assessed by alterations of cerebral cortex and liver; MDA level and SOD activity in brain and liver were measured to evaluate free radicals. RESULTS $AlCl_3$ (100 mg·kg^{-1}, ip, 50 d) resulted in a decreased ability of learning and memory in water maze task, lowered automatic action numbers, neuronal-hepatic-pathological changes and free radicals abnormal alterations, as compared with control group. The dose of SSF 50, 100 and 200 mg·kg^{-1} could significantly reverse above pathological changes in toxic mice caused by aluminum. CONCLUSION SSF could reduce cognitive deficits and automatic dyskinesia, improve neuronal-hepatic pathological changes and free radicals abnormal alterations.

P050049

Effects of Capsule Yi-Zhi on learning and memory disorder and beta-amyloid peptide induced neurotoxicity in rats

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Objective To investigate the effects of Yi-Zhi Capsule (YZC) on learning and memory disorder and β-amyloid protein (Aβ) induced neurotoxicity in rats. Methods Various doses of YZC were administered to Sprague-Dawley (SD) rats for 8 days, twice a day. Then scopolamine hydrobromide (Sco) intraperitoneal injection was performed on each rat and the MORRIS water maze test and step-though test was carried out respectively. Primary rat cortex neurons were cultured in vitro for 7 days and then, serum containing YZC was added to neurons before or after the addition of Aβ25-35. MTT assay and test of level of LDH in the culture media was performed. Results Compared with control group, rats in Morris water maze test presented significantly decreased time in finding the platform, and in step-though test, the latent period rose and the error number decreased. Moreover, in cultured primary neurons, the dramatic drop of LDH level and the high A scores rising in MTT test. Conclusions YZC presented promising effects on learning and memory dysfunction and Aβ induced neurotoxicity in vitro.

Key words: learning and memory disorder, beta-amyloid peptide, neurotoxicity

P050050

Antidepressant-like effect of the ethanolic extract of Xiaobuxin-Tang, a traditional Chinese herbal prescription in animal models of depression

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Xiaobuxin-Tang is a traditional Chinese herbal prescription which was recorded in a silk scroll unearthed from Mogao Caves of Dunhuang. Ancient literature and clinical studies indicate it can remit depressive disorder. The aim of the present study was to investigate its antidepressant effect by animal depression models. We adopted three behavioral despair models and acutely administrated the ethanolic extract of Xiaobuxin-Tang by p.o. As a result, the extract at dose of 300 mg/kg and 600 mg/kg significantly decreased the duration of immobility time in mice forced swimming test; Also, the extract at dose of 1200 mg/kg significantly decreased the duration of immobility time in rat forced swimming test. Furthermore, the extract at dose of 600 mg/kg had the same effect in mice tail suspension test. The extract (300-1200 mg/kg) also increased the accumulative number of the 5-HTP induced head twitch response in mice. These results firstly indicate that the ethanolic extract of Xiaobuxin-Tang exerts antidepressant-like effect, which may be related to the potentiation of brain serotonergic neurotransmission.

P050051

Central Nervous System Activity of Purine Alkaloids from Camellia assamica var. kucha in Mice

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Aim: We investigated the central nervous system activities of theacrine (1,3,7,9-tetramethyluric acid), a purine alkaloid which is abundantly present in Camellia assamica var. kucha in mice. Method: Ambulatory activity, pentobarbital-induced sleep and forced swimming test were used to evaluate the central nervous system activities. Result: Caffeine treatment led to marked decrease of the immobility time at the doses of 10 and 30 mg/kg, while theobromine had no significant effect. Although the decreased immobility time was also observed in theacrine at the same dose, its effect was slighter than caffeine. Caffeine (10 and 30 mg/kg) and theacrine (30 mg/kg) markedly increased the ambulatory activity of mice. However, either theobromine (10 and 30 mg/kg) or theacrine (10 mg/kg) had no remarkable effect. Theacrine could significantly prolongate the sleeping time of mice induced by pentobarbital, but caffeine and theobromine decreased the sleeping time in the same schedule. Conclusion: These results indicated that theacrine showed central nervous system action was different from caffeine and theobromine.

Keywords: theacrine; purine alkaloid; Camellia assamica var. kucha; Central nervous system

P050052

Heroin craving induced by reward and withdrawal leads to the relapse to heroin use in rats

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In humans, conditioned effects of opioid withdrawal may contribute to drug craving. However, the opiate withdrawal that enhances the drug seeking is still unclear. In the present experiments, rats were initially trained to self-administer heroin (50 μg/kg/infusion) in 4 hours daily sessions. The sessions were completed for 1, 5, 10, and 14 days, respectively. To observe the drug seeking behavior induced by associated reward learning and withdrawal, we determined the drug seeking 1 day or 14 days of forced abstinence after the termination of heroin self-administration. Linear regression showed that the drug seeking elicited by conditioned cues at 1 day or 14 days was positive related to the training number of heron self-administration. After 14 days of forced abstinence, the all groups showed more vigorous drug-seeking behavior than those after 1 day of withdrawal. The drug seeking induced by conditioned cues were increased following the pretreatment with naltrexone at 1 day of termination of drug, but not changed at 14 days of withdrawal. The present studies demonstrate the two forms of craving induced by primary reward learning and withdrawal cause the drug seeking behavior.

P050053

Amnesia induced by beta-amyloid peptide in dopamine D_3 knock-out (KO) mice is affected by a cannabinoid CB_1 receptor antagonist

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The dopamine D_3 receptors subtype, belonging to the D_2-like group, is mostly located in brain regions regulating cognitive processes and emotion. Increasing evidence suggests a dynamic multilevel dopaminergic and endocannabinoid interaction critically implicated in various neurophysiological responses. Aim of the present study was to assess the effects of CB_1 receptor blockade on memory deficit induced in D_3 knock-out mice (KO) by pretreatment with BAP(1-42). Different groups of mice were injected i.c.v. with 400 pMol BAP(1-42) and 14 days later tested in a step-through passive avoidance paradigm. The CB_1 receptors antagonist rimonabant (1 mg/kg), was injected intraperitoneally (i.p.) for 11 or 7 days. D_3 KO mice control group showed a better performance than wild type (WT) mice. Both groups pretreated with BAP(1-42) exhibited a worsening of passive avoidance response. D_3 KO mice treated with the CB_1 receptor antagonist for 11 days exhibited a better performance than WT in passive avoidance paradigm. Different results were found after 7 days of treatment.

These results suggest that dopamine and cannabinoid system could be involved in the performance of D_3 KO mice in the passive avoidance paradigm.

P050054

Anxiolytic-like effects and motor coordination activity in rats and mice in two models.

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The anxiety is an answer of fight-flight and the OMS, DSM-IV, and ONG it is one of the 10 main causes of disability with motor coordination decrease. The purpose of this work is to determine if the MEL, the DIAZ and the BUS, have anxiolytic-like effect in relationship with the motor coordination activity in rats and mice. We use model Rota-Rod for rats, they are separated in groups: strong, Vehicle, MEL1, MEL2, DIAZ and BUS, the rats were placed in borosilicate box and it was evaluated: micturition, number of fecal skittles, nibble and piloerección; later on, they underwent the Rota-Rod and they were administered Flumazenil (FLU), 60 sec later underwent the Rota-Rod. The mice were placed in a borosilicate box and was evaluated, of spheres buried totally, later on they were administered FLU. In conclusion: 1) The MEL produce an "anxiolytic-like effect of the MEL, DIAZ and BUS, in rats and mice, 2) The MEL and DIAZ diminish the motor coordination significantly in rats, 3) The BUS have not effect on the motor coordination in rats and 4) The FLU produces an antagonistic effect when DIAZ and MEL are administered.

Key Words: Anxiety, Melatonin, Motor Coordination
Supported by DGAPA: IN205905

P050056

Mefenamic acid attenuates intracerebroventricular streptozotocin-induced cognitive deficits in the rat: a behavioral analysis

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Intracerebroventricular (ICV) injection of streptozotocin (STZ) in rats is followed by long-term and progressive deficits in cognitive performance in rats. Epidemiological studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs) could delay or slow the clinical expression of SAD. Therefore, the beneficial effect of mefenamic acid (MA) was investigated on ICV STZ-induced learning, memory, and cognitive impairment in male rats. For this purpose, rats were injected with ICV STZ bilaterally, on days 1 and 3 (3 mg/kg). The STZ-injected rats received MA (30 mg/kg/day, i.p.) starting from day 5 post-surgery for two weeks. The learning and memory performance was assessed using passive avoidance paradigm, and for spatial cognition evaluation, radial eight arm maze (RAM) task was used. MA-treated STZ-injected rats show higher correct choices and lower errors in RAM than vehicle-treated STZ-injected rats. MA administration also significantly attenuated learning and memory impairment in treated STZ-injected group in passive avoidance test. These results demonstrate MA efficacy against cognitive deficits caused by ICV injection of STZ in rats.

Key words: Mefenamic acid, Spatial cognition, Alzheimer

P050057

The effect of chronic oral administration of Nigella sativum on the contractile reactivity of thoracic aorta of male diabetic rats

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Therapeutics, especially medicinal plants are of high value in preventing vascular complications of diabetes mellitus. Considering the anti-diabetic effect of Nigella sativum, this research study was conducted to evaluate the effect of oral two-month administration of Nigella sativum on the contractile reactivity of aorta in diabetic rats. Male Wistar rats were divided into control, black seed-treated control, diabetic, and black seed-treated diabetic groups. The treatment groups received oral administration of black seed-mixed pelleted food (6.25%) for two months. After two months, contractile reactivity of aortic rings to KCl and norea-

drenaline was determined. There was a cumulative dose - dependent effect for these two agonists in aortic rings from all groups. In addition, the maximun contractile reactivity was significantly higher in diabetic group as compared to control one ($p < 0.001$). Meanwhile, this response was lower in black seed - treated diabetic group in comparison with untreated diabetic group ($p < 0.05$). Chronic oral administration of Nigella sativum could attenuate enhanced vascular responsiveness in diabetes mellitus.

Key words: Nigella sativum, Aorta, Diabetes Mellitus, Rat

P050058

Effects of Taurine on Rat Behaviors in Three Anxiety Models

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In our previous studies using an elevated plus - maze test in mice, taurine was shown to present an anxiolytic - like effect after single and repeated administration (Chen et al., 2004). The aim of the present study was to investigate the anxiolytic and behavioral effects of taurine on rats in the open field, hole - board, and social interaction test compared to the positive control diazepam. Taurine (14, 42, and 126 mg/kg, i.p.) was administered 30 min before the tests. In the social interaction and hole - board tests, taurine (42 mg/kg) significantly increased social interaction time and the number and duration of head - dipping. In the open - field test, taurine (126 mg/kg, i.p.) presented anxiolytic - like effects by increasing the number of center entries, time spent in the central area and the anti - thigmotactic score while having no effect on the locomotor activity. Results from these experiments suggest that taurine produces an anxiolytic - like effect in these animal models and may act as a modulator or anti - anxiety agent in the central nervous system.

P050059

The ASIC1a antagonist PcTX - 1 reduces fear - related behavior

Patricia Westmoreland, Matthew Coryell, Zlatan Kurjkovic, Mikail Schnizler, Michael Welsh and John Wemmie

Current medications for anxiety achieve remission in only 40% of cases. Identifying novel pharmacological targets in animal models of fear may lead to therapeutic advances. The acid sensing ion channel (ASIC1a) is important in fear - related behavior. In mice, deletion of the ASIC1a gene significantly reduces fear. In order to test whether ASIC1a antagonists have a similar effect, we used the ASIC1a antagonist PcTX - 1. When we found that PcTX - 1 blocks ASIC1a - mediated currents in transfected CHO cells, we tested its effect on fear - related behavior. We administered PcTX - 1 or artificial cerebrospinal fluid into the mouse brain by intracerebroventricular cannula and assessed the fear - response to the predator odor trimethylthiazoline (TMT) and in the open field test. Consistent with an anxiolytic effect, in wild type mice PcTX - 1 diminished TMT - evoked freezing significantly and also increased center time in the open field. PcTX - 1 had no significant effect on these behaviors in the ASIC1a null mice. Thus, inhibition of ASIC1a with PcTX - 1 attenuates the fear response in wild type mice. These results suggest that pharmacological inhibition of ASIC1a may provide a novel way to reduce anxiety in patients.

P06. Neuropharmacology (Neuropathic pain)

P060001

Enhanced antinociceptive effects of morphine in histamine H_2 receptor gene knockout mice

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The involvement of supraspinal histamine H_2 receptor in antinociception by morphine was examined using histamine H_2 receptor gene knockout (H_2KO) mice and histamine H_2 receptor antagonists. Antinociception was evaluated by assays for thermal (hot - plate, tail - flick and paw - withdrawal tests) and chemical (capsaicin tests) stimuli. Thresholds for pain perception in H_2KO mice were higher than wild - type mice. Antinociceptive effects of (i.c.v.) administered morphine were enhanced in the H_2KO mice compared to wild - type mice. Intracerebroventricular co - administration of morphine and cimetidine produced significant antinociceptive effects in the wild - type mice when compared to morphine or cimetidine alone. Furthermore, zolantidine, a selective and hydrophobic H_2 receptor antagonist, enhanced the effects of morphine in all nociceptive assays examined. These results suggest that histamine exerts inhibitory effects on morphine - induced antinociception through H_2 receptors at the supraspinal level. Our present and previous studies suggest that H_1 and H_2 receptors cooperatively function to modulate pain perception in the central nervous system.

Keywords: Antinociception; Histamine H_2 receptor; knockout mice

P060002

The kinetic distribution in rat brain nedei and the transport through rat neuron of berberine in Coptidis Rhizoma alkaloids

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Coptidis Rhizoma alkaloids and its main component, berberine, have multiple cerebral bioactivities and have been used for the treatment of cerebral diseases in clinic. After intravenous administration of Coptidis Rhizoma alkaloids at a dose of 10.2 mg/kg containing 3 mg/kg berberine to rats, the results showed that berberine could reach hippocampus, striatum, thalamus and cortex, quickly distribute to them, slowly eliminate from them, which suggests that berberine might directly act on certain regions of brain neuclei to provide a neuroprotective effect. Simultaneously, berberine could be determined in brain infusion saline after infusion of lateral ventricle in rats. These results indicated that berberine could penetrate through the blood brain barrier. And, it proved that berberine could be transported from blood to intestinal in animal models firstly. The mechanisms of transport through cortical neuron for berberine should be of facilitative transport, and organic cation transporter might be involved in the process. Berberine were exported out of neuron mediated by P - glycoprotein and it was a active transport.

P060003

Inhibitory Action of Penicillin Antibiotics on the Enkephalinase Enzyme in the Guinea Pig Ileum

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It has been shown by biochemical enzymatic study that Penicillin antibiotics are able to act as competitive reversible inhibitors of enkephalinas enzyme. In this study we evaluated the effect of Penicillin antibiotics on the enkephalinas enzyme in the guinea pig ileum. Guinea pig ileum was used in normal Tyrode solution. The ileum was stimulated at 0.1 HZ frequency and the isotonic contraction of this muscle was recorded by a Narco physiograph. Stimulation of guinea pig ileum at 10 HZ resulted in Naloxone sensitive depression of the twitch contractions of this muscle which shows the release of endogenous opioid peptides. After several minutes this depressive effect was reversed by enkephalinas enzyme. Addition of Penicillin antibiotics during the 10 HZ stimulation potentiated the depressive effect of endogenous opioid peptides in a dose dependent manner. IC_{50} of Ampicillin, Nafcillin and Cloxacillin was calculated as 4.8×10^{-8} M, 1.4×10^{-8} M, 7.4×10^{-9} M respectively. Our result shows that the Penicillin antibiotics potentiate the depressive effect of 10 HZ stimulation of guinea pig ileum by inhibition of enkephalinas enzyme.

Key Words: Penicillin, Enkephalinas, Ileum, Opioid Peptides.

P060004

Presynaptic Mechanism Underlying cAMP - Induced Synaptic Potentiation in Medial Prefrontal Cortex Pyramidal Neurons

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The cAMP has been recently proposed to participate in regulating prefrontal cortical cognitive functions, but yet little is known about how it dose so. Here, we used forskolin, an adenylyl cyclase activator, to examine the effects of cAMP on excitatory synaptic transmission in the medial prefrontal cortex (mPFC) using whole - cell patchclamp recordings from layer V pyramidal cells in vitro. We found that bath application of forskolin significantly increased the amplitude of excitatory postsynaptic currents (EPSCs) in a concentration - and age - dependent manner. This enhancement was completely abolished by coapplication of PKA inhibitor and p42/p44 MAPK kinase inhibitor, but not application of either drug alone. The augmentation of EPSCs by forskolin was accompanied by a reduction of the synaptic failure rate, coefficient of variation and paired - pulse ratio of EPSCs. These results indicate that cAMP acts presynaptically to elicit a synaptic potentiation on the layer V pyramidal neurons of mPFC through converging activation of PKA and p42/p44 MAPK signaling pathways.

Key words: cAMP, PKA, p42/p44 MAPK
This work was supported by research grant NSC94 - 2321 - B - 006 - 008.

P060005

Neuroprotective profile of pinocembrin attenuates glutamate - induced cell death in rat cortical neurons via CREB function modulations

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AIM: To investigate neuroprotective profile of pinocembrin, a natural compound extracted from Chinese propolis, on cultured rat cortical neuron against glutamate neurotoxicity. METHODS: Neuron was determined on cell death by LDH release and stained with Hoechst 33342. Mitochondria were assessed on function and membrane potential level. Cellular expression of c - Fos, CREB, pCREB and PP2B was evaluated by immunoblotting assay. RESULTS: Neuron was damaged by glutamate, with dying rate as 67.6 ± 3.2 %, and LDH increased to 127.5 ± 10.5 U·L^{-1}. Mitochondria were injured with function decreased to 51.3 ± 8.6 % and lowered membrane potential level. Pinocembrin improved neuron morphology and decreased LDH value. Pinocembrin also increased pCREB/CREB value and level of c - Fos, which was CRE - dependently coded. In addition, pinocembrin treated group had a decreased PP2B expression level and activity. CONCLUSION: Pinocembrin protected neuron against glutamate by improving CREB/CREB value and c - Fos expression. Its modulation on PP2B provided a possible reason of the elevated pCREB/CREB level. It was for the first time elucidating molecular mechanisms of pinocembrin for neuroprotection profile on neuron from glutamate neurotoxicity.

P060006

Resistance to morphine tolerance in rats deleted of TRPV1 - expressing sensory neurons

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Systemic administration of resiniferatoxin (RTX), an ultrapotent analogue of capsaicin, destroys TRPV1 - expressing afferent neurons and their central terminals in the spinal dorsal horn. We have shown recently that loss of TRPV1 - expressing afferent neurons eliminates presynaptic μopioid receptors present on TRPV1 - expressing afferent neurons, but paradoxically potentiates the analgesic potency and duration of μopioid agonists. In this study, we examined if removal of TRPV1 - expressing afferent neurons influences the development of opioid tolerance. Morphine tolerance was induced by daily intrathecal injection of 10 μg of morphine for 10 days or by subcutaneous implantation of a morphine (75 mg) pellet. The development of morphine tolerance was measured by daily testing the paw withdrawal threshold in response to a mechanical noxious stimulus applied to the hindpaw of rats treated with RTX or the vehicle. Loss of TRPV1 - immunoreactivity was confirmed in the dorsal root ganglia and spinal cord dorsal horn in RTX - treated rats. In vehicle - treated rats, the effect of intrathecal or systemic morphine on the paw withdrawal threshold was gradually diminished within 7 days. We found that the antinociceptive effect produced by intrathecal and systemic morphine remained significantly in RTX - treated rats at the time the morphine analgesic effect was lost in vehicle - treated rats. Thus, this study demonstrates that loss of TRPV1 - expressing sensory neurons attenuates the development of morphine tolerance. These data suggest that the biochemical pathways responsible for the termination (receptor desensitization, internalization, and sequestration) of the μopioid actions may be different between TRPV1 - and non - TRPV1 - nociceptive sensory neurons.

Supported by NIH grants GM64830 and NS45602.

P060007

Effects of nonspecific HCN channel blocker CsCl on learning and memory in rats

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AIM To study the effects of CsCl on synaptic plasticity, spatial learning and memory. METHODS Rats were injected intraperitoneally with CsCl (120 mg/kg, once per day for 30 d), Morris water maze was used to measure spatial memory performance, the evoked population spike (PS) was recorded in hippocampus CA3 region in vivo. Electron microscopy was applied to explore ultrastructural pathologic features of CA3 region, and high performance liquid chromatography (HPLC) with fluorescence detection was used to measure the content of glutamate in hippocampus. RESULTS CsCl resulted in spatial learning and memory impairment, inhibited the induction of long - term potentiation (LTP); the synaptic vesicles were decreased after high frequency stimulation (HFS) compared with Saline group. The content of glutamate in saline group was increased in hippocampus after HFS, but CsCl could decreased it. CONCLUSION CsCl decreased glutamate release, inhibited the induction of LTP, and impaired the spatial learning and memory of rats.

KEY WORDS: CsCl; LTP; Glutamate;

ACKNOWLEDGEMENT This work was supported by Natural Science Foundation of China (No.30371639).

P060008

Investigation of anxiolytic effects of the hydro - alcoholic extract of Mentha Pipereta in Mice

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Previous studies indicated that extracts of the aerial parts (leaf and stem) of Mentha Pipereta (MP) have sedative effects. This study was designed to evaluate anxiolytic effects in different doses of the hydro alcoholic extracts of MP in mice. In this study, fifty male albino mice (25 - 30 gr) were used (n = 50). Also we used of model of Elevated Plus Maze (EPM) for assessment of anxiety. Hydro alcoholic extracts of MP (50, 100, 200 and 500 mg/kg) or saline (10 ml/kg) were injected IP 30 min before of test. At the first time for increasing activity animals have put inside the black wall box for 5 min. Then animal transfer to the EPM and evaluation their anxiety reaction that including of number and percent of time spent in open arm. Results indicated that injection of extract in doses of 50, 100 and 200 reduced of reaction anxiety and with compare to saline group in the test group animals have more number of entrances and spent more percent time in open arm ($P < 0.05$). Whenever doses 500 was not significantly effects. It is concluded that the extract of MP plays an important role in fear and anxiety and hypnotic which is related to dose.

P060009

ASSESSMENT OF FORCED - SWIM AND TAIL SUSPENSION TESTS IN JUVENILE RATS AS MODELS OF ANTIDEPRESSANT DRUG EFFICACY FOR CHILDHOOD AND ADOLESCENT DEPRESSION

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A valid and reliable behavioral model of juvenile depression would be useful to develop specific treatments for childhood and adolescent depression. We tested day 21 male Sprague - Dawley rats in the forced - swim (FST) and the tail suspension (TST) tests. Animals received IP injections of either saline or antidepressant 24, 6, and 1 h prior to the behavioral tests. In the FST rats treated with selective serotonin reuptake inhibitors (SSRIs) showed a decrease in immobility and an increase in swimming behavior. Rats treated with tricyclic antidepressants (TCAs), displayed no decrease in immobility compared to saline controls. This is in contrast to TCA treated adults which display a decrease in immobility and increase in climbing behavior. In the TST rats treated with a SSRI or TCA exhibit a decrease in immobility compared to controls. In conclusion, for day 21 rats, drug treatment with an SSRI is effective for reversing behavioral despair for both the FST and TST models of depression. Drug treatment with a TCA is only effective for reversing behavioral despair in the TST. The drug response in the FST models the response of children and adolescents to TCA and SSRI treatment.

Support: MH66959 (DBB); HFF (HKH).

P060010

Down - regulation of noradrenaline transporter induced by chronic desipramine and the counteraction by coadministration with local anesthetics.

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Involvement of chronic inhibition of monoamine transporter in the brain with respect to sensitization to cocaine (Co) - and local anesthetics - induced seizures was studied in mice. Daily administration of desipramine (DM) which is an inhibitor of noradrenaline transporter (NET) for 5 days decreased locomotor activity induced by methamphetamine, increased the incidence of appearance of lidocaine (LC) - induced convulsions and decreased that of Co - induced convulsions. These changes induced by repeated administration of DM were reversed by co - administration of LC with DM. [^{3}H] noradrenaline (NA) uptake into hippocampus region isolated from chronic DM treated mice was significantly decreased and the decrease in NA uptake was reversed by co - administration of LC with DM. Daily treatment of Co increased [^{3}H] NA uptake into hippocampus. These results

suggest that down-regulation of hippocampal NET induced by chronic administration of DM may be relevant to DM-induced sensitization of LC convulsions. Inhibition of Na^+ channels by local anesthetics may regulate DM-induced down-regulation of NET function.

Noradrenaline transporter, desipramine, local anesthetics

P060011

Effects of Environmental Estrogenic Pollutants on Catecholamine Biosynthesis

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Environmental estrogenic pollutants are compounds that have estrogenic effects on fetal reproductive systems. We report here the effects of environmental estrogenic pollutants on catecholamine synthesis in cultured bovine adrenal medullary cells. Treatment of cultured adrenal medullary cells with p-nonylphenol and bisphenol A at 10 nM for 3 days stimulated ^{14}C-catecholamine synthesis from [^{14}C]tyrosine and tyrosine hydroxylase activity, an effect that was not inhibited by ICI 182,780, an antagonist of estrogen receptors. Significant effects of pnonylphenol on ^{14}C-catecholamine synthesis were observed at 0.1 nM that is 45 times lower than that of the international regulatory standard (4.5 nM). Short-term treatment of cells with 10 nM p-nonylphenol for 5-10 min also activated tyrosine hydroxylase and mitogen-activated protein kinase (MAPK). These findings suggest that short-term and long-term treatment of cells with estrogenic pollutants at environmental concentrations stimulates catecholamine synthesis and MAPK through a nuclear estrogen receptor-independent pathway.

This research was supported by a Grant-in-Aid (17590111) for Scientific Research (C) from the MEXT.

P060012

Role of $GABA_B$ receptors in the control of synaptic inputs to spinal dorsal horn neurons in rat model of diabetic neuropathy

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We determined the effect of the $GABA_B$ receptor agonist baclofen on the glutamatergic and GABAergic/glycinergic inputs to spinal dorsal horn neurons in a rat model of diabetic neuropathy using wholecell voltage-clamp recordings in spinal lamina II neurons in this study. The effect of baclofen (1-50 μM) on the frequency of glutamatergic EPSCs evoked from the dorsal root was significantly reduced in diabetic compared to control rats. The basal frequency of mEPSCs was significantly higher in diabetic than contrd rats, but baclofen had a similar inhibitory effect on mEPSCs in both groups. Also, baclofen similarly inhibited spontaneous GABAergic and glycinergic IPSCs in both control and diabetic rats. Interestingly, the basal frequency of GABAergic mIPSCs was significantly elevated, while that of glycinergic mIPSCs was significantly decreased in diabetic than control rats. Baclofen inhibited the frequency of GABAergic and Glycinergic mIPSCs in both control and diabetic groups. These data suggest that the $GABA_B$ receptor function at primary afferent terminals is decreased in the spinal cord of diabetic rats.

key words: $GABA_B$ receptors; Neuropathic pain; Spinal cord; Dorsal horn neuron

P060013

Effect of ANEPIII, a novel recombinant neurotoxic polypeptide, on Sodium currents in primary cultured rat hippocampal and neocortical neurons

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The effects of ANEPIII, a novel recombinant neurotoxic polypeptide originally from Buthus martensi Karsch, on sodium currents were studied in primary cultured rat hippocampal and neocortical neurons by using the whole-cell patch clamp recording techniques. ANEPIII decreased the sodium currents in a voltage-dependent manner, which appeared as a shift of the current-voltage relation to positive potentials. The effect was reversible after washing. The concentration-responsiveness measured in hippocampal and neocortical neurons revealed an IC_{50} value of 214.76 nM and 124.57 nM at a potential of -30 mV and -20 mV, respectively. For the different types of neurons, the shift of the current-voltage relation was distinct and was 9.7 mV in hippocampal neurons, and 5.7 mV in neocortical cells with 1000 nM ANEPIII. Furthermore, the time constant for recovery from inactivation was also prolonged by 1000 nM ANEPIII. Taken together, our results demonstrated that ANEPIII in submicromolar concentration was a voltage-dependent, reversible blocker of sodium currents in hippocampal and neocortical neurons, which, at least in part, contributed to the anti-neuroexcitatory properties of this peptide.

Keywords: ANEPIII; Antineuroexcitatory; Sodium channel; Whole-cell clamp-patch

P060014

Effective Components Group of Traditional Chinese Medicine Prescription NaoDeSheng Protects Against Rat Focal Cerebral Ischemia

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The purpose of this study was to investigate the effects of the Effective Components Group (ECG) of NaoDeSheng (NDS) on permanent cerebral ischemia-induced brain injury in rats. Male Sprague-Dawley rats were subjected to a permanent middle cerebral artery occlusion (pMCAO), and then were randomly assigned to one of the following treatment conditions: Nimodipine (0.012 g/kg), Naodesheng tablet (1.075 g/kg), total extracts (0.23 g/kg), ECG high dose (0.07 g/kg), ECG middle dos (0.02 g/kg), ECG low dose (0.007 g/kg), or vehicle. Treatment was initiated immediately at 2 h after the occlusion of the middle cerebral artery and repeated at 4, 24 h (experiment 1), or this treatment was continued for the following 7 days (experiment 2) as a daily oral administration. Infarction size and water content in the brain were evaluated at 26 h after pMCAO (experiment 1). Alterations in the neurological deficits, oxidative stress and apoptosis were measured at 7 days post-stroke (experiment 2). The results revealed that ECG could reduce ischemia-induced brain injury significantly, which was associated with its roles in extenuating the oxidative stress and the occurrence of apoptosis. Combined with previous results, all of these data suggest that the ECG of NDS could attenuate stroke-induced impairments, which reflects that effective components group-guided methodology is a feasible tool to improve the neuoroprotective properties of Traditional Chinese Medicine prescription NDS in rat focal cerebral ischemia.

Key words: ECG; NDS; ischemia; neuroprotection; apoptosis

P060015

Reduction of Neuro 2a cell apoptosis by lactate acid pre-treatment in hypoxia-ischemia/reoxygenation

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Lactate has been considered for many years to be a useless and harmful end-product of anaerobic glycolysis. Recently, large numbers of reports have demonstrated that lactate showed significantly a role of recovery of neurons function after hypoxia-ischemia in vivo and in vitro. However, the underlying mechanism is not clear, and the effects of lactate on neuronal apoptosis have not been known well. The aim of this study was to investigate the effects of lactate on Neuro 2a cell apoptosis in 4 hours of hypoxia-ischemia followed by 24 h of reoxygenation model. Notably, pre-treatment with lactate during hypoxia and reoxygenation increased Neuro 2a cell viability assessed by MTT from 75.37% to 98.07% ($p < 0.05$) consistent with decreased levels of lactate dehydrogenase (LDH) release from 42.08% to 21.31% ($p < 0.01$). Flow cytometric analysis revealed that Neuro 2a cell apoptosis rat reduced from 24.92% to 16.56% ($p < 0.05$) in a dose-dependent manner at concentrations ranging from 5 to 15 mM. It is concluded that lactate may play an important role in recovery of neurons function through preventing neuronal apoptosis due to hypoxia-ischemic brain injury.

P060016

Effects of vigabatrin on absence-like seizures and tonic convulsions in spontaneously epileptic rats

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To study the effects of vigabatrin on absence-like seizures and tonic convulsions in spontaneously epileptic rats (SERs). METHODS Electrcorticogram and depth electroencephalographic activity in hippocampus of SERs were recorded with implanted electrodes after administration of vigabatrin. RESULTS The number of absencelike seizures was significantly reduced from 100% to (54.5)%, (41.9)% and (34.4)% ($P < 0.01$, compared with control) at 3 h, 4 h, 5 h after

vigabatrin (100 mg·g^{-1}) administration. When vigabatrin was administered at a dose of 250 mg·g^{-1}, the frequency of tonic convulsions also significantly decreased from 100 % to (68.13) %, (39.13) % and (21.6) %, respectively ($P < 0.01$, compared with control). The inhibitory effects of vigabatrin on tonic convulsions could be antagonized by bicuculline, a GABA(A) receptor antagonist. CONCLUSION Vigabatrin is effective for treatment of absence-like seizures and tonic convulsions in SERs.

Key words: vigabatrin; spontaneously epileptic rat; absence-like seizures; tonic convulsions

P060017

Effects of aspirin on apoptosis of neurocytes and expression of HSP70 after cerebral ischemia-reperfusion in rats with different decapitate time-point

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OBJECTIVE This study, we design to investigate the protective effects of aspirin (ASA) on neurocytes after cerebral ischemia-reperfusion injury (CIRI) in rats for 24 h or 72 h. METHOD Right middle cerebral artery was occluded by inserting a thread through internal carotid artery for 2 h, and then reperfused. 60 mg·kg^{-1} doses of aspirin were ig administrated at reperfusion 0 h and 6 h. The brain injured area was estimated by TTC staining. Apoptosis of neurocytes were detected by TUNEL method. Immunohistochemical staining method was used for HSP70 detection in brain tissue. RESULTS After CIRI 24 h, the brain injured area, apoptosis of neurocytes, and expression of HSP70 were significently increased. With use of ASA, the brain injured area, and apoptosis of neurocytes were dramatically reduced, no significant difference in expression of HSP70 was discovered. After CIRI 72 h, compare with 24 h, all of them were reduced. With use of ASA, the brain injured area, and apoptosis of neurocytes were significantly reduced, no expression of HSP70 was discovered in brain tissue. CONCLUSION ASA improved the brain injury after CIRI either 24 h or 72 h by inhibited stress reaction and reduced apoptosis.

P060018

Two families of mGluR5 allosteric potentiators act through binding to two distinct sites of the receptor.

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Allosteric potentiators of metabotropic glutamate receptor subtype 5 (mGluR5) have been predicted as novel antipsychotic and cognition-enhancing reagents. Three families of mGluR5 allosteric potentiators have been discovered from distinct structural families: DFB, CPPHA and CDPPB. Little is known about the action sites of these compounds. MPEP is a well-characterized antagonist of mGluR5. Previous studies indicate the binding of a MPEP site ligand is displaced by DFB and CDPPB but not CPPHA. Here we show the potencies of CDPPB family compounds as mGluR5 potentiators are closely correlated with their affinities at the MPEP binding site. In addition, Schild analysis suggests that a MPEP site ligand antagonizes potentiation effects of a CDPPB analog competitively but blocks CPPHA effects non-competitively. A point mutation that eliminates MPEP binding also disrupts potentiation by CDPPB related allosteric potentiators but not CPPHA. Meanwhile, we have also identified mutations that reduce CPPHA elicited potentiation but not CDPPB's. Together, these data suggest that CDPPB and related compounds act at the MPEP site, while CPPHA acts through a distinct site.

P060019

Molecular cloning and expression patterns of zebrafish receptor protein tyrosine phosphatase σ

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Recent researches indicate that DLAR, a receptor protein tyrosine phosphatase (RPTP), regulates active zone formation at the neuromuscular junction in Drosophlia. However, in vertebrate, functions of three homologous RPTPs (LAR, PTPδ and PTPσ) in synapse formation in vivo remain to be elucidated. To investigate the role of RPTPs in synapse formation in living zebrafish embryos, we characterized zebrafish PTPσ. Database search for zebrafish PTPσ genes at the Ensembl Zebrafish Genome Server website revealed the presence of two zebrafish counterparts of mouse PTPσ designated as PTPδa and PTPδb. Using a cDNA library prepared from adult zebrafish brain, we cloned the entire coding sequence of zebrafish PTPδa by RT-PCR and 5' and 3' RACE. Deduced amino acid sequence of zebrafish PTPδa shared 62%, 66% and 70% identity with mouse LAR, PTPδ and PTPσ, respectively. In situ hybridization analyses showed that the PTPδa mRNA was expressed widely in the nervous system of developing zebrafish embryos including the olfactory placode. Microinjection of olfactory neuron specific double-cassette vectors for dominant negative PTPδa and synaptic markers will reveal the role of PTPσ in synapse formation in vivo.

P060020

The Protective Effect of Taurine on Reperfusion Injury after Focal Cerebral Ischemia in Rats

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Aim: To investigate the protective effects and mechanisms of taurine on cerebral injury induced by brain schemia/reperfusion in rats. Methods: A middle cerebral artery occlusion model was established in Wistar rats before they were divided into three groups: sham group, ischemia-reperfusion (I/R) group and I/R with taurine treatment group. After ischemia 1 hour and reperfusion 24 hours in each model, the change in cerebral infarct volume, water content, pathologic alteration in brain tissues and the expression of proteins were determined. Results: The cerebral infarct volume percentage was 0, (13.32±3.18) %, (9.21±2.24) % in three groups respectively and it was reduced by 30.83 % in taurine treatment group compared with pure I/R group ($P < 0.05$). The brain water content was also notably decreased in taurine treatment group ($P < 0.05$). Likely, the ischemic neuronal damage was relieved and the expression of Cytochrome C、Bax and NF-κB protein were downregulated with taurine treatment while the expression of Bcl-2 was up-regulated. Conclusions: Taurine has a neuroprotective effect on reperfusion injury after focal cerebral ischemia in rats.

P060021

Effects of Stilbene-glycoside on Learning and Memory Function, inflammation chang and expression of Glycogen synthase kinase 3β of brain in Dementia Model Mice

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Object To observe the effect of 2,3,5,4'-tetrahydroxy stilbene-2-β-D-glycoside (TSG) on learning and memory ability, content of interleukin-6 and expression of Glycogen synthase kinase 3β in dementia model mice induced by β-amyloid (Aβ). Method The model group was administered Aβ1-40 into the right lateral ventricle, and the therapy group were administered TSG for 8 weeks by gastrogavage. All the mice of different groups were tested with Morris water maze and step-through test. Then the mice were killed and the radioimmunoassay was used to assay the content of interleukin-6, and the expression of GSK3β was determined with immunohistochemistry method. Results The model mice showed worse ability in learning and memory compared to control mice. The cortical IL-6 cotent increased and the expression of GSK3β increased in model mice compared to normal control; While TSG improved the learning and memory disability of model mice, reduced cortex IL-6 content and expression of GSK3β. Conclusions TSG could improve the learning and memory disability of model mice, decrease cortex IL-6 content and expression of GSK3β, suggesting that TSG may have a promising application prospect in treatment of dementia disease such as AD.

Key words: 2,3,5,4'-tetrahydroxy stilbene-2-β-D-glycoside, Alzheimer's disease, β-amyloid,, Dementia, Learning and Memory, Alzheimer'disease

P060022

Effects of AST and AS-I on memory loss of mice induced by hydrocortisone

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To explore the effects and their mechanisms of Astragalosides (AST) and Astragals Saponin I (AS-I) on memory loss of senescent mice induced by hydrocortisone. Rotating rod test and step-down type passive avoidance test were performed to determine the effects of AST and AS-I on memory loss of senescent mice treated Hydrocortisone. Electron microscope was used to observe the ultra-

microstructure of thymus and dorsal hippocampus neurons. The study showed that Hydrocortisone induced obvious memory impairment of senescent mice accompanied with atrophy of the thymus and hippocampus. AST and AS-I was shown to antagonize HC-induced atrophy of thymus and hippocampus of 20-month mice, as well as to restore their impairment of memory, indicating that AST and AS-I have protective effect on HC induced atrophy of thymus and hippocampus of senescent mice which was related to its improvement of brain function and immunomodulatory effects.

Key Words: AST, AS-I, Hydrocortisone
Acknowledgments: Supported by the Nature Science Foundation of Anhui Province (No. 00144414), the "Shiwu" Technology Special purpose Foundation of Anhui Province (No. 01803016) and Department of Anhui Province Education (No:2005hbz18)

P060023

Repeated L-DOPA treatment increases c-fos and BDNF mRNAs in the subthalamic nucleus in the 6-OHDA rat model of Parkinson's disease

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The subthalamic nucleus is, together with striatum, a major input region of the basal ganglia and is dysfunctional in Parkinson's disease. This study used the unilateral 6-OHDA rat model of Parkinson's disease to examine effects of single and repeated injections with L-DOPA on the levels of two activity-dependent genes, c-fos and BDNF, in the subthalamic nucleus and, for comparison, in striatum. No differences in the expression of c-fos or BDNF mRNAs in the subthalamic nucleus or striatum were found in saline-treated rats. In rats treated with a single injection of L-DOPA, the only significant effect was an induction of c-fos in the dopamine-depleted striatum. Repeated L-DOPA treatment increased c-fos as well as BDNF in the dopamine-depleted subthalamic nucleus. This treatment also increased c-fos expression in striatum. It is concluded that repeated treatment with L-DOPA strongly elevated c-fos and BDNF mRNA levels in the subthalamic nucleus. These molecular adaptations may reflect changes in neuronal plasticity and efficacy that underlie some therapeutic actions and/or side-effects of L-DOPA in Parkinson's disease.

Key words: c-fos, BDNF, subthalamic nucleus, Parkinson's disease

P060024

Does Nicotine/Tobacco Smoking Release Dopamine in Human Brain Nucleus Accumbens?

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The dopamine (DA) hypothesis of brain reward involving nucleus accumbens (Nac) is widely accepted. Nicotine has been shown by many to release DA in rodent Nac (see Di Chiara and Imperato, 1988; Zocchi et al., 2003). Acute nicotine releases DA mostly in the Nac shell, and in pretreated animals in the core (Benwell and Balfour, 1992; Cadoni and Di Chiara, 2000; Nisell et al, 1997) found preferential DA release in Nac shell after acute and chronic administration. Data from our research using PET methods for monkeys and humans indicate that nicotine/tobacco smoking produces a relatively small release of brain DA. Furthermore, the precise location of DA release measured indirectly with displacement of [^{11}C]raclopride in overnight abstinent smokers who smoke average nicotine cigarettes in the ventral striatum. The data to support this conclusion are the subject of this report. Tobacco smokers are exposed to daily nicotine for years, whereas daily nicotine exposure of rodents is usually for weeks (Malin, 2001). Brain neurotransmitter systems have less time to adapt to nicotine exposure in such animal studies compared to tobacco smoker studies.

Key Words: Nicotine, Tobacco, Dopamine, Release

P060025

Gamma-vinyl GABA inhibits cocaine-primed relapse by a DA-independent mechanism.

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It was reported that gamma-vinyl GABA (GVG), an irreversible GABA transaminase inhibitor, inhibits the acute rewarding effects of cocaine. In the present study, we investigated whether and how GVG inhibits cocaine-primed relapse in rats. Systemic administration of GVG (2.5-300 mg/kg i.p.) dose-dependently inhibited cocaine-primed relapse. However, the mechanism appears to be DA-independent, because GVG pretreatment failed to block cocaine-induced increases in extracellular dopamine (DA) in the accumbens. GVG alone also failed to alter extraellular DA. In contrast, GVG pretreatment produced an additive or synergistic increase with cocaine on extracellular glutamate, and dose-dependently elevated extracellular GABA levels. Finally, GVG-induced increase in glutamate is tetrodotoxin-dependent, while GVG-induced increases in GABA was partially blocked by blockade of type 1 GABA transporters. Together, the present study, for the first time, demonstrates that GVG inhibits cocaine-primed relapse by a mechanism correlated to GVG-induced increase in GABA and/or glutamate, but not to a decrease in cocaine-induced increase in DA.

Key Words: gamma-vinyl GABA, cocaine, dopamine

P060026

Parkinson's disease model in vitro establishment by overexpressing rat c-Jun N-terminal Kinase in SH-SY5Y

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Objective: More and more evidences suggest that c-Jun N-terminal kinase (JNK) pathway is activated in Parkinson's disease (PD). JNK3, largely restricted to brain, is a subtype of MAPK family and its phosphorylation can result in neuron apoptosis. Here we established a kind of model via overexpressed rat JNK3 in SH-SY5Y (SH-SY5Y-rJNK3) to find or study candidate for PD. Methods: The pc-DNA3.1-his/C-rJNK3 vector was established and stably transfected into SH-SY5Y overexpressing JNK3. SH-SY5Y-rJNK3 was selected by Western blotting analysis. Then the growth rate and the sensitivity to MPP+ of SH-SY5Y-rJNK3 were further evaluated by morphological observation and MTT assay. Results: There were morphological differences between SH-SY5Y and SH-SY5Y-rJNK3. The result of MTT showed that there were little differences between growth rate of both. Stimulated by MPP+, the SH-SY5Y-rJNK3 made more morphological changes in 100 μM MPP+ than SH-SY5Y, and the results of MTT also demonstrated that SH-SY5Y-rJNK3 was more sensitive to MPP+ compared to SH-SY5Y with lower cell viability.

Key words: Parkinson's disease JNK3 MTT MPP+
Acknowledgements: This work was funded by the National Basic Research 973 Program Grant of China (2004CB518906).

P060027

The Effects of Sophoridine on the Positive Cells Glu and GABA Immunoreaction in CNS of Rats

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Abstract: objective: To study the effects of sophoridine on the positive cells Glu and GABA immunoreaction in cortex and hippocampus of rats and the mechanism of its central pharmacological effects. Method Immunohistochemistry method and micrographic analysis technique were employed to monitor the effect of SR on the alterations of positive cells Glu and GABA immunoreaction n cortex and hippocampus of rats. Result SR administrated icv (0.2 mg/rat) surprisingly increased the number of positive cells Glu immunoreaction but decreased the number of positive cells GABA immunoreaction ($P<0.05$, $P<0.01$). Conclusion The expression imbalance of Glu and GABA in CNS caused by SR may be one of the mechanisms in which LSR leads the effects of excitation in CNS.

key words: sophoridine (SR); glutamase (Glu); gammaaminobutyric acid (GABA); central nervous system (CNS); rats

P060028

Melatonin improves the viability of 293T cells stably expressing hyperphosphorylated tau

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Hyperphosphorylated tau is the predominant protein component of Alzheimer's paired helical filaments (PHFs) and neurofibrillary tangles (NFTs). To investigate whether melatonin prevents cells from the damage caused by hyperphosphorylated tau, 293 cells were used to stably overexpress EGFP-tau and okadaic acid (OA), a protein phosphatase inhibitor, was used to induce tau hyperphosphorylation. Cell viability was determined by MTT assay. It was found that the morphology of cells appeared round and the viability of cells decreased after exposure

to OA (100 nM) for 4 h. The viability of cells was restored to the normal after treatment with melatonin at the concentration of 10-4 mol/L for 24 h. However, the morphology of cells was not improved by melatonin during the period of observation. This suggests that melatonin has protective effect on the viability of cells stably overexpressing hyperphosphorylated tau.

Key words: tau; melatonin; AD

P060029

The expression of type IIIN voltage-gated sodium channel is up regulated in spontaneously epileptic rat brain hippocampus

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AIM To investigate the expression of mRNA coding voltage-gated sodium channel (VGSC) α-subunits in spontaneously epileptic rats (SERs). METHODS Total RNA were extracted from neocortex, dentate gyrus, CA1 and CA3 hippocampus, and all types of VGSC α-subunits were obtained by PCR A protocol after reverse transcription. The mRNA expression detections of type I, IIA, IIN, IIIA and IIIN VGSC α-subunits were operated respectively after PCR B, C and D. RESULTS All types of VGSC α-subunits in SERs expressed a little higher than those in control group in neocortex, dentate gyrus, CA1 and CA3 of hippocampus but had no significant difference ($P > 0.05$). Relative proportion of VGSC α-subunits I, IIand III mRNAs in adult brain areas had also no significant difference ($P > 0.05$) between the control rats and SERs. However, restriction map analysis showed that IIIN increased significantly in SERs than that in control group ($P < 0.01$). CONCLUSION The expression of type IIIN VGSC α-subunits was up-regulated in SERs brain hippocampus.

Key words: mRNA; sodium channel; α-subunit; spontaneously epileptic rat

P060030

Effecet of Morphine on Deep Dorsal Horn Projection Neurons Depends on Spinal GABAergic and Glycinergic Tone: Implications for Reduced Opioid Effect in Neuropathic Pain

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The μopioid agonist morphine has distinct effects on spinal dorsal horn neurons in the superficial and deep laminae. However, it is not clear if the inhibitory effect of morphine on dorsal horn projection neurons is secondary to its potentiating effect on inhibitory interneurons. In this study, we tested the hypothesis that removal of GABAergic and glycinergic inhibitory inputs attenuates the effect of morphine on dorsal horn projection neurons and the reduced spinal GABAergic tone contributes to attenuated morphine effect in neuropathic pain. Single-unit activity of deep dorsal horn projection neurons was recorded in anesthetized normal/sham controls and L5 and L6 spinal nerve-ligated rats. Spinal application of 10 μM morphine significantly inhibited the evoked responses of dorsal horn neurons in both normal/sham controls, and this effect was abolished by the specific μ opioid antagonist. However, the effect of morphine on dorsal horn projection neurons was significantly reduced in nerve-injured rats. Furthermore, topical application of the $GABA_A$ receptor antagonist bicuculline (20 μM) almost abolished the effect of morphine in normal/sham control rats but did not significantly attenuate the morphine effect in nerve-injured rats. On the other hand, the glycine receptor antagonist strychnine (4 μM) significantly decreased the effect of morphine in both nerve-injured and control animals. These data suggest that the inhibitory effect of opioids on dorsal horn projection neurons depends on GABAergic and glycinergic inputs. Furthermore, reduced GABAergic tone probably contributes to diminished analgesic effect of opioids in neuropathic pain.

P060031

Effects of corticosterone on cytosolic adenylate kinase in the rat hippocampal neurons cultured in vitro

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An increasing number of studies are revealing that prolonged exposure to elevated glucocorticoid levels has been associated with deficits in learning, memory and retrieval. However, the mechanisms involved in this detrimental effect are not well understood. In this work, 5 days after treated with 10^{-5} M corticosterone, cytosolic adenylate kinase (AK1) activity in the rat hippocampal neurons cultured in vitro was determined by the method of High Performance Liquid Chromatography. AK1 levels and expression were also investigated by using immunoblotting and semi-quantitative reverse transcriptase-polymerase chain reaction, respectively. The results showed that 10^{-5} M corticosterone could decrease AK1 activity and levels, as well as downregulate AK1 mRNA levels in contrast to 10^{-7} M corticosterone. These data suggested that exposure to elevated glucocorticoid levels might induce a decrease of AK1 activity by downregulating mRNA levels, indicating that a balance of adenylates at ATP-consuming and ATP-generating intracellular sites might be destroyed. Based on these results, we hypothesized that an abnormity of energy balance might be a mechanism by which corticosterone treatments influence memory.

P060032

GABA mediated induction and maintenance of long-term potentiation(LTP) at perforant pathway (PP) fibers—hippocampus CA3 region synapse

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To investigate the role of GABA in the induction and maintence of long-term potentiation(LTP) at perforant pathway (PP) fibers—hippocampus CA3 region synapse, we examined the concentration of GABA in hippocampus at 90 min after the establishment of LTP by HPLC with fluorescence detection. Effects of GABA and $GABA_A$ receptor antagonist bicuculline methbromide (BMB) on LTP were observed. We found that (1) the content of GABA in LTP-induction rats obviously decreased ($P < 0.01$). (2) GABA 200 nmol at 5 min before tetanic stimulation (TS), the PS amplitudes were significantly decreased. This effect of GABA on LTP induction were attenuated by BMB. (3) GABA 200 nmol at 30 min after TS, the TS-induced LTP effects were completely reversed. This effect of GABA on LTP maintenance was attenuated by BMB. (4) BMB 1 nmol at 5 min before giving test stimulation, the PS amplitudes obviously increased and near to LTP-induction group. The results suggested that GABA mediated the induction and maintenance of LTP at PP fibers—CA3 region synapse.

Key words: GABA; CA3 region; long-term potentiation (LTP); hippocampus
Acknowledgement: Project supported by the National Natural Science Foundation of China (No 30371639)

P060033

Brasilein protects the brain against the focal cerebral ischemia reperfusion injury

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Brasilein (6a,7-dihydro-3,6a,10-trihydroxy-benz[b]indeno[1,2-d]pyran-9(6H)-one) is a compound obtained in a large amount from Caesalpinia sappan ethanol extracts with a high purity of about 98%. In the rat MCAO (Middle Cerebral Artery Occlusion) experiment, we found Brasilein(2.5 mg/kg) can reduce the brain infarction area by 31.7% ($P < 0.05$), which indicates that Brasilein is a potential therapeutic compound for acute stroke. In vitro experiments show that Brasilein can protect the Neu2a cells from the OGD (oxygen-glucose deprivation) injury. Brasilein can also suppress the nitric oxide release of macrophage RAW264.7 cells and murine microglial BV2 cells induced by lipopolysaccharide. Based on the above results, we will try to understand the mechanisms of this protective effect of Brasilein to cerebral ischemia reperfusion in the future.

P060034

New goal in ischaemia stroke therapy: rHu-EPO nasal application.

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The rHu-EPO neuroprotective actions have been broadly studied in experimental models. Clinical trials with satisfactory results have been carried out. In this paper, we showed rHu-EPO nasal application diminished the cerebral damage after ischemia in Mongolian gerbils, suggesting a new therapeutic alternative for neuroprotection in stroke. These results constitute the first report of rHu-EPO nasal drops arrival to the brain, having a neuroprotective effect, demonstrated by a significant improvement in behavior, motor activity, neurological condition, growth curve, cerebral edema decrease and with a hipocampo CA1 cell higher survival. The nasal way for stroke treatment offers the following advantages: a quick arrival to the lesion place; molecule arrival to poor or not irrigated areas of the CNS; elimination of surgical risks or other possible implications given by traumatic ways; alternative way of access to the brain without damaging it, and use for treatment

and/or vascular brain illness prevention.

Key words: Neuroprotection, Erythropoietin, nasal way, Mongolian gerbil, ischaemia brain.

P060035

Superoxide anion- and nitric oxide-associated mitochondrial dysfunction in neuronal apoptosis after spinal cord injury

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Activation of the mitochondria-dependent signaling cascades mediates apoptosis. Superoxide (O_2^-) and Nitric oxide (NO) are important factors leading to mitochondrial impairment. The present study delineated the roles of O_2^-, NO and mitochondria in the execution of neuronal apoptosis after spinal cord injury. A complete spinal cord transaction (SCT) at level of thoracic segment 8 of Sprague-Dawley rats resulted in DNA fragmentation. SCT also caused cytochrome c release and nuclear translocation of mitochondrial apoptosis inducing factor (AIF) in a temporal profile that was preceded by increase in O_2^- production and NO up-regulation. We also found that application of the superoxide dismutase mimetic, tempol, or NO scavenger, carboxy-PTIO, into the epicenter of the injured spinal cord significantly preserved the bioenergetic capability of the mitochondria, leading to inhibition of the SCT-induced apoptosis. Together these results suggest that after SCT, oxidative stress of the enhanced productions of O_2^- and NO caused reduction in the mitochondrial respiratory enzyme activities, leading to the mitochondrial-associated activation of both caspase-independent and caspasedependent neuronal apoptosis.

P060036

Establishment a PD associated model in vitro-α-synudein-overexpressing SH-SY5Y cells

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α-Synuclein is the primary component of Lewy's Body, which is the pathological hallmark of the Parkinson's disease (PD). PD is a multifactor-caused neurodegenerative movement disorder, and the mechanism of PD is vague. There are some genetic and environmental factors associated with PD. α-Synuclein has close relationship with PD and numberless researches have pay attention to its function in PD, but we still don not know whether α-synuclein is the causative agent or the result of other toxicant. In order to investigate relationship between α-synuclein and PD, Cells SH-SY5Y were transfected with pcDNA3.1-his/c-α-syn and selected with G418. The SH-SY5Y-α-synuclein cells lines were confirmed by Western-blot assay and immunofluorescence study. And we found SH-SY5Y-α-synuclein cells were more sensitive to the damage induced by MPP+, which indicated indirectly that the overexpressed α-synuclein was harmful to the SH-SY5Y cells. So the results offer a useful model similar to PD for research the mechanism of PD and selecting an available compound for treating PD.

Key Words: α-synuclein; PD; MPP+

Acknowledgement: This work was supported by 973 project (Grant No. 2004CB518906).

P060037

Substance P receptor expression in human skin keratinocyteS and fibroblasts

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Background: There is increasing evidence that neuropeptide, especially substance P (SP), may be involved in the pathogenesis of cutaneous allergic inflammation (CAI). Aim: This study was performed to investigate the expression of SP receptor (Neurokinin-1 receptor) in human epidermal keratinocytes and dermal fibroblasts and their potential influence in CAI. Methods: HaCaT cells, a human epidermal keratinocyte cell line, and dermal fibroblasts were cultured. The expression of NK-1 receptor protein was examined by immunohistochemistry technique, and the mRNA level was detected by semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR). The modulation of NK-1 receptor expression in HaCaT cells and fibroblasts was detected by flow cytometry and Western blotting analysis. Results: NK-1 receptor exists in HaCaT cells and fibroblasts. The expression of NK-1 receptor mRNA in fibroblasts was weaker than that in HaCaT cells. SP and IFN-γ significantly up-regulated the expression of NK-1 receptor. [D-Arg1, D-Trp7, 9 Leu11]-Substance P (Spantide I), a pan-specific NK-1 receptor antagonist, degraded the expression of NK-1 receptor stimulated by SP. Conclusions: HaCaT cells and fibroblasts can express NK-1 receptor at protein and transcription levels, and the expression was modulated by SP, IFN-γ and Spantide I. That indicated the keratinocytes and fibroblasts were involved in the regulation of skin immune and the NK-1 receptor may play an important role in the pathogenesis of cutaneous allergic inflammation.

Key words: Substance P receptor; HaCaT cell line; fibroblast; cutaneous allergic inflammation

Acknowledgements: We thank Dr. Q.S. Mi and Prof. J. Gu for kindly providing the HaCaT cell lines. This work was supported by the Natural Science Foundation of China (Grants No. 30271553 and No. 30572269).

P060038

Intrathecal nicotine has the analgesic effect on the tibial nerve transection (TNT)-induced neuropathic pain

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Since it has been indicated that stimulation of nicotinic acetylcholine receptors (nAChRs) induce an antinociceptive action, we attempted to characterize the action of nicotine at spinal level on the mechanical allodynia in a neuropathic pain model developed by tibial nerve transection in this study. We found that intrathecal nicotine, RJR-2403, a selective α4β2 nAChR agonist, and choline, a selective α7 nAChR agonist, produced analgesic effects on the nerve injury-induced allodynia. This action of nicotine was significantly suppressed by intrathecal pretreatment of a non-selective nicotinic antagonist mecamylamine, a selective α4β2 nAChR antagonist dihydro-β-erythroidine or a selective α7 nAChR antagonist methyllycaconitine. Pretreatment of intrathecal strychnine, a glycine receptor antagonist, blocked the antinociception induced by nicotine. These results suggest α4β2 and α7 nAChR systems via enhancing glycinergic neuron in spinal level exert the inhibition of nociceptive transduction in neuropathic pain.

Keywords; nicotine, allodynia, nicotinic acetylcholine receptors, glycine

P060039

Effect of cerebral ischemia on brain mast cells in vivo and in vitro

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The purpose of this study was to investigate the effect of cerebral ischemia on mast cells. The number of thalamic mast cell in rats decreased significantly at 1 h, 2 h, 4 h and 7 d after transient cerebral ischemia except at 1 d when the number just increased to the same level of sham control groups. However, at 1 d following ischemia the number of mast cell in the middle aspect of the thalamus increased which was twice as that of other regions in the thalamus. Histamine contents increased significantly in the thalamus and striatum after ischemia. In in vitro ischemia, mast cells were exposed to oxygen-glucose deprivation (OGD). From OGD 2 h, the degranulation percentage of mast cell increased and showed a progressive further increase, associated with a similar change in lactate dehydrogenase (LDH) release. The histamine release was elevated significantly from 1 h of OGD exposure. These results indicate that brain mast cells may participate in the pathological process after cerebral ischemia.

Key words: Cerebral ischemia, Histamine, Mast cell

This project was supported by the National Natural Science Foundation of China (30371638, 30472013, 30572176)

P060040

Antioxidants in Stroke: Increased NADPH Oxidase Expression and Superoxide Generation after Endothelin-1-Induced Stroke in Conscious Rats

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Oxidative stress causes the progression of brain injury after ischaemic stroke. We found that potent antioxidant flavonols, given after stroke, reduce cerebral infarct size and improve recovery of neurological function in rats. We have now investi-

gated the function of NADPH oxidase, an important source of superoxide in artery disease, in the brain after stroke. We examined mRNA expression of the crucial NADPH subunits, Nox1, Nox2 and Nox4 after endothelin-induced vasoconstriction of the middle cerebral artery in conscious rats. Superoxide was detected in situ with dihydroethidium (DHE) fluorescence. From 0.25 to 7 days after stroke, Nox2 increased markedly more in the ipsilateral cortex and striatum than the contralateral side. In contrast, Nox4 increased only transiently in the cortex at 6 h, and Nox1 did not change throughout. DHE fluorescence decreased in the ischaemic core at 24 h in both cortex (41 ± 2 %) and striatum (43 ± 3 %), but it increased in the ischaemic penumbra, partly in inflammatory cells. Thus the transient increase in Nox4 in ischaemic and penumbral regions may trigger progressive oxidative brain damage and may be a target for rescue after stroke.

Keywords: NADPH oxidase stroke superoxide

P060041

Protective effect of carnosine on NMDA-induced neurotoxicity in differentiated rat PC12 cells

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The role of carnosine in excitotoxic neuronal cell death was investigated in differentiated PC12 cells. MTT assay, Hoechst 33342 and propidium iodide staining showed that carnosine suppressed excitotoxic neuronal injuries in time- and concentration-related manners. The effect of carnosine was antagonized by H_1 antagonist pyrilamine, but not by H_2 antagonists cimetidine. Carnosine produced no appreciable effect on histidine decarboxylase (HDC), and increased both synthesis and release of histidine and histamine. However, alphafluoromethylhistidine, an HDC inhibitor, only partially reversed the protection of carnosine on neuronal cell death and histamine level. Additionally, carnosine decreased glutamate release secondary to NMDA insult. These results indicate that carnosine can effectively protect against NMDA-induced neurotoxicity in PC12 cells, and its protection may be due to the activation of histamine H_1-receptors via two different mechanisms, one being carnosine's direct action, and the other being indirectly mediated by histaminergic pathway.

Keywords: Carnosine; Histamine; NMDA; Neurotoxicity

This Project was supported by the National Natural Science Foundation of China (30371638, 30572176).

P060042

Cerebrovascular and serotoninergic mechanisms of antimigraine drugs

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It is known that the vascular factors play an important role in migraine pathogenesis, it appeared interesting, therefore, to evaluate the role of serotonergic system in the cerebrovascular action of the antimigraine drugs. The regional and local cerebral blood flow was recorded using the ultrasonic flowmeter and the laser Doppler flowmeter. The antiserotonin cerebrovascular action was demonstrated not only by methysergide and dihydroergotamine, but also by nicergoline, and less stronger one was produced by propranolol and tofenamic acid. The novel antagonist of serotonin tropoxin was shown to completely eliminate or significantly reduce the constrictory action of serotonin on the cerebral vessels of intact and ischemised animals and acts as blocker of brain 5HT$_2$-receptors. The antimigraine drug and agonist of 5HT1B/D-receptors sumatriptan in most experiments increased the constrictory action of serotonin upon the cerebral vessels. Along with that, sumatriptan strongly increased the cerebral circulation and was not inferior to nimodipine and pirrolidone in this respect.

Partial financial support of the Russian Foundation for Basic Research (projects No 04-04-48608 and No 05-04-08132) is acknowledged.

P060043

Lesion of the tuberomammillary nucleus attenuates postictal seizure protection in rats*

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To determine whether the tuberomammillary nucleus (TM) is involved in postictal seizure protection (PSP) in rats, we tested effects of bilateral electrolytic lesions of TM on intermittent maximal electroshock (MES)-induced seizures. In sham rats, intermittent MES resulted in PSP, with a progressive decrease in both seizure pattern score and duration of tonic fore- and hindlimb extension with each successive seizure. The TM lesions weakened PSP. α-Fluoromethylhistidine (100 μg) mimicked the TM lesion-induced attenuation of PSP. Neurochemical studies revealed that the TM lesions decreased basal histamine levels in the cortex, brainstem and hypothalamus, but had no significant effect on basal glutamate and GABA levels. Moreover, intermittent MES induced a persistent decrease of brain histamine levels in both sham and TM lesioned rats. These results indicate that the TM may function as an inhibitory neural substrate during the intermittent MES procedure and the intrinsic histaminergic system may play an important role in the mechanisms of PSP.

Keywords: epilepsy, histamine

* This Project was supported by the National Natural Science Foundation of China (30371638, 30572176).

P060044

Multiple actions of dimethylphytosphingosine and dimethylsphingosine in 1321N1 astrocytes

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N,N-Dimethyl-D-erythro-sphingosine (DMS) has inhibitory actions on protein kinase C and sphingosine kinase. In the present study, we investigated effects of DMS and dimethylphytos phingosine (DMPH) on human 1321N1 astrocytes. We determined variations of intracellular Ca^{2+} concentration and pH by fluorescence spectrophotometer using Fura-2 and BCECF, respectively. Both sphingolipids increased intracellular Ca^{2+} concentration and cytosolic pH significantly in a dose-dependent manner. Treatment of cells with DMPH and DMS for 24 h reduced viability of cells largely and concentration-dependently, as evaluated by MTT assay. Finally, in the experiment using [^{3}H] glutamate, DMPH and DMS inhibited glutamate uptake by 1321N1 astrocytes. In summary, DMPH and DMS increased intracellular Ca^{2+} concentration and pH, evoked cytotoxicity and inhibited glutamate uptake in 1321N1 astrocytes.

Keywords; dimethylphytosphingosine; dimethylsphingosine; glutamate uptake

This work was supported by a grant (R05-2004-000-10165-0) from Korea Research Foundation.

P060046

Roles of histamine receptors on NMDA-induced necrosis in cultured cortical neurons

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Using histamine receptor ligands, roles of histamine receptors on NMDA-induced necrosis was investigated in rat cultured cortical neurons. Within 3 hours of intense NMDA insults, most of neurons died by necrosis. Pretreatment with histamine reduced this injury, and which was antagonized by H_2 receptor antagonists cimetidine, not by H_1 receptor antagonists pyrilamine. The H_2 receptor agonist amthamine also produced protection, which was prevented by cimetidine not by pyrilamine. 8-Br-cAMP mimicked the protection. Additionally, the adenylyl cyclase inhibitor SQ-22536 and the PKA inhibitor H-89 reversed the protection of histamine. H_3 receptor antagonists thioperamide and clobenpropit attenuated the injury, which was antagonisted by H_3 receptor agonist and $GABA_A$ receptor antagonist, not by pyrilamine and cimetidine. Further study demonstrated that thioperamide and clobenpropit could increase GABA release, which was also inhibited by SQ-22536 and H-89. These results indicate both H_2 receptor/PKA and H_3 receptor/PKA/GABA release pathways participate in NMDA-induced necrosis.

Keywords: histamine; NMDA; necrosis

Supported by the National Natural Science Foundation of China (30371638, 30572176).

P060047

Low-frequency stimulation of piriform cortex, but not tuberomammillary nucleus inhibits amygdaloid kindling seizure in rats

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The present study was examined the effect of unilateral Low-frequency stimulation(LFS) of the central piriform cortex(cPC) and tuberomammillary nucleus (TM) on amygdaloid kindling seizure in rats. The ipsilateral or contralateral cPC received LFS(15 min train of 0.1 ms pulses at 1 Hz and 50-150uA) immediately after termination of once daily kindling stimulation in the amygdala. LFS of either the ipsilateral or contralateral cPC suppressed the progression of seizure stages and reduced afterdischarge duration. The suppression induced by LFS was due to the retardation of progression from stage 0 to stage 1 and stage 3 to stage 4 seizures. In addition, the suppressive effect of LFS did not disappear when the stimulation was stopped. However, LFS of TM produced no effect. These findings indicate that the unilateral LFS of the cPC may have an artiepileptogenic effect, and may be helpful for the exploring on effective and long-lasting therapies for human temporal lobe epilepsy.

Key words: Low-frequency stimulation; Piriform cortex

Supported by the National Natural Science Foundation of China (30371638, 30472013).

P060049

Promoting effects of beta-carotene from Dunaliella bardawil on learning and memory in mice and rats

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The effects of β-carotene (β-C) on learning and memory in mice and rats were studied by using stepdown test and Y-maze test. In mice, β-C (12.5, 25 and 50 mg/kg, ip) exerted markedly promoting effects on step-down test, and could remarkably antagonize the memory impairment induced by scopolamine (1 mg/kg, ip), and 20% alcohol (10 ml/kg, po). But β-C could not antagonize the impairment induced by sodium nitrite (120 mg/kg, sc). β-C also had significant effects on Y-maze tests. These results suggested that β-C, as an antioxidant, could improve the ability of learning and memory of mice and rats.

Key words: β-carotene; learning; memory; behavior, animal

Acknowledgements: This work was supported by Nation Science and Technology Ministry 86 Program (85-08-07).

P060050

Chronic morphine treatment-induced increment of P-glycoprotein activity via opioid-receptor independent mechanism

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P-glycoprotein (P-gp), one of the components of blood-brain barrier, is known to transport morphine at the luminal membrane of brain capillary endothelial cells. In this research, we determined the effect of chronic morphine treatment on the morphine distribution to the brain, and on the expression and activity of P-gp in mice. We found that brain content of morphine in chronic morphine treated mice was significantly lower than that in chronic saline treated mice, while there was no significant difference in blood concentration of morphine. In addition, the P-gp expression and the basal P-gp ATPase activity in chronic morphine treated mice were significantly higher than that in chronic saline treated mice. Furthermore, morphine concentration, which maximally activated the P-gp ATPase in vitro, was lower in chronic morphine treated mice than in chronic saline treated mice. Finally, the effect of chronic morphine treatment on P-gp expression was not inhibited by naloxone, an opioid receptor antagonist. These results suggest that chronic morphine treatment may modulate the function of P-gp via opioid-receptor independent mechanism.

Key words: chronic morphine, Pglycoprotein, blood-brain barrier

P060051

Effect of tetramethylpyrazine on neuropathic pain mediated by P2X3 receptor

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To investigate the effects of tetramethylpyrazine(TMP) on neuropathic pain induced by P2X3 receptor. Chronic constriction injury(CCI) model was adopted. SD rats(male, n=24) had been randomly divided into blank(Ⅰ), sham(Ⅱ), CCI+TMP(Ⅲ), and CCI(Ⅳ) group. Mechanical withdrawal threshold and thermal withdrawal latency were measured and P2X3 immunoreactivity in L4/L5 spinal cord was detected by immunohistochemistry. At day 14 after operation, the mechanical withdrawal threshold and thermal withdrawal latency in group Ⅳ were lower than group Ⅰ, Ⅱ and Ⅲ, while the expression of P2X3 receptor in L4/L5 spinal cord of Ⅳ group was higher than group Ⅰ, Ⅱ and Ⅲ. The mechanical withdrawal threshold, thermal withdrawal latency and the expression of P2X3 receptor in L4/L5 spinal cord showed no significant difference among group Ⅰ, Ⅱ and Ⅲ. The expression of P2X3 receptor in L4/L5 spinal cord of group Ⅲ was higher than group Ⅰ and Ⅱ, but it was lower than group Ⅳ. Conclusion: TMP can alleviate neuropathic pain induced by P2X3 receptor.

Key words: P2X3 receptor; tetramethylpyrazine; neuropathic pain.

Acknowledgment - This work was supported by the National Natural Science Foundation of China (№30260030) to S.D. Liang.

P060052

Effect of rosmarinic acid against the PC12 cell injures inducd by glutamate

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To investigate the effect and mechanism of Rosmarinic acid(Ros A) against PC12 cell injury induced by glutamate. With the model of PC12 cell injury induced by glutamate(1-20 mmol·L^{-1}), the live viability of cell was observed by 3-(4,5 dimethyhhiazol-2-y1)-2,5diphenyl-tetrazolium bromide (MTT) assay; the change of cell shape was observed by nuclear staining with Acridine orange (AO); and the cell apoptosis was detected by flow cytometric analysis (FCM); The expre-ssion of bcl-xl and bax gene were determined by reverse transcription polymerase chain reaction (RT-PCR). After one hour's pretreatment of Ros A (100 μmol·L^{-1}), the cell survival of PC12 cells was promoted, and the apoptotic rate of PC12 cells was decreased marked-ly, the expression of bcl-xl gene was increased and the expression of bax gene was decreased. Ros A can resist to injury of PC12 induced by glumatame, The possible mechanism of it is related to regula-tion of the expression of bcl-xl and bax genes.

KEY WORDS: Ros A; PC12 cells; anti-apoptosis; bcl-xl

Project supported by the Department of Human health(204062) and Department of Hunan Education(03A040).

P060053

Studies on effects of chiral drug R-(-)-phencynonate hydrochloride on antimotion sickness

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R(-)-phencynonate hydrochloride is a eutomer of phencynonate hydrochloride (CPG), which is a new central anticholinergic anti-motion sickness drug. In this paper, we study the antimotion effects on two animal models:(1) Unanesthesized rabbit, with the cholinesterase of the right side of cerebrum and brain stem including the vestibule inhibited by paraoxon, (2) unanesthesized cat, im physostigmine. The results show that R(-)-CPG is more potent than CPG and S(+)-configuration. The other pharmacological activities are assessed in three individual experiments: (1) potentiating the effect of subthreshold hypnotic dose of sodium pentobarbital, (2) inhibiting oxotremorine-induced salivation and (3) inhibiting the contractile response to carbachol. The results demonstrate that R(-)-CPG is equivalent with CPG in inhibiting salivation and contraction of smooth muscle, but less potent than CPG in the central inhibitory effect. The radio-ligand receptor-binding assay reveals the selectivity of R(-)-CPG to M1, M3 and M4 receptors.

Key words: optical isomers; muscariric receptors; motion sickness.

Acknowledgement: The work was supported by National Natural Science Foundation of China (No.203900508)

P060054

Effect of tramadol administered concomitantly with pentoxyphylline on pain behavior and paw edema in rats.

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Activated glia is implicated in generating and maintaining pathological pain. Pentoxyphylline (PTX) inhibits glial activation and reduces release a variety of neuroactive substances. The study were aimed at evaluation of antinociceptive and anti-inflammatory effect of tramadol (TRAM) administered together with PTX in rat formalin (FT) and carrageenan tests (CT).

Total number of paw flinches was recorded in the first (I) and the second (II) phase of FT after i.p. injections of TRAM (5, 10 mg/kg) or/and PTX (50, 100 mg/kg). In CT paw edema was evaluated after TRAM (10 mg/kg) or/and PTX (100 mg/kg).

TRAM (5, 10 mg/kg) and PTX (100 mg/kg) induced significant antinociceptive effect in both phases of FT. PTX (100 mg/kg) significantly improved TRAM (10 mg/kg) effect in both phases of FT. TRAM given separately or together with PTX significantly reduced paw edema (15.4 and 16.8%, respectively), unlike PTX injected separately.

Concomitant administration of TRAM with PTX may help to achieve more effective treatment of both acute and persistent pain states. TRAM exerts both antinociceptive and anti-inflammatory effects.

Key words: tramadol, pentoxyphylline, formalin, carrageenan.

Supported by MHE (502-1-08).

P060055

Monoamine Oxidase Inhibitory Properties of Areca Nut

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Areca nut is known since the pre-Christian era and is still very popular chewing nut in different area of the world. In folk medicine Areca nut has an important place. Our earlier studies indicated that the Areca extract and its fractions possess antidepressant properties. Monoamine oxidase (MAO, EC 1.4.3.4) exists in two forms MAO-A and -B. Inhibitors of both forms have therapeutic value. The present investigation was undertaken to assess Areca extract, its fractions and sub fraction for their MAO-A and -B inhibiting properties.

Monoamine oxidase activities were determined using rat brain synaptosomes in the presence and absence of Areca extract, its fractions and sub fractions. One of the metabolites of determination, H_2O_2, was measured flourometrically. Among all the tested compounds methanol sub fraction was found to be most potent inhibitor of both MAO-A (IC_{50} = 102 ± 8.3 µg/ml) and -B (IC_{50} = 707 ± 23.4 µg/ml). In conclusion, Areca extract, its hexane and dichloromethane fractions may possess only MAO-A inhibitory activity. However, its sub fractions have both MAO-A and -B inhibiting properties.

Key words: Areca nut; synaptosomes; monoamine oxidase inhibition

P060056

Nicotinic receptors play a key role in methylenedioxymethamphetamine (MDMA)-induced neurotoxicity in mice.

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Due to previous results (*), we studied the role of alpha7 nicotinic receptors (7nAChRs) on MDMA effects and neurotoxicity in mice. Methyllycaconitine (MLA), specific 7nAChRs antagonist, prevented MDMA-induced dopaminergic neurotoxicity ([^{3}H] WIN35428 binding) (31.2 ±8.1%, MDMA vs 114 ± 15.8% MLA + MDMA, $P < 0.01$) and microglial activation ([^{3}H] PK11195 binding) (165.8 ±21.2% MDMA vs 107.9 ±10.8% MLA + MDMA, $P < 0.05$). In mice striatum, MDMA 100 uM induced intrasynaptosomal ROS production (136.5 ±6.2%), releasing vesicular dopamine (DA). This effect was DA, calcium and MAO-B dependent, pointing to endogenous DA as the source of ROS. MLA and alpha-bungarotoxin antagonized this effect and prevented the decrease in DA uptake induced by MDMA (from 73 to 11%). Effect on glutamate receptors was ruled out. From all these results it can be deduced that coordinate activation of 7nAChR together with DA transporter blockade and displacement of DA from intracellular stores promotes neurotoxicity that can be prevented by MLA. So, 7nAChR have a key role in MDMA neurotoxicity in mice.

Key words: MDMA, alpha7 nicotinic receptors

Work supported by grants: SAF2005-0573, SGRE00793, PI050486. (*) Escubedo et al. JPET (2005) 315:658-667

P060057

Protective effects of LDQ injection on cerebral ischemia reperfusion injury in rats

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Objective: To study the protective effects of LDQ injection on cerebral ischemia reperfusion injury in rats. Methods: The model of rat focal cerebral ischemia-3h/reperfusion-24h was induced by middle cerebral artery occlusion (MCAO). After 24h reperfusion, the behavior, infarct size and water content of ischemic rats were evaluated. The content of malondialdehyde (MDA), the activity of superoxide dismutase (SOD) in brain tissue, and the content of nitric oxide (NO), the activity of nitric oxide synthase (NOS) in plasma were measured. Results: LDQ injection (1, 2g/kg) could significantly improve the behavior of ischemic rats and markedly decrease their infarct size and water content. The drug could enhance the SOD activity and reduce the MDA content in rats' brain tissue at the same time. Besides, it could also decrease the content of NO and inhibit the activity of NOS in plasma. Conclusion: LDQ injection has protective effect against focal brain damage induced by occlusion reperfusion in middle cerebral artery of rats. This effect may be related to increasing antioxidase activities, decreasing lipid peroxidative damage.

Key Words: LDQ; Cerebral ischemia; Reperfusion

P060058

Modulation of Ca^{2+} signals by phosphatidinositol (PI)-linked novel D1 dopamine receptor in primary cultured hippocampal neurons: role in neuroplasticity.

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The present work was designed to characterize the Ca^{2+} signal regulated by PI-linked D1 dopamine receptor (D1DR) in primary cultures of hippocampal neurons by employed calcium imagine technique. The results indicated that PI-linked D1DR agonist SKF83959 induced long-lasting increase of basal $[Ca^{2+}]i$ in a time- and dose-dependent manner. The sustained elevation of $[Ca^{2+}]i$ depended on both the intracellular calcium release (initial phase) and calcium influx (late component). Depletion of intracellular Ca^{2+} by thapsigargin abolished SKF83959-stimulated Ca^{2+}, indicating that release of Ca^{2+} from intracellular stores is essential for triggering the late phase of Ca^{2+} influx. Removal of extracellular Ca^{2+}, SKF83959 induced increase in late phase of $[Ca^{2+}]I$ was diminished. We further showed that activation of PLC/IP3 was responsible for the drug-induced Ca^{2+} release from intracellular stores. Moreover, we demonstrated that L-type Ca^{2+} channel and NMDA receptor operated Ca^{2+} channel contributes to SKF83959-induced Ca^{2+} influx. Lastly, we demonstrated that stimulation of Ca^{2+} by SKF83959 appears to be the underlying mechanism for PI-linked D1DR-stimulated LTP.

Key words: dopamine receptor, calcium.

P060059

Effect of intrathecal injection of Glutamate-antagonist on neuropathic pain model rat

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Excitatory amino acids (EAAs) mediate nociceptive inputs to the spinal cord. Recently, G-protein coupled metabotropic glutamate receptors (mGluRs) are important modulators of synaptic transmission in the mammalian CNS and have been implicated in various forms of neuroplasticity and nervous system disorders. We determined the effect of intrathecal (i.t.) injection of NMDA and mGluRs antagonists in the rats with thermal hyperalgesia. Thermal hyperalgesia was introduced by chronic constraction of the left sciatic nerve (CCI) with chromic gut-suture in rat. I.t. injection of Group I mGluR antagonist CPCCOEt (1, 5mM) and MPEP (10, 30mM) increased the withdrawal latency (analgesia) both CCI and Sham-operated rats. I.t. injection of Group II mGluR antagonist EGLU (3, 10mM) and Group III mGluR antagonist CPPG (10, 30mM) did not show significant effect. On the other hand, NMDA R-antagonists MK-801 (0.5, 5mM) and AP-5 (0.1, 1mM), and AMPA-Kinate R-antagonist NBQX (0.1, 1mM) increased the withdrawal latency These results suggest that spinal NMDA and Group I mGluR antagonists have a role of thermal nociceptive pro-

cessing, but other mGluRs Groups have not.

P060060

Endocannabinoid levels are regulated by a novel phospholipase D in rat brain culture

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Fatty acid ethanolamides are released from N-acyl-phosphatidylethanolamines (N-acyl-PEs) by the hydrolysis of a phosphodiesterase of the phospholipase D type. The endocaonnabinoid anandamide (AEA) and other bioactive long-chain fatty acid ethandlamides are formed from their precursor N-acyl-PEs by a specific phospholipase D (NAPE-PLD) in the brain as well as other tissues. However, the characterization of NAPE-PLD is still incomplete. In this study, we examined NAPE-PLD mRNA expression levels by Q-PCR and in-situ hybridization, suggesting that the expression of NAPE-PLD corresponds, at least in part, to endocannabinoid distribution in the brain. Mbreover, we infected rat brain organotypic slices with the viral mediating overexpression of NAPE-PLD and NAPE-PLD shRNA. The results showed that infection with the overexpression NAPE-PLD virus increased the release of AEA, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), but not 2-arachidonoyl-glycerol (2-AG). By the contrast, the infection of NAPEPLD shRNA virus showed a decrease of endocannabinoid levels. These studies indicate that NAPE-PLD plays an important role on endocannabinoid regulation in the central nervous system.

P060061

Effects of ethanol dependence and withdrawal on the levels of neurosteroids in the rat nucleus accumbens

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AIM: To investigate the effects of ethanol dependence and withdrawal on the concentration of neurosteroids in the rat nucleus accumbens. METHODS: Neurosteroids were isolated and extracted separately in a two-step procedure, Then a clean-up step was performed by solid-phase extraction (SPE), then all steroids were derivatized and analyzed by HPLC/MS using selected-ion monitoring. RESULTS: Compared with control group, chronic ethanol administrations resulted in a marked decrease in the concentrations of PREG, DHEA and AP ($P < 0.05$) respectively in male rat nucleus accumbens. The concentrations of PREGS and DHEAS decreased ($P < 0.05$), respectively. Ethanol withdrawal induced a significant decrease in the concentrations of DHEAS and PREGS ($P < 0.05$) respectively compared with control group, and induced a significant increase in the concentrations of AP ($P < 0.05$) compared with dependent group. CONCLUSION: Ethanol dependence and withdrawal affected the concentrations of neurosteroids in the rat nucleus accumbens, which suggests that endogenous neurostroids might be related to the development of ethanol dependence and withdrawal.

KEY WORDS ethanol dependence; neurosteroids; nucleus accumbens HPLC/MS

P060062

Protective effects of icariin on brain dysfunction induced by lipopolysaccharide in rat model

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Objective: To observe the protective effects of Icariin on studying and memory in the rat model with brain inflammation induced by Lipopolysaccharide and explore the active mechanisms. Methods: The rat model with brain inflammation was induced by injections of lipopolysaccharid into the lateral ventricle. The abilities of spatial learing and memory in rats were tested by Morris water maze. The expressions of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and cyclooxygenase-2 (COX-2) were observed by immunohistochemistry (IHC). The mRNA levels of TNF-α, IL-1β and COX-2 were quantitated and analysed by real-time RT-PCR, respectively. Results: The groups treated by Icariin ($30\ mg \cdot kg^{-1} \cdot d^{-1}$, $60\ mg \cdot kg^{-1} \cdot d^{-1}$, $120\ mg \cdot kg^{-1} \cdot d^{-1}$) had significantly shorted in escape latency and searching distance compared with model group. Icariin decreased the content and the mRNA levels of TNF-α, IL-1β and COX-2 in hippocampus of the rats in treatment groups at the doses of $30\ mg \cdot kg^{-1} \cdot d^{-1}$, $60\ mg \cdot kg^{-1} \cdot d^{-1}$ and $120\ mg \cdot kg^{-1} \cdot d^{-1}$ ($P < 0.01$ respectively), in which the effects were in a dose-dependent manner. Conclusion: Icariin can improve the ability of spatial learing and memory of rats with the brain inflammation induced by lipopolysaccharid, in which may be due to decrease the expressions of COX-2, TNF-α and IL-1β.

Key words: icariin; TNF-α; IL-1β; lipopolysaccharid; learning and memory; COX-2

P060063

Protective effects of resveratrol on rat model with Parkinson's disease induced by 6-OHDA

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To observe the effects of resveratrol (Res) on rat model with Parkinson's disease (PD), the model was induced by injecting 6-hydroxydopamine (6-OHDA) into the dexter striatum of SD rats. Apomorphine was used to induce turning behavior, and the rotational frequency (RF) of valid rats was 15 ± 4 cycles/min. The model rats were randomly divided into five groups: Res ($10, 20, 40\ mg \cdot kg^{-1} \cdot d^{-1}$), VitE ($50\ mg \cdot kg^{-1} \cdot d^{-1}$) and model control.

Mbreover, sham control and normal administration (Res $20\ mg \cdot kg \cdot d-1$) were also used. Res and VitE were administered by gavage. The levels of mRNA and expressions of COX2 and iNOS were determined by RT-PCR and Western blot respectively. The results showed that the RF of rats was obvious variance between therapeutic groups and model control at tenth week ($P < 0.01$). However, the RF of model control was aggravated after ten weeks ($P < 0.01$). Res decreased the levels of mRNA and expressions of COX2 and iNOS in SN and striatum. It is concluded that Res can have protective effect on PD rats induced by 6-OHDA, which may be relate to inhibition of inflammatory cytokine release.

Key words: resveratrol; 6-OHDA; rat; rotational behavior

P060064

Protective effect and mechanism of GABA on Ca^{2+} overload induced by oxygen-glucose deprivation in cultured human oligodendroglioma cell

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It is well known that both glutamate and GABA releasing increase during cerebral ischemia and the excitatoxicity of glutamate is one of the important factors which cause cerebral damage. In order to investigate the role of GABA and it's probable mechanism, human oligodendroglioma cells were bubbled with a mixture of 95% N_2 and 5% CO_2 in glucose deprived (oxygen-glucose deprivation, OGD) artificial cerebral spinal fluid to produce ischemic-like model. Laser scanniong confocal microscope was used to detect real-time changes of $[Ca^{2+}]i$. It was showed that Ca^{2+} influx increased dramatically when the cells were put in OGD artificial cerebral spinal fluid and preincubation with GABA for 5 min could significantly reduce the Ca^{2+} overload induced by OGD. The above action of GABA could be blocked by $GABA_B$ receptor antagonist phaclofen but not $GABA_A$ receptor antagonist bicuculline. These results suggest that GABA might play it's protective role on human oligodendroglioma cell ischemia via blocking $GABA_B$ receptor.

Key words: cerebral ischemia, GABA, Ca^{2+} overload, human oligodendroglioma cell

P060065

Functional proteomics of lymphocytes is related to energy failure in acute stroke.

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A study on energy-linked enzymes of lymphocytes was performed in healthy blood donors (groups of ages 20-86 yrs) and in acute stroke patients aged 51-86 yrs.

Catalytic activities of hexokinase-HK, lactate dehydrogenase-LDH (glycolysis); citrate synthase-CS, malate dehydrogenase-MDH (Kreb's cycle, TCA); Complex I-III, Complex II and Complex IV (Mtochondrial Electron Transfer Chain, ETC) and glutamate dehydrogenase-GLDH were assayed.

The HK and CS increased starting at 31 (male) and 51 (female) years, Complex II at 51 (male) or 61 (female) yrs; ETC enzymes increased starting at 31 yrs, only in male. In stroke male patients, LDH, CS increased; GLDH and Complex IV decreased; in female, HK, CS, MDH, Complex I-III increased, while Complex IV decreased (ANOVA test).

Thus, stroke strongly modified the activities of these biological markers of energy metabolism on peripheral cells differently for gender and age, more in female than in male. Glucose metabolism and ATP synthesis are primary affected also in lymphocytes, reflecting as a mirror the cerebral metabolic dysfunctions observed in

stroke patients, allowing to elaborate a systematic model to study neurological (and psychiatric) diseases and drugs' actions.

P060066

Gamma-aminobutyric acid (GABA) as a partial agonist at specific GABA-A receptor subtypes

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GABA is the main inhibitory neurotransmitter in the mammalian brain and its fast actions are mainly mediated by GABA-gated ion channels, the GABA-A receptors. GABA is supposed to fully activate all GABA-A receptor subtypes. However, it is known that there are some GABA-A receptors, in which GABA has atypically low efficacy e.g. to displace the ionophore ligand $^{[35S]}$TBPS from certain regions of the brain (Sinkkonen et al., Mol Brain Res. 86:168-78, 2001). We have used a Thy1alpha6 mouse line with forebrain expression of alpha6 subunits under the Thy1 promoter to show atypically low GABA efficacy especially in the CA1 region of the hippocampus, which has ectopic alpha6beta subtype expression. In this brain region of the mutant mice, the full agonist action of gaboxadol was inhibited by GABA. Similar interaction was observed also in the thalamus and cerebellar granule cell layer of both the mutant and wild-type mice. The data indicate that there are populations of GABA-A receptors, in which GABA seems to be only a partial agonist, and by which potent sedative actions of gaboxadol might be mediated.

Key words: extrasynaptic, autoradiography, gaboxadol, THIP.
Supported by the Academy of Finland.

P060067

Developmental Regulation of Insulin-Degrading Enzyme in Long-Term Hippocampal Cell Culture

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More and more evidence suggested that insulin-degrading enzyme, also called IDE, is responsible for the degradation of amyloidogenic proteins and peptides, especially β-amyloid (Aβ) which plays a central role in the pathogenesis of Alzheimer's disease. However, little is known about the cellular and molecular regulation of IDE, although several researches showed that the steady-state level of IDE protein diminished as a function of age. In the present study, the protein and mRNA levels of IDE were evaluated respectively by quantitative Western blotting, Immunocytochemistry and RT-PCR in a long-term neuron culture system. The results of our study will reveal the phase in which the major regulation of IDE during aging occurs and indicate a possible regulatory mechanism.

Key words: Insulin-degrading enzyme, β-amyloid, Alzheimer's disease, hippocampal neurons

P060068

D-serine transport in neurons and astrocytes

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D-Serine is an endogenous agonist of N-methyl-D-aspartate receptors that plays an important role in regulation of synaptic function and may be important in excitotoxicity. We are interested in how extracellular Dserine levels are regulated and therefore investigated D-serine uptake kinetics in cultured cortical neurons and astrocytes. Cells were exposed to $^{3}H^{-}$D-serine with varying amounts of unlabeled D-serine and intracellular ^{3}Hcontent measured following uptake. Uptake was linear with time and predominantly Na^{+}-dependent in both neurons and astrocytes. Both cell types displayed low affinity D-serine uptake and stained positive for low affinity Na^{+}-dependent transporters ASCT1 and ASCT2. In addition, D-serine uptake in both cell types was inhibited by amino acids known to be substrates for ASCT1 and ASCT2. Neurons also stained for the high affinity transporter, asc-1, but no evidence of functionally was found. These data contributes to our understanding of Dserine transport and therefore provide valuable insight into how extracellular D-serine levels are regulated.

Key words: D-serine, transport, neuron, astrocyte.
Acknowledgement: Funded by Ajinomoto Amino Acid Research Program.

P060069

Protective Effects of Dendrobium alkaloid in the Nerve Cell Injured by Ischemia/Reperfusion

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This study examined the protective effects of alkaloid extracted from Dendrobium nobile Lindl. a preciouson Chinese medicinal herb, on neurons treated by ischemia/reperfusion in vitro. The primary culture cerebral cortical neurons of Wistar rat were studied during the different periods of oxygen-glucose deprivation/reperfusion with oxygen and glucose. MTT assay was used to determine cell viability, and the activity of lactate dehydrogenase (LDH) leaked from neurons were measured. During the different period of oxygen-glucose deprivation (1,2,4h) or oxygen-glucose deprivation (2h)/reperfusion with oxygen and glucose (3, 12,24h) incubation in vitro,the neuronal viability was decreased, and the percentage of LDH leakage was increased. Dendrobium alkaloid (final concentration 0.025 mg·L^{-1}, 0.25 mg·L^{-1}, and 2.5 mg·L^{-1}, respectively) can attenuate neuronal damage, in which the absorbance of MTT was increased and leakage of LDH was decreased during the different period of oxygen-glucose deprivation or reperfusion with oxygen and glucose in the primary culture neurons. Morphologic changes of the neurons and cell apoptosis were measured, as well as concentration of intracellular free calcium and mitochondrial membrane potential (MMP) evaluated respectively at the time of oxygen-glucose deprivation (2h)/reperfusion with oxygen and glucose(12h). The changes of expressions of cysteine aspartyl proteinase 3(caspase-3) and cysteine aspartyl proteinase 12(caspase-12) were observed by real-time reverse transcription-polymerase chain reaction (RT-PCR). Cell apoptosis and intracellular free calcium concentration significantly elevated, and the disruption of MMP were induced by oxygen-glucose deprivation (2h)/reperfusion with oxygen and glucose (12h). Treatment with Dendrobium alkaloid (final concentration 0.025 mg·L^{-1}, 0.25 mg·L^{-1}, and 2.5 mg·L^{-1}) decreased cell apoptosis and inhibited intracellular free calcium concentration elevation, increase MMP in concentration-dependent manner, reducing cell morphologic changes, significant decrease were found at the expressions of caspase-3 and caspase-12. In summary, this study demonstrates that Dendrobium alkaloid has significantly protective effects on primary cultured neuronal damages induced by oxygen-glucose deprivation/reperfusion with oxygen and glucose. This protection appears to be due to stabilizing MMP and cell activity. It also inhibited the onset of cell apoptosis, which may be related with its effected for inhibiting the calcium overload and decreased the expressions of caspase-3 and caspase-12.

Keywords: Dendrobium alkaloid; cerebral ischemia/reperfusion; neuron damage; calcium; mitochondrial membrane potential; caspase-3; caspase-12

P060070

Ganglioside/Calmodulin-Dependent Protein Kinase II (CaMKII) Signals Triggering Cytoskeletal Actin Reorganization in Neurons

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Cell surface glycoconjugates are thought to play roles in neural development and functions. GT1b ganglioside, which is located at synaptic areas, is the bioactive cell surface glycoconjugate. Recent study showed that CaMKII have important roles in stabilizing the dendritic architecture.

We employed a novel fluorescent imaging system to monitor CaMKII activity by exposure to nanomolar level of GT1b in primary cultured rat hippocampal neurons or neuroblastoma-glioma hybridoma(NG108-15) cells using a fluorescence-labeled peptide substrate and found that GT1b stimulated Ca^{2+}/CaMKII in a few seconds. The treatment was accompanied by peripheral actin polymerization and filopodia formation in cells described above within 2 min, induced by cdc42, a member of Rho family GTPases, is related to the initiation of dendritogenesis in addition to filopodia formation. CaMKII inhibitors blocked both CaMKII activation and subsequent filopodia formation. Furthermore, long-term exposure to GT1b accelerates dendritogenesis indicating physiological roles of the signals in neuronal differentiation and maturation.

Keywords: ganglioside, CaMKII, cdc42, dendritogenesis
Acknowledgements: We thank Dr. Yuhe Yuan for her valuable advice and comments on the manuscript, Prof. Hideyoshi Higashi for technical help and encouragement. This work was supported by 973 program(2004CB518906).

P060071

A centrally-acting antitussive rescues impairment of learning and memory caused by prenatal diethylstilbestrol exposure of mice

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We have previously reported that centrally-acting antitussives inhibited G protein-coupled inwardly rectifying K^{+}(GIRK) currents mediated by activation of 5-HT_{1A} receptors, and that the antitussives ameliorated overactive bladder and difficulty in urination in conscious rats with cerebral infarction. In this study, we investigated whether or not the antitussives rescue impairment of brain function caused by prenatal diethylstilbestrol (DES) exposure, since the antitussives appear to have a stabilizing effect on disturbance of brain functions. [Methods] DES was orally given at 0.1 μg/30 μl/animal once a day for the 11th to 17th days of gestation in ddY strain mice. Cloperastine (CP) was subcutaneously given at 10 or 30 mg/kg twice a day from 32 to 41 days after birth of male mice.

Passive avoidance response (PAR) test was performed at 6 week-old of the offspring. [Results] CP significantly prolonged latency of PAR, and increased the level of phosphorylated CaMKII in the hippocampus compared to that of control. In conclusion, the antitussives might rescue impairment of learning and memory caused by prenatal exposure to endocrine disruptors such as DES, possibly through inhibition of GIRK channel.

P060072

The Effect of Melatonin on the Lipide and Protein Peroxidation in the Forebrain of Rats under Acute Hypoxia.

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The effect of melatonin on the contents of products of lipide peroxidation (malonic dialdehyde) and protein peroxidation (dinitrophenylhydrazone derivatives), and activity main antioxidant enzyme of neurones (glutathione peroxidase) in cerebral cortex and hippocampus of juvenile 5-6 weeks old male rats was investigated under conditions of acute hypobaric hypoxia. Melatonin was administered intraperitoneally in the dose of 1 mg/kg of body weight 30 minutes prior to hypoxia modulation. Acute hypoxia was achieved by aspirating the air to the pressure equivalent to an altitude of 12,000 metres. Euthanasia of rats was accomplished 30 minutes after hypoxia modulation. It has been established that melatonin raised the activity of glutathione peroxidase, reduced the intensity of lipide peroxidation at the acute hypoxia especially in a hippocampus. At the same time the melatonin administration enhanced the intensity of protein peroxidation in a hippocampus. Thus, the administration of melatonin under acute hypoxia prevents the strengthening of lipide peroxidation, raises the activity of some antioxidant enzymes, but enhances the intensity of protein peroxidation in several brain structures.

Key words: melatonin, hypoxia, peroxidation, brain.

P060073

Effect of methandic extract of Matricaria Chamomilla L. on seizures induced by picrotoxin in mice.

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Matricari Chamomilla L. is a well known medicinal plant for its effects as carminative, analgesic and anticonvulsant agent. The present investigation is an attempt to establish a scientific basis for the use of the plant as antiepileptic in Iranian traditional medcine.

Dried seeds of Matricari Chamomilla L. were extracted by methanol. Mice were pretreated with the exract via intraperitoneal injection. After 20 minutes of pretreatment, animals received 12 mg/kg picrotoxin in order to induce seizures. Latency time for beginning of seizures, duration of seizures death time and mortality rate were investigated.

The results showed that lantency time for begining of seizure was increased in the experimental groups pretreated with the extract. This increase was significant at the dose of 200 mg/kg ($P < 0.05$). This dose, in addition, delayed the death time in mice ($P < 0.01$) which was even more effective than phenobarbital (40 mg/kg).

The results of this study indicated that the extract of Matricaria chamomilla L. was effective on generalized seizure induced by picrotoxin.

Key words: Matricaria Chamomilla L., Seizures, Picrotoxin, Anticonvulsant

P060074

the role of orexin 1 receptors in CA1 region of adult male rat hippocampus on spatial learning and memory

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Orexin containing neurons in the lateral hypothalamic area (LHA) prouduce orexin-A (hypocretin-1) and orexin-B (hypocretin-2) and send their axons to the hippocampus, which predominantly expresses orexin 1 receptors (OX1R) showing a higher affinity to orexin-A. Recent studies have shown central administration orexin-A on learning and memory but literature concerning the role of orexinergic system in cognition remains controversial. Here, we examined the effect of pre-training, post-training and pre-probe trial intrahippocampal administration of SB334867-A (1.5, 3, 6 µg/0.5 µl), a selective OX1R antagonist, on acquisition, consolidation and retrieval in a single-day testing version of Morris water maze (MWM) task. Compare with control group, SB334867-A impaired aquisition, consolidation, and retrieval of MWM task. This drug had no effect on escape latency of a non-spatial visual discrimination task. Therefore, it seems that endogenous orexins, especially orexin-A, plays role in spatial learning and memory Processing in the rat.

Key words: Orexin, Hippocampus, spatial learning and memory, Rat

P060075

Identification of a presynaptic cannabinoid CB_1 receptor in the guinea-pig atrium and partial sequencing of the guinea-pig CB_1 receptor

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In superfused guinea-pig atrial pieces preincubated with 3H-noradrenaline, the electrically evoked tritium overflow was inhibited by the cannabinoid receptor agonist WIN 55,212-2. The effect of WIN 55,212-2 1 micro M (inhibition by 41%) was attenuated by the CB_1 antagonist rimonabart 0.032 micro M and abolished by rimonabart 0.32 micro M; rimonabant had no effect by itself. Since the guinea-pig proves to be particularly suited for the identification of presynaptic CB_1 receptors, we examined its nucleotide sequence, using primers of the closely related species Agouti taczanowskii, the partial sequence of which has been published by Murphy et al. (2001) (GenBank AY011590). We determined a partial sequence (330 amino acids) of the guinea-pig (Cavia porcellus) including the 1st to 6th transmembrane domain (TMD) and six amino acid residues of the 7th TMD (GenBank DQ855990). The homology was 99% (Agouti taczanowskii), 95% (man) and 96% (rat or mouse). In conclusion, noradrenaline release from the guinea-pig atrium is subject to inhibition by presynaptic CB_1 receptors. The guinea-pig CB_1 receptor shows a high homology to the CB_1 receptor of humans and rodents.

Key words: Guinea-pig CB_1 receptor sequence

P060076

Effects of rotenone and MPTP on CNS dopaminergic system in mice

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Insecticide rotenone and methyl phenyl tetrahydropyridine (MPTP) are known to inhibit mitochondrial complex I and induce neurodegenerative changes which may kill dopaminergic neurons. In the present study, effects of rotenone and MPTP on behavior and dopaminergic parameters in the cortex (Cor) and striatum (Str) were examined after repeated administration in mice. Rotenone 0.5-16 mg/kg and MPTP 8-24 mg/kg for 7 or 14 days did not induce catalepsy. Rotenone at 0.5-16 mg/kg twice a day for 7 days or once a day for 14 days reduced binding of [3H] haloperidol (DA-R) 24 hrs after the last dosage in Cor or Cor and Str, respectively. MPTP 10 mg/kg reduced the DA-R in the Str 24 hrs after chronic dosage for 7 days but not acute one. Rotenone at 10.7 or 16 mg/kg twice a day for 14 days and MPTP at 10 mg/kg 4 times at every 1 hr decreased the contents of DA metabolites except DA in the Str 24 hrs after the last dosage. The contents in Str recovered 7 days after the last dosage of rotenone but not of MPTP. It is concluded that rotenone reversibly and MPTP irreversibly affect the dopaminergic system nonselectively in both Str and Cor and selectively in Str, respectively.

Key words: Rotenone, MPTP, Dopaminergic

P060077

Methamphetamine and methylenedioxymethamphetamine interact with and upregulate alpha-7 nicotinic receptors in NGF-differentiated PC12 cells

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Previous work from our group pointed to a key role of alpha-7 nicotinic receptors (7nAChR) in amphetamine derivatives-induced oxidative stress and neurotoxicity. The aim of the present work was to demonstrate the interaction of methamphetamine (Meth) and methylenedioxymethamphetamine (MDMA) with 7nAChR in NGF-differentiated PC12 cells. Specific binding of [3H] methyllycaconitine, a specific 7nAChR antagonist, was displaced by the two drugs, showing MDMA a higher affinity. Also, Meth and MDMA (300 µM) increased the ability of nicotine to displace this binding. Meth and MDMA displaced [3H] nicotine binding to intact PC12 cells with IC_{50} values of 160 and 80 µM, respectively. It can be deduced that these drugs directly interact with 7nAChR either exerting a positive allosteric modulation or activating it. In addition, as described with pretreatment with nicotine, pretreatment with Meth and MDMA resulted in up-regulation of 7nAChR which was maximum after 48 h at a concentration of 300 µM (increase about 65% and 100%, respectively) and was already apparent after a 6h-treatment.

Work supported by grants: SAF2005-0573, SGRE00793, PI050486.
Keywords: Methamphetamine, MDMA, alpha-7 nicotinic receptors.

P060078

Differential effects of distinct classes of N-methyl-D-aspartate receptor antagonists on seizures and synaptic neurotransmission

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Too much and too little NMDAR activation can cause pathophysiology. Electro-

physiological granule cell layer recordings from hippocampal slice cultures revealed that chronic NMDAR blockade with the high - affinity competitive antagonist, D- APV, or moderate - affinity uncompetitive antagonist, memantine, exacerbated bicuculline (BMI) - and 0 mM Mg^{2+} - induced electrographic seizures. The NR2B selective antagonist, Ro256981, reduced both types of seizures. Next, we examined the mechanisms underlying the differential effects. Treatment with the NR2A - selective antagonist, NVPAAM077, reduced BMI - but increased 0 mM Mg^{2+} - induced seizures, suggesting a role for subunit selectivity. Granule cell membrane properties were unaltered, so cannot account for differential effects. Treatment with D- APV increased mEPSCs but reduced mIPSCs.

Ro256981 and memantine modestly increased mEPSCs, but NVPAAM077 had no effect. Ro256981 had little effect on mIPSCs; analyses of NVPAAM077 or memantine effects on mIPSCs are underway. Thus, plasticity in glutamatergic circuits or GABAergic control may contribute to differential effects of NMDAR antagonists although clear associations are not yet apparent. = Support: NS045964 & PR030035

P060079

Alterations of Signal Molecules and Proopiomelanocortin Gene Expression in the Hypothalamus of Mice Induced by Intraplantar Formalin.

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We examined POMC mRNA and beta - endorphin expression in mouse hypothalamus elicited by intraplantar formalin. POMC mRNA increased at 2 hr after injection, and returned to control level at 10 hr in the arcuate nu. of hypothalamus. In the tail - flick test, formalin attenuated opioids - induced antinociception. Increase of POMC was also observed after systemic morphine and attenuated by naltrexone. Thus, formalin - induced increase of hypothalamic POMC may be mediated by endogenous opioid system. We further examined alterations of signal molecules. pERK was increased within 30 min and remained at a high level up to 10 hr in the arcuate nu.. pCaMKII was increased within 2 hr but decreased at 10 hr. However, POMC mRNA expression was reduced by pretreatment with PD98059 or KN93. Furthermore, pIkB was increased at 2 hr and remained at high level up to 10 hr. Using confocal IF, we confirmed that cells contain beta - endorphin after formalin also express pERK, pCaMKII and pIkB. In conclusion, POMC mRNA expression in arcuate nu. of hypothalamus induced by intraplantar formalin may be mediated by pERK as well as pCaMKII. Furthermore, NFkB pathway may play an important role in the regulation of POMC gene expression.

P060080

Role of prefrontal dopamine system in the antidepressant - like effect of combination of sulpiride and fluvoxamine

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Combination therapy of antipsychotic sulpiride, a dopamine (DA) - D2 receptor antagonist, and serotonin (5 - HT) reuptake inhibitors (SSRIs) is clinically effective for treatment - resistant depression in Japan. This study examined whether coadministration of sulpiride and fluvoxamine, an SSRI, has an antidepressant - like effect and studied, using in vivo microdialysis technique, the effects of the coadministration on the release of amine neurotransmitters in the brain. In the tail - suspension test, sulpiride and fluvoxamine alone did not affect the immobility time of mice, but coadministration of these drugs reduced significantly the immobility time. Sulpiride at low doses did not affect DA, 5 - HT and noradrenaline (NA) release in the frontal cortex, while fluvoxamine caused no change in DA release, a marked increase in 5 - HT release and a slight increase in NA release in the cortex. Under the conditions, coadministration of these drugs caused a significant increase in cortical DA release, but did not affect 5 - HT and NA release. These results suggest that combination of sulpiride and fluvoxamine has an antidepressant - like effect and that cortical DA system may play a key role in the antidepressant - like effect.

P060081

Effect of Drug - induced Ascorbic Acid Release in the Striatum and the Nucleus Accumbens in Hippocampus - lesioned Rats

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The mechanism of ethanol, morphine, methamphetamine (MAP), nicotine - induced ascorbic acid (AA) release in striatum and nucleus accumbens (NAc) is not well understood. Our previous study showed that the glutamatergic system was involved in the addictive drug - induced AA release in NAc and striatum. Furthermore, frontal decortication eliminates drug - induced ascorbic acid release in the striatum but not in the NAc. In the present study, the roles of the hippocampus in drug - induced AA release in the striatum and NAc were studied by using microdialysis coupled to high performance liquid chromatography with electrochemical detection (HPLC - ECD). Ethanol (3.0 g/kg, i.p.), methamphetamine (3.0 mg/kg, i.p.), and nicotine (1.5 mg/kg, i.p.) significantly stimulated AA release in the striatum and NAc, respectively. Morphine (20 mg/kg, i.p.) significantly stimulated AA release in the striatum, but not in the NAc. After hippocampus lesion by kainic acid, AA release induced by ethanol, methamphetamine, and nicotine could be eliminated in NAc, but not in the striatum. These results suggest that the hippocampus might be a common and necessary area in addictive drug - induced AA release in the NAc, which also imply that different pathways might be involved in drug - induced AA release in the striatum and the NAc of the rats.

Key words: Ascorbic acid; Striatum; Nucleus accumbens; Ethanol

P060082

Acetaldehyde - induced Changes in the Amino Acid Extracellular Microdialysate Content of the Anterior Cingulate Cortex

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The extracellular changes of amino acids (glutamate, taurine and GABA) in the anterior cingulate cortex (ACC) of freely moving rats by intraperitoneal acute acetaldehyde injections (20 mg/kg and 100 mg/kg) were studied using the microdialysis technique coupled with high performance liquid chromatography (HPLC) and fluorescent detection. Glutamate levels decreased significantly after both doses of acetaldehyde, taurine increased significantly with the higher acetaldehyde dose, the inhibitory amino acid, GABA, had no changes at any time points assayed. These findings suggest that the ACC was in an inhibitory state after acetaldehyde injection. These data provided the first evidence on acetaldehyde - induced changes in extracellular amino acids content in ACC.

Keywords: Acetaldehyde; Amino acid; Anterior cingulate cortex; Microdialysis

P060083

Plasma concentration and muscarinic receptor binding characteristics of novel antichdinergic agents directed toward the therapy of overactive bladder

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Anticholinergic agents (oxybutynin: Oxy) are widely used for the treatment of overactive bladder (OAB). However, its use is often limited by the systemic side effects (dry mouth). Therefore, the effectiveness of novel dosage forms (Oxy transdermal system) and intravesical Oxy has been currently studied. The present study was performed to measure simultaneously muscarinic acetylcholine receptor (mAChR) binding in rat tissues, plasma levels and salivation after transdermal and intravesical Oxy, compared with oral administration. Transdermally administered Oxy binds significantly to bladder mAChRs and it does not produce a long - lasting occupation of the submaxillary gland mAChRs observed by oral administration. Such distinction in submaxillary gland mAChR binding characteristics after transdermal Oxy may be attributable to the absence of rapid rise of plasma drug concentration. Intravesical Oxy binds selectively to bladder mAChRs. Furthermore, the inhibition of salivation due to transdermal and intravesical Oxy was significantly weaker than that by oral administration. In conclusion, transdermal and intravesical Oxy have been shown to be more advantageous than oral Oxy for treating patients with OAB.

P060085

STRESS - INDUCED ACTIVATION OF THE KAPPA OPIOID SYSTEM IN THE MOUSE STRIATUM: IN VIVO AND IN VITRO SIGNALING MECHANISMS

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Stress induces release of the kappa opioid dynorphin to potentiate the behavioral response to cocaine and reinstate craving. We examined the underlying mechanisms by assessing the role of MAPK pathways in the mouse striatum and in pri-

mary cultures of striatal cells. Following 2 days swim stress both p38 and ERK1/2 MAPK showed 2-fold increased phosphorylation in mouse striatum. This increase was blocked by the KOR antagonist norBNI (10 mg/kg) and not evident in KOR knockout mice. p38 MAPK was activated from 10 min to 1 hr after stress and returned to baseline at 6 hrs. Stress activated KOR in GFAP-IR astrocytes and GABA-IR neurons in striatum. Cultured primary striatal astrocytes and neurons both showed increased p38 MAPK phosphorylation after KOR agonist (U50) treatment that was blocked by norBNI and not evident in KOR-/- cultures. U50 treatment of astrocytes loaded with Fluo-4 induced a 4-5 fold increase in intracellular calcium that was blocked by norBNI and not evident in KOR-/- cultures. These data suggest that stress leads to KOR-mediated activation of distinct MAPK and calcium signaling pathways in mouse striatum.

(Supported by F32 DA20430, and RO1 DA16898)

Key Words: Opioid peptides, drug abuse

P060086

Effect of adenosine A1 receptor antagonist 8-cydopentyl-1,3-dipropylxanthine (DPCPX) on memory and its influence on cholinergic nerve

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Objective: To study the effect of blocking adenosine A1 receptors on memory and the relation with cholinergic nerve. Method: By step through test, biochemical assay, bioassay method and electrophysiological technique, the influences of selective adenosine A1 receptor antagonist DPCPX icv on memory, brain AChE activity and ACh concentration of mice, and LTP induced by HFS (200 Hz) in the dentate gyrus of hippocampus of anesthetized rats, were studied. Result: DPCPX (0.3～0.015 μg) could improve scopolamine (Scop, 3 mg·kg^{-1}, ip)-induced memory impairment. DPCPX (0.3, 0.15 μg) could inhibit the brain AChE activity. In vitro test, DPCPX (30 μg ml^{-1}) could inlibit the brain AChE activity. After icv DPCPX (0.3 μg) significantly increased the brain ACh concentration of mice. DPCPX (0.03 μg) could reverse the inhibitory effect of Scop (3 μg, icv) on LTP. Conclusion: DPCPX could influence the levels of central cholinergic neurotransmitter and improve the Scop-induced memory impairment. Its mechanism may be related to the inhibition of brain AChE activity.

Key Words: 8-cyclopentyl-1,3-dipropylxanthine; adenosine A1 receptor; cholinergic nerve; memory.

This project was supported by Natural Science Fundation of Hebei Province.

P060087

Mildronate attenuates AZT-induced and partial sciatic nerve ligation-evoked hyperalgesia in rodents

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Sensory neuropathy due to mitochondrial impairment is recognized as a common side effect of the anti-HIV drug azidothymidine (AZT). The aim of the present study was to investigate the effect of mildronate, a mitochondria-targeted drug, on hyperalgesia caused by AZT and peripheral nerve injury. Continuous treatment with mildronate (100 mg/kg/day i.p.) reduced hyperalgesia (tail flick test) in Balb/c mice caused by 2-week administration of AZT (50 mg/kg/day i.p.). In Wistar rats, traumatic mononeuropathy was induced by partial ligation of the sciatic nerve, mechanonociceptive threshold of the paws was measured by analgesimetry.

Development of mechanical hyperalgesia was observed from postoperative day 3 (PD3) up to PD12. In control group, hyperalgesia developed on PD7 and lasted throughout the whole observation period. Mildronate (100 and 200 mg/kg/day i.p. for 12 days) prevented the development of hyperalgesia. These data suggest mildronate as a promising drug for the treatment of AZT-induced toxic polyneuropathy, as well as traumatic mononeuropathy after nerve injury.

Key words: mildronate, AZT, mechanical hyperalgesia, rodents

Acknowledgements: LCS Grant 05-1418, ESF Grant ESS2004/3

P060088

Sensitization of TRPV1 through G-protein-coupled metabotropic receptors

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One important aspect of TRPV1 regulation concerns the mechanisms by which the inflammatory mediators in damaged tissues sensitize TRPV1. TRPV1 can be phosphorylated by several kinases including PKA, PKC, Ca^{2+}/CaM-dependent kinase II or Src kinase. There has been extensive work demonstrating that activation of a PKA-dependent pathway by inflammatory mediators influences capsaicin- or heat-mediated actions in sensory neurons. PKC-dependent phosphorylation of TRPV1 occurs downstream of activation of Gq-coupled receptors by several inflammatory mediators including ATP, bradykinin, prostaglandins and trypsin or tryptase.

PKC-dependent phosphorylation of TRPV1 caused not only potentiation of capsaicin- or proton-evoked responses but also reduced the temperature threshold for TRPV1 activation so that normal body temperature were capable of activating TRPV1, thereby leading to the sensation of pain. Direct phosphorylation of TRPV1 by PKC was proven using, and two target Ser residues were identified. Phosphorylation of TRPV1 by different kinases seems to control TRPV1 activity through the dynamic balance between the phosphorylation and dephosphorylation.

Key words: inflammation, TRPV1, phosphorylation

P060089

Paeoniflorin attenuates chronic cerebral hypoperfusion-induced learning dysfunction and brain damage in rats

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Paeoniflorin (PF), a major constituent of peony root, was proved to be neuroprotective in middle cerebral artery occlusion model. In this study, we investigated whether PF could attenuate chronic cerebral hypoperfusion induced learning dysfunction and brain damage in rat. 7 weeks after permanent, bilateral occlusion of the common carotid arteries, the rats were tested in the Morris water maze. PF ameliorated cerebral hypoperfusion related learning dysfunction and prevented CA1 neuron damage. Chronic cerebral hypoperfusion increased the immunoreactivity of astrocytes and microglias in hippocampus, which was prevented by PF. Cerebral hypoperfusion also increased expression of nuclear factor-kB (NF-kB), mostly in astrocytes, but not in neurons. In the presence of PF, NF-kB immunostaining was diminished in hippocampus. The data from this study demonstrated that PF attenuated cognitive deficit and brain damage induced by chronic cerebral hypoperfusion and the neuroprotective effect of PF might involve in suppressing neuroinflammatory reaction in brain.

Key Word: Paeoniflorin; glial cell; nuclear factor-kB; chronic cerebral hypoperfusion.

P060090

A NEW REVERSE PHASE HPLC METHOD FOR EXTRACTION, SEPARATION AND QUANTIFICATION OF MELATONIN, CARBAMAZEPINE EPOXIDE AND CARBAMAZEPINE SIMULTANEOUSLY IN SERUM SAMPLES.

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Carbamazepine is one of the commonly prescribed anticonvulsant in India. The active metabolite of carbamazepine-carbamazepine-10-11 epoxide and recently, the pineal hormone, melatonin have also exhibited anticonvulsant effects. Waters millennium 2010 chromatography manager with a 515 HPLC pump and Waters 24879 dual absorbance UV detector was used. A 25 μl of sample and standard were injected, and the contents of melatonin, carbamazepine epoxide and carbamazepine calculated. Chromatographic separation was achieved by Merck C18 reverse phase. It was quantitated subsequently by exposure to UV light at 210 nm. The retention times of melatonin, CBZ epoxide, and CBZ were 6.3 min, 7.5 min, and 13.9 min respectively. The Mobile Phase consisted of Water: Acetonitrile (70:30) at pH 3.0 adjusted with Orthophosphoric acid at the flow rate of 1 ml/min. Standard curves of carbamazepine, carbamazepine epoxide, and melatonin were obtained. The Limits of detection of melatonin is about 800pg; carbamazepine epoxide 500pg and carbamazepine 1300pg. A new HPLC method has been developed for simultaneous extraction, separation and quantification of melatonin, carbamazepine epoxide and carbamazepine in serum samples.

P060091

Sensory Modulation of Midbrain Dopamine and non-Dopamine Neurons

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To study the mechanisms of peripheral sensory inputs on midbrain dopamine

(DA) and non-DA neuron activity. We recorded firing activity using single unit recording in paralyzed rats with gallamine and artificial respirator. In non-anesthetized rats, the firing activity of many DA neurons showed a slow oscillation (SO) that followed precisely the frequency of the respirator. This, however, was not observed in chloral hydrate anesthetized rats. Several lines of evidence further suggested that the SO is caused by sensory inputs activated by the artificial respiration. First, the SO largely disappeared when sensory inputs were blocked by chloral hydrate injection. Secondly, the response was not present in all DA neurons recorded. Finally, the response was almost completely blocked when the spinal cord at the level of the foramen magnum were transected. Spinal transection at the level of C4 or below produced little effect on the SO. Combined, these results suggest that the activity of midbrain DA neurons and neighboring non-DA neurons can be profoundly influenced by the peripheral nervous system.

Acknowledgement: This work was supported by MInistry of Science and Technology and National Natural Science Foundation

P060092

Effects of Agmatine on the Proliferation of progenitor Cells from Neonatal Rat Hippocampus

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AIM: To study the effects of agmatine on the proliferation ability of neural progenitor cells from neonatal rat hippocampus. METHODS: Hippocampus of neonatal rat was isolated and made into single-cell suspension, which was cultured in serum-free medium. The survival rate of neural precursor cells incubated with various concentrations of agmatine and efaroxan was tested by CCK-8 kit assay and ^{3}H-thymidine incorporation assay. RESULTS: Cells could continuously proliferate and cultured as floating neurospheres. Agmatine at 1 μmol·L^{-1} and 10 μmol·L^{-1} enhanced the survival rate of neural precursor cells by CCK-8 kit assay, and imidazoline-1 receptor antagonist efaroxan blocked the proliferation effect of agmatine. The same results were observed by ^{3}H-thymidine incorporation assay. CONCLUSION: Agmatine were found to increase the proliferation of neural precursor cells and efaroxan can block the proliferation effect. It suggested that imidazdoline-1 receptor may be related to the proliferation effect.

Key words: neural precursor cells; proliferation ability; agmatine; imidazoline-1 receptor

P060093

ACTION POTENTIAL BURSTS IN CENTRAL SNAIL NEURONS ELICITED BY PROCAINE: ROLES OF IONIC CURRENTS

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The role of ionic currents in the procaine-elicited action potential bursts was studied in an identifiable RP1 neuron of the African snail, Achatina fulica Ferussac, using the two-electrode voltage-clamp method. The RP1 neuron generated spontaneous action potentials and bath application of procaine (10 mM) reversibly elicited action potential bursts (BoP) of the central RP1 neuron in a concentration-dependent manner. Tetraethylammonium chloride (TEA) and tacrine did, while 4-aminopyridine did not, elicit the BoP. Pretreatment with U73122 blocked the BoP elicited by procaine. Voltage-clamp studies revealed that procaine at 10 mM decreased (1) the Ca^{2+} current, (2) the Na^{+} current, (3) the delayed rectifying K^{+} current, (4) the Ca^{2+}-activated K^{+} current and (5) the fast-inactivating K^{+} current. U73122 did not affect the delayed rectifying K^{+} current of the RP1 neuron; however, U73122 decreased the inhibitory effect of procaine on the delayed rectifying K^{+} current. It was concluded that procaine elicited BoP in the central snail RP1 neuron and the effect was closely related to the delayed rectifying K^{+} current and phospholipase C activity of the neuron.

P060094

Characterization of brain cyclooxygenase involved in CRF-induced central activation of sympathoadrenomedullary outflow in rats

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Prostaglandins (PGs) have been shown to be generated either by cyclooxygenase-1 (COX-1) or by cyclooxygenase-2 (COX-2) in the brain. We previously reported that intracerebroventricularly (i.c.v.) administrated CRF (corticotropin releasing factor) activates the sympatho-adrenomedullary outflow by brain PGs-mediated mechanisms in rats (Eur J Pharmacol, 2003). Then in the present study, we tried to characterize which type of COX is involved in the CRF-induced responses in urethane-anaesthetized rats. I.c.v. administrated CRF (1.5 nmol/animal) increased plasma noradrenaline and adrenaline. The CRF-induced increase of plasma catecholamines was reduced by cycloheximide (an inhibitor of protein synthesis) (30 μg/animal). The CRF-induced elevation of plasma catecholamines was attenuated by pretreatment with NS-398 (250 μg and 500 μg/animal, i.c.v.), a highly selective inhibitor of COX-2. On the other hand, pretreatment with SC-560 (250 μg and 500 μg/animal, i.c.v.), a highly selective inhibitor of COX-1, was relatively without effect. These results suggest that central COX-2 is involved in the CRF-induced activation of central sympatho-adrenomedullary outflow in rats.

P060095

Effect of Osthol on Memory Impairment Induced by β-amyloid peptide

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OBJECTIVE: To observe the effect of osthol on learning and memory impairment induced by β-amyloid peptide (β-AP). METHODS: Aggregated β-AP (25～35) 3 μl (1.0 mmol L^{-1}) icv once to mice was used as an Alzheimer's disease (AD) animal model. Mice were administered with osthol (15, 7.5 mg kg^{-1}, ip) for 10 days after β-AP icv, and saline as control. Then learning and memory abilities of mice were detected through Morris water maze. RESULTS: In mice, aggregated β-AP (25～35) could induce obvious learning and memory impairment in Morris water maze test 11 days after β-AP icv. During a six-day water maze training, Osthol significantly improved the learning and memory impairment induced by β-AP ($P < 0.05$ or $P < 0.01$). Osthol decreased the latencies and the distance in mice and improved the corresponding changes in search strategies. The crossing annulus times of β-AP group was 1.50 ±0.99. Those of osthol groups were 2.80 ±0.79 and 3.09 ±1.23 ($P < 0.01$). CONCLUSION: osthol could improve the memory impairment induced by aggregated β-AP (25～35) in Morris water maze test.

KEY WORDS: osthol; β-amyloid peptide; memory; Morris water maze.

P060096

Involvement of spinal cholecystokinin in the attenuation of morphine-induced antinociception following electroacupuncture in rat

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Our previous study has demonstrated that electroacupuncture (EA) produces antinociceptive effect, whereas the antinociception of intrathecal, but not intracerebroventricular, morphine is paradoxically attenuated after EA stimulation, indicating that EA activates two opposing systems (i.e., opioid and anti-opioid systems). In this study, we examined the involvement of cholecystokinin (CCK) in the anti-opioid property following EA stimulation in the spinal cord. EA was applied to ST-36 acupoints, and pain thresholds were assessed by the hind-paw pressure test in male Sprague-Dawley rats. The amount of mRNA expression of CCK and its receptors (CCK-1 and CCK-2) in the spinal cord were determined by reverse transcriptase-polymerase chain reaction.

The attenuation of morphine-induced antinociception after EA was significantly reversed by proglumide, CCK receptor antagonist. And the expression of CCK and CCK-2 receptor mRNA in the spinal cord was markedly increased after EA stimulation. These results suggest that CCK mediated-neural systems in the spinal cord may be involved in the attenuation of antinociceptive effect of morphine after EA.

Key words: electroacupuncture; opioid; anti-opioid; cholecystokinin

P060097

Desensitization of inhibitory presynaptic bradykinin receptors in rat sympathetic neurons

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Activation of presynaptic B2 receptors by bradykinin (Bk) reduces the release of previously incorporated [3H]noradrenaline from primary cultures of rat superior cervical ganglia. Here, we investigated their desensitization.

Bk (1uM) reduced [3H] overflow triggered by 40 mM K^+ by 28.9, 15.2, and 4.5 % when present for 2, 4, or 8 min prior to the stimulation. In cultures treated with phorbol-12-myristate-13-acetate (PMA, 1 uM for 24 h) to reduce protein kinase C (PKC), these values of inhibition were 59.9, 55.3, and 30.1 %. In the presence of the PKC inhibitor bisindolylmaleimide I, Bk elicited inhibition of tritium overflow by 46.9, 33.3, and 14.1 %. In perforated patch recordings of whole-cell Ca^{2+} currents, Bk reduced current amplitudes, but this inhibition was lost when the peptide was present for more than 3 min. In neurons treated with PMA or in the presence of the PKC inhibitor, the inhibition was maintained for more than 5 min in the presence of Bk.

In conclusion, inhibitory B2 receptors of sympathetic neurons desensitize within minutes through an activation of PKC.

Key words: bradykinin, desensitization, presynaptic, calcium channel.

Supported by the Austrian Science Fund, FWF (P15797)

P060098

The roles of cysteinyl leukotrienes and their receptors in PC12 cell death induced by serum deprivation

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Cysteinyl leukotrienes (CysLTs, including LTC4, LTD4 and LTE4) are potent inflammatory mediators, and their receptors include CysLT1 and CysLT2. CysLTs and their receptors are involved in brain injuries. However, whether they mediate the neural cell apoptosis is unknown. To determine this mediation, we performed permanent transfection of CysLT1 or CysLT2 into PC12 cells, and observed the sensitivity to serum deprivation (SD)-induced apoptosis as detected by flow cytometer, MTT reduction assay and double fluorescent staining with Hoechst 33258 and propidium iodide. We found that over-expression of CysLT2 inhibited SD-induced PC12 cell apoptosis. Pranlukast, a CysLT1 antagonist, and Bay u9773, a non-selective antagonist, did not antagonize this change. Interestingly, the receptor agonist, LTD4, protected against SD-induced apoptosis in all PC12 cells (wild-type, CysLT1- and CysLT2-transfected cells); the protective effect was inhibited partially by pranlukast and completely by Bay u9773. Thus, we conclude that CysLTs protect PC12 cell against SD-induced apoptosis through CysLT1 and CysLT2 and that the over-expressed CysLT2 plays more important role.

P060099

Distinct roles of Cysteinyl leukotriene receptor type 1 and type 2 on PC12 cell injury induced by oxygen glucose deprivation

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Cysteinyl leukotrienes (CysLTs) are involved in brain injury after ischemia. Two subtypes of cysteinyl leukotriene receptors, CysLT1 and CysLT2, have been identified and cloned. However, which receptor subtype mediates the ischemic injury remains unknown. To determine this mediation, we performed a permanent transfection to increase CysLT1 and CysLT2 expressions in PC12 cells. Cell death was induced by oxygen glucose deprivation (OGD) and was detected by using flow cytometer and double fluorescent staining with Hoechst 33258 and propidium iodide. OGD-induced cell death was mainly apoptosis. Over-expression of CysLT1 decreased and over-expression of CysLT2 increased OGD induced cell death, indicating that the expression level of the two receptors changed cell sensitivity to OGD injury. CysLT1 antagonist pranlukast (10-6M) protected all PC12 cell from OGD injury, whereas another CysLT1 antagonist montelukast and a nonselective antagonist Bay u9773 did not. Agonist LTD4 (10-8 M) did not mimic OGD injury because it induced much weaker injury. Thus, CysLT1 and CysLT2 play distinct roles in OGD injury in PC12 cells; CysLT1 attenuated while CysLT2 facilitated the injury.

P060100

N-methyl-D-aspartate-mediated neuronal injury via cysteinyl leukotriene receptor 1 in mice

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Excitotoxicity plays a determent role in cerebral injury. Cysteinyl leukotrienes (CysLTs), potent inflammatory mediators, and their CysLT1 receptor are also involved in cerebral injury. The purpose of this study was to determine whether CysLT1 receptor is involved N-methyl-D-aspartate (NMDA) induced excitotoxic injury in mouse brain. Brain injury and the changes in CysLT1 receptor expression were induced by NMDA microinjection (50-150 nmol) into the cerebral cortex, and the effects of pranlukast (0.01 and 0.1 mg/kg), a CysLT1 receptor antagonist, ketamine (30 mg/kg), an NMDA receptor antagonist, an antioxidant edaravone (9 mg/kg) were observed. We found that NMDA induced brain injury, and increased CysLT1 receptor mRNA or protein expression that was mainly localized in neurons. All the agents attenuated NMDA-induced injury, and pranlukast (0.1 mg/kg) and ketamine inhibited the increased CysLT1 receptor expression, but edaravone did not affect the expression. Therefore, the up-regulation of CysLT1 after NMDA treatment and inhibition of NMDA induced responses by CysLT1 receptor antagonist indicating that increased CysLT1 receptor is involved in NMDA excitotoxicity.

P060101

The involvement of 5-Lipoxygenase in acute and late brain injuries after focal cerebral ischemia in rats

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Evidences show that 5-lipoxygenase (5-LOX) is involved in cerebral ischemia. However, its distribution and enzymatic activity after ischemia remain unknown. To determine the 5-LOX changes after brain ischemia, in rats with transient focal cerebral ischemia we observed brain injury and 5-LOX changes from 3 h to 14 days after reperfusion. We found that 5-LOX mRNA and protein levels were increased in the neurons in the ischemic cores 12-24 h, and in the proliferated astrocytes in the boundary zone 7-14 days after reperfusion. The level of cysteinyl-leukotrienes, 5-LOX metabolites, was largely increased 3 to 24 h and mildly increased again 7 days after reperfusion; however, the level of LTB4, another metabolite, was increased mildly 3 h after reperfusion but largely 7-14 days after reperfusion. 5-LOX inhibitor caffeic acid attenuated neurological deficits and neuron loss in the ischemic core 24 h and the injuries 14 days after reperfusion; it also inhibited 5-LOX enzymatic activity. Thus, we conclude that 5-LOX is activated after focal cerebral ischemia, and mediates neuron injury in the acute phase and astrocyte proliferation in the late phase.

P060102

Minocycline protects rat brain against focal cerebral ischemia via inhibiting 5-lipoxygenase activation

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Minocycline possesses anti-inflammatory activity in the central nervous system and protective effects on cerebral ischemia. As a pro-inflammatory molecule, 5-lipoxygenase (5-LOX), a key enzyme metabolizing arachidonic acid to leukotrienes, play a role in ischemic brain injury. In this study, we determined whether minocycline attenuates brain injury via inhibiting 5-LOX expression and activation after the middle cerebral artery occlusion (MCAO) in rats. We found that minocycline (45 mg/kg, injected intraperitoneally for 3 days) attenuated neurological deficits, and reduced infarct volume and neuron loss 72 h after 30 min of MCAO. In addition, 5-LOX mRNA and protein expressions, and the levels of 5-LOX metabolites (LTB4 and cysteinyl-leukotrienes) in the ischemic cortex were increased after MCAO. The increased 5-LOX was primarily localized in the neurons in ischemic core, and in the astrocytes and macrophage/microglia in the boundary zone. Minocycline significantly inhibits 5-LOX expression and production of LTB4 and cysteinyl-leukotrienes. These results indicate that the protective effect of minocycline may be, at least partly, mediated via inhibiting 5-LOX activation.

P060103

Effects of cysteinyl leukotrienes on the edema and expression of aquaporin 4 in cultured astrocytes

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Cysteinyl leukotrienes (including LTC4, LTD4, LTE4) are potent inflammatory mediators that increase brain blood barrier permeability and brain edema after various central diseases. The receptors for cysteinyl leukotrienes have been identified and cloned; their subtypes are CysLT1 and CysLT2. On the other hand, aquaporin-4 (AQP4) is the most abundant aquaporin in the brain and is involved in the water balance after brain injury. To determine whether AQP4 involved in the brain edema is modulated by cysteinyl leukotrienes, we observed the effects of LTC4 in primary cultured astrocytes. LTC4 (10^{-8} and 10^{-7} M) significantly increased cell size and upregulated AQP4 protein levels 24 h after exposure. Bay u9773, a non-selective CysLT receptor antagonist, abolished LTC4-induced AQP4 up-regulation and ameliorated the cell enlargement, while pranlukast, a selective CysLT1 receptor antagonist, showed no effect. These results indicate that AQP4 may be modulated by cysteinyl leukotrienes through activating CysLT2 receptor.

P060104

The effect of swim stress on tolerance and swim stress-inuced analgesia and its interaction with histaminergic system in mice

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The goal of the study is to investigate the effect of swim stress on the tolerance and antinociception induced by swim stress and its interaction with histaminergic system. During our experiments it was observed that three minutes of 20 water swim stress causes antinociception. Repeating of three-minute swim stress for three days causes a reduction in swim stress induced antinociception. It was observed that administration of morphine (50 mg·kg^{-1}) for three days to induce tolerance reduced the antinociception in the acute phase but increased it in the chronic phase of formalin test. Naloxone as a mu receptor antagonist had no effect on the swim stress-induced tolerance. Chlorpheniramine as an H_1 receptor antagonist caused an increase in the antinociception induced by swim stress in the chronic pain phase. Chlorpheniramine and cimetidine (as an H_2 receptor antagonist) both increased morphine-induced antinociception in the chronic pain phase. Hence we suggest that both H_1 and H_2 receptors are involved in the antinociception and tolerance induced by swim stress.

Key words: Formalin test, Tolerance, Antinociception

P060105

Effects of WIN55,212-2 on voltage-gated sodium channels in trigeminal ganglion neurons of rats

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WIN55,212-2 is a potential cannabinoid receptor agonist. This study was conducted to investigate the effects of WIN55,212-2 on voltage-gated sodium currents (INa) in cultured trigeminal ganglion neurons of rats. Whole-cell patch clamp techniques were used. The results showed WIN55,212-2 0.01 μmol/L could enhance INa slightly by 11.5 ±4.7% ($n = 8$, $P < 0.05$), this effect couldn't cancelled by AM251, the CB_1 receptor antagonist. However, WIN55,212-2 could inhibit INa in concentration dependent manner at higher concentration. The inhibition rate were 17.4 ±6.0%, 22.5 ±7.8%, 43.9 ±9.4%, 73.9 ±6.7%, respectively by 0.1, 1, 10, 100 μmol/L WIN55,212-2 ($n = 7$, $P < 0.05$ or $P < 0.01$). This inhibitory effect could be cancelled partly by 1 μmol/L AM251 ($n = 7$, $P < 0.05$). Both WIN55,212-2 0.01 μmol/L and 10 μmol/L produced a slight leftward shift on the activation curve of INa ($n = 7$, $P < 0.05$). WIN55,212-2 0.01 μmol/L had no obvious effect on the stable inactivation curve of INa ($n = 7$, $P > 0.05$), but WIN55,212-2 10 μmol/L affected it to a 5 mV negative shift ($n = 7$, $P < 0.05$). We concluded that WIN55,212-2 had bidirectional effects on INa. It might act on different receptors, and the CB_1 receptor participated in its modulation on INa.

P060106

MK801 blocks acquisition but not expression of conditioned opiate withdrawal in acute-dependent rats

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Limited preclinical research has been conducted investing the neurobiologyical mechanism underlying the negative motivational component of withdrawal from acute morphine dependence. It has been shown that NMDA receptors involve in anxiety and opiate dependence. Therefore, the present study extended these findings by examining MK801's effect in the acquisition and expression of conditioned affective reaction of morphine withdrawal employing a conditioned place aversion (CPA) paradigm in rats. Those data indicate that MK801 blocked the acquisition of CPA when administered prior to naloxone on each conditioning trial, but was ineffective in blocking the expression of CPA when administered prior to the test session. Those effects of MK-801 were caused neither by hyperactivity nor by the impairment of learning and MK801 produced no place conditioning by themselves in either morphine-nave or morphine-exposed subjects. These results indicate that NMDA antagonist may play a role in the negative affective aspect of withdrawal from acute dependence, and in part suggest that the acquisition and expression of CPA may involve different neurobiological mechanisms.

Keywords: MK801; withdrawal; place aversion; morphine

P060107

Heme oxygenase-1 protects MPTP-induced dopaminergic neuronal death

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MPTP constitutes the best-characterized toxin paradigm for Parkinson's disease, faithfully replicating most of its clinical and pathological hallmarks. Heme oxygenase (HO) catalyzes the conversion of heme to biliverdin with the release of iron and carbon monoxide. HO^{-1}, a stress-responsive enzyme, has previously been shown to protective the cells from several oxidative stress. In this study, the protective effects of HO^{-1} on the MPTP-induced dopaminergic neuronal death in striatum and substantia nigra and generation of Parkinsonism.

Preconditioning with 8 mg/kg MPTP (s.c.) induced expression of HO^{-1} in striatum and substantia nigra in C57BL/6 mice. MPTP (40 mg/kg, s.c.)-induced dopaminergic neuronal death in striatum and substantia nigra was significantly attenuated by preconditioning with 8 mg/kg MPTP. MPTP-induced decreases of behavioral parameters (locomotor activity, motor coordination and grip strength) were significantly attenuated by preconditioning. These results suggest that HO^{-1} has a protective effects against MPTP-induced dopaminergic neuronal death in striatum and substantia nigra and HO^{-1} can inhibit the generation of Parkinsonism.

P060108

Effect of extracts of astragalus on hippocampal delayed neuronal death in rats

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The global cerebral ischemia-reperfusion model was established by four-vessel occlusion 10 min and 7d reperfusion to study the effect of extract of astragalus (EA) on hippocampal delayed neuronal death in rats. Electron microscope was used to observe the ultramicrostructure of dorsal hippocampal neurons. Light microscope was used to survey the structure of hippocampal neurons and to count the number of normal neurons in CA1 sector. Glial fibrillary acidic protein (GFAP) was detected by immune histochemistry. Compare with ischemia-reperfusion group (I/R), EA can improve the ultrastructure of hippocampal neurons, suppress the decrease of normal neurons in CA1 and degrade the expression of GFAP. The number of GFAP positive cells in I/R group was 69 ±10.7, in EA (20, 40, 80 mg·kg^{-1}) groups, 53 ±5.6, 39 ±7.1 and 46 ±7.6 respectively. The results show that EA can restrain hippocampal delayed neuron death of global ischemia and 7d reperfusion in rats. It maybe suppress hyperplasy of astrocytes in hippocampal CA1 sector.

Key Words: extract of astragalus; delayed neuron death

Supported by the Nature Science Foundation of Anhui Province (No. 00144414) and the "Shiwu" Technology Foundation of Anhui Province (No. 01803016).

P060109

γ- Schisandrin Inhibits Expression of Amyloid β- Peptide 42 in M146L Cell

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Objective: To investigate the effect of γ- schisandrin (γSc) on the production of $A\beta_{42}$ secreted by CHO cells (M146L) transfected by both APP gene and PS- 1 gene of the patient with Alzheimer's disease. Methods: M146L cells were treated by γSc (1.67, 5, 15 $\mu g \cdot ml^{-1}$). CCK- 8 was used to assess cell viability. ELISA was carried out to determine the alteration of $A\beta_{42}$. Weston blot was used to test C99. β- Secretase (βS) and γ- secretase (γS) assay kit were used to detect βS and γS activity. Results: The CCK- 8 test indicated that different concentrations of γSc had no effects on cell activity and survival, and the ELISA test showed that the quantity of $A\beta_{42}$ secreted by the M146L cell in γSc- treated groups decreased obviously as compared with solvent control. The Weston blot test indicated that the C99 in γSc- treated groups did not increase, but in these groups their γS activities decreased obviously. Conclusions: The γSchi can inhibit expression of Aβ42 in M146L cell and its target is likely to be γS.

Key words: γ- Schisandrin; Alzheimer's disease; amyloid β- protein; secretase

P060110

Ketamine enhances the effect of peripheral electrical stimulation on mechanical allodynia in rat model of neuropathic pain

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Our previous studies have shown that 2 Hz peripheral electrical stimulation (PES) produced analgesia via releasing endogenous opioid peptides which activate μ opioid receptors. The present study aimed to examine whether ketamine, an NMDA receptor antagonist, can enhance anti- allodynia effects induced by 2 Hz PES in the rat model of neuropathic pain following spinal nerve ligation (SNL). The mechanical withdrawal threshold was determined with the method described by von Frey filaments. The results are as follows: (1) PES itself or i.p. injection of ketamine reduced the mechanical withdrawal threshold. (2) Although injection of ketamine at low dose (1.0 mg/kg) alone did not influence mechanical withdrawal threshold, combination of ketamine at this dose with PES had much more potent anti- allodynia effect than that induced by PES alone. (3) The anti- allodynia effect of PES combined with ketamine could be reversed by i.p. injection of naloxone (2.0 mg/kg). These results suggested that ketamine could potentiate anti- allodynia of PES in neuropathic pain, and endogenous opioid system might be involved in this processing.

P060111

Anti- inflammatory and Neuroprotective Effect of KJ0530 in Cerebral Ischemic Insult: Down- regulation of Rho GTPases

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In cerebral ischemic insult, a chemoattractant monocyte chemoattractant protein 1 (MCP- 1) is produced by various kinds of cells such as endothelial cells and microglia. Over- expression of MCP- 1 is associated with recruitment of inflammatory cells into the lesion and may lead to modulating the degree of ischemic brain injury.

In the present study, KJ0530 (1 mg/kg, i.v. injection) attenuated the cerebral ischemic injury and reduced the

recruitment of ED1- positive microglia/ macrophages in rat brain ischemic lesion. We found that KJ0530 (as low as 10 nM) inhibited the migration activity of microglia through the down- regulation of Rho GTPases (including Rac, Cdc42 and Rho), chemotactic sensing and directed motility. Currently, the intracellular signaling molecules regulating the expression of Rho GTPases are under investigation. Understanding the exact neuroprotective mechanism of KJ0530 may provide a therapeutic strategy for anti- inflammatory response in neurodegenerative diseases.

P060112

Postischemic Treatment With Total Saponins of Panax Notoginseng Attenuates Brain Inflammation After Focal Cerebral Ischemia Reperfusion in Rats

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Objective: To investigate the effect of Total saponins of panax notoginseng(PNS) on brain inflammation after focal cerebral ischemia reperfusion in rats when the treatment was delayed at 4 hours after the onset of ischemia.

Methods: Focal cerebral ischemia model in rats were induced by middle cerebral artery occlusion(MCAO) for 2 hours and followed by 24 hours reperfusion. PNS 25 or 50 mg/kg were administered at 4 hours after the onset of ischemia. After 24 hours reperfusion, brain edema, neutrophil infiltration, the level of interleukin- 8(IL- 8) and the expression of ICAM- 1 and P- selectin in the cerebral ischemic tissue were measured with dry- wet weight, myeloperoxidasse (MPO) activity, radioimmunoassay and immunohistochemistry, respectively.

Results: PNS 50 mg/kg reduced brain edema, decreased the level of IL- 8, inhibited neutrophil infiltration and the expression of ICAM- 1 and P- selectin($P < 0.01$) compared with MCAO model group.

Conclusion: PNS attenuated brain inflammation following cerebral ischemia reperfusion in rats when treatment was delayed at 4 hours after the onset of ischemia.

Key words: Total saponins of panax notoginseng, Cerebral ischemia, Neutrophil, Cell adhesion molecules

P060113

Effects of xanthine on adenosine A1- receptor responses in rat hippocampus

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We have observed that the free radical- generating mixture of xanthine and xanthine oxidase (X/ XO) can suppress the inhibitory effects of adenosine on synaptic transmission in the hippocampus, but that this action can be mimicked by xanthine alone. We have now clarified the mechanism of these interactions by using the new, potent and highly selective inhibitor of xanthine oxidase, 1- (3- cyano- 4- neopentyloxyphenyl) pyrazole- 4- carboxylic acid (Y- 700). Field excitatory postsynaptic potentials (fEPSPs) were recorded in the CA1 region of rat hippocampal slices. X/ XO induced a long- lasting increase of fEPSP slope and significantly reduced the presynaptic inhibitory effect of adenosine. Both these actions were prevented by Y- 700 at a concentration of only 200 nM. Similarly the superfusion of xanthine alone increased fEPSP slope and reduced sensitivity to adenosine but these effects were also prevented by Y- 700. The results indicate that the antagonism of adenosine responses by X/ XO or by xanthine alone are entirely attribute to the activity of the added or endogenous XO activity, probably generating free radicals.

Keywords: Adenosine; Xanthine; Hippocampus; Superoxide

P060114

SKF83959 stimulates Ca^{2+} signals in primary cultured hippocampal neurons

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The present work was to characterize the Ca^{2+} signal regulated by PI- linked D1 dopamine receptor agonist SKF83959 in primary cultured hippocampal neurons by calcium imaging. The results indicated that SKF83959 induced a long- lasting increase of basal $[Ca^{2+}]i$ in a time- and dose- dependent manner. The sustained elevation of $[Ca^{2+}]i$ was depend on both intracellular Ca^{2+} release and Ca^{2+} influx. 1 μM thapsigargin abolished SKF83959- induced stimulation of Ca^{2+}, indicating that the initial phase of Ca^{2+} increase from intracellular stores triggered the late phase of Ca^{2+} influx. Activation of PLCβ IP3 was responsible for the drug- induced Ca^{2+} release from intracellular stores. Both Cd2+ and nifedipine largely attenuated SKF83959- induced $[Ca^{2+}]i$ increase. 10 μM APV but not 10 μM CNQX reduced SKF83959- induced late phase of $[Ca^{2+}]i$, indicating that L- type calcium channel and NMDA receptor channel contributed to PI- linked D1 receptor- regulated $[Ca^{2+}]i$ changes.

Key words: SKF83959; dopamine receptor; Ca^{2+} signal

Acknowledgement: This work was supported by grants from National Distinguished Young Scientists of China (30425024) and NSFC- RGC Joint Foundation (30418016) to Dr. Chen J.

P060115

NO and ATP co- mediate the non- adrenergic, non- cholinergic (NANC) relaxation in the human colon and rat ileum

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Two P2 purinoceptor antagonists PPADS and suramin were used to assess the possible role of ATP in two intestinal preparations.

Methods. Isolated organ experiments were performed on the human sigmoid colon circular muscle strip (mucosa - free) and the rat ileal longitudinal muscle - myenteric plexus strip. Atropine and guanethidine were used to maintain NANC conditions. Both kinds of strip were pre - contracted and field stimulated (EFS).

Results. An inhibition of the NO synthesis reduced the NANC relaxation in both preparations. The responses were further inhibited (in most cases fully abolished) by the P2 purinoceptor antagonists PPADS (50 micro M) or suramin (100 micro M). The purinoceptor antagonists alone caused only weak inhibitions.

Conclusions. NO and an endogenous P2 purinoceptor stimulant (probably ATP) co - mediate the NANC relaxation in these preparations. A supra - additive relationship between NO and ATP is proposed.

Acknowledgements. This study was supported by Hungarian ETT and OTKA grants.

P060116

Effects of ATP and alpha, beta - methylene ATP (ABMA) and their inhibition by PPADS in the non - stimulated and field - stimulated guinea - pig ileum

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The effects of the putative neurotransmitter ATP and of its stable analogue and P2 purinoceptor agonist ABMA were studied in the guinea - pig ileum in vitro, under basal and stimulated conditions (electrical field stimulation).

Results: Three motor effects of ATP were detected: (a) relaxation of the pre - contracted ileum, (b) quick cholinergic contraction, (c) atropine - resistant slower contraction of non - precontracted preparations. ABMA only caused cholinergic contraction. All these effects were significantly inhibited by the P2 purinoceptor antagonist PPADS, in a specific manner. The effect of ABMA showed a marked tachyphylaxis. Tachyphylaxis to ABMA caused a reduction of cholinergic contractions in response to electrical stimulation.

Conclusions: ATP may be involved in the regulation of intestinal movements. ABMA stimulates myenteric cholinergic motoneurons through P2 purinoceptors. These neurons probably have purinergic inputs through ABMA - sensitive (and - desensitized) receptors, which contributes to the contractile effect of field stimulation, i.e., the cholinergic twitch of the guinea - pig small intestine includes a presynaptic purinergic component.

Supported by Hungarian OTKA, ETT grants.

P060117

The involvement of central cholinergic system in the analgesic effect of intracerebroventricularly injected CDP-choline in acute pain models of rats

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In the present study, intracerebroventricularly injected CDP - choline (0.5, 1, 2 micromoles) induced dose and time dependent antinociceptive effects in thermal and mechanical paw withdrawal tests and acetic acid writhing tests in rats. Equimolar dose of CDP - choline (1 micromole) and choline (1 micromole) caused similar antinociceptive effects while cytidine (1 micromole) produced small, transient but statically significant antinociceptive effect. Mecamylamine, MLA and HC - 3 pretreatments completely antagonized CDP - choline induced antinociception in acute thermal and mechanical tests while HC - 3, MLA, mecamylamine and atropine pretreatments partially blocked the antinociceptive effect of CDP - choline in the acetic acid writhing test. CDP-choline did not impair motor performance of rats evaluated by rota - rod test. These results indicate that centrally administered CDP - choline induced dose and time dependent antinociception in rats by activating mainly central cholinergic nicotinic receptors through the activation of presynaptic cholinergic mechanisms.

Key Words: CDP - choline, antinociception, acute pain, cholinergic

Acknowledgement: Thanks to the Research Fund of Uludag University (T - 2003/ 37)

P060119

Analgesic and sedative effects of the polysaccharide extract from Plantago sp - an experimental study

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The present study is an experimental research of possible analgesic and sedative effects of the polysaccharide extract from Plantago sp. After extraction of the polysaccharidic fraction, studies of experimental pharmacology were conducted on mice.

The writhing test for the analgesic effect and the exploration test for the sedative effect were used.

The polysaccharide extract had a rapid analgesic effect (at 15 minutes) which lasted shortly (max. 30 minutes). A dose - effect relationship was present. The sedative effect was significant at 30 and 120 minutes after the test solution administration, but wasn't significant at 60 minutes. A dose - effect relationship was present.

The polysaccharidic fraction from Plantago lanceolata has a rapid, short and dose - dependent analgesic effect.

The same fraction has a slower and longer dose - dependent sedative effect. It is possible to exist more than one substance with a sedative effect contained in the polysaccharide extract.

Keywords: Plantago sp., analgesia, sedation.

P060120

Phorbol 12 - myristate 13 - acetate (PMA) induced ear inflammation in transient receptor potencial vanilloid 1 (TRPV1) receptor transgenic mice

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The aim of our study was to examine the participation of sensory neurogenic components especially the role of TRPV1 receptors in PMA - induced ear inflammation using TRPV1 receptor transgenic mice. Inflammation was induced by smearing of PMA dissolved in acetone. Ear thickness was measured by micrometer. Myeloperoxidase (MPO) activity, IL - 1b content and histopathological changes were detected. A group of animals received systemic resiniferatoxin (RTX) pretreatment. PMA - induced oedema formation, MPO content and histopathological scoring did not show difference in TRPV1 + / + and - / - animals but oedema formation after contralateral acetone treatment was decreased in TRPV1 - / - mice. The local IL - 1b concentration in the contralateral acetone - treated ears was significantly enhanced. This effect was attenuated in RTX - pretreated mice. We conclude that potentiating action of PMA on contralateral acetone - induced ear oedema might be due to the release of IL - 1b which sensitizes the capsaicin - sensitive afferents. PMA - induced ear swelling has a strong neurogenic but TRPV1 independent component itself.

Keywords: PMA, ear, inflammation, TRPV1

Grants: The Wellcome Trust, OTKA - T046729, RET 008/ 2005

P060121

Inhibitory effect of the selective sst_4 receptor agonist J - 2156 on nocifensive behaviour in acute and chronic pain models of mice and rats

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Somatostatin released from capsaicin - sensitive sensory nerves exerts systemic anti - nociceptive actions presumably via the somatostatin receptor subtype 4 (sst4). In the present study the anti - nociceptive effects of a novel, sst_4 selective peptidomimetic compound, J - 2156 (1 - 100 μg/ kg i.p.) were examined. J - 2156 inhibited nocifensive behaviour of Balb/ c mice in the second, acute inflammatory phase of the formalin test. Adjuvant - evoked chronic inflammatory mechanical allodynia was decreased in Lewis rats treated with J - 2156 throughout 21 days. Partial sciatic nerve ligation - induced mechanical hyperalgesia in Wistar rats was inhibited by J - 2156 on the 7th postoperative day. These findings show that J - 2156 potently inhibits acute chemonociception and diminishes chronic inflammatory and neuropathic mechanical allodynia and hyperalgesia, therefore, provides novel perspectives for analgesic therapy.

Keywords: somatostatin, adjuvant - induced inflammation, traumatic mononeuropathy

Grants: OTKA F-046635, T-043467; RET-008/2005.

P060122

Functional changes of P2X3 and P2X2/3 receptors in dissociated small DRG neurons under neuropathic condition induced by spinal nerve ligation in rats

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This study is aimed to explore the upregulation and mechanism of P2X receptor responses to ATP in freshly dissociated DRG neuron of neuropathic rats. The L5-6 DRG of the spinal nerve ligation model were removed, dissociated and plated in culturing media for >12 h. Isolectin IB4 antibody was used to identify P2X positive small DRG neurons. Whole-cell recordings at -60 mV were made to measure P2X response to fast perfusion of ATP. Compared to naive, the neuropathic DRG neurons showed greater amplitude of responses to ATP.

Pretreatment of 1 mM staurosporine for 5 min decreased ATP-induced response in neuropathic DRG neurons to 67.1 ±6.69%. Neuropathic DRG neurons also exhibited longer duration of response with channel kinetic resembling that of a mixed P2X3 and P2X2/3 responses in >80% of the neurons tested, whiles naive DRG neurons predominantly showed P2X3 like response. The data indicate that P2X3 and P2X2/3 receptor mediated response of DRG neurons to ATP is dramatically potentiated under neuropathic conditions. The mechanism of this potentiation may be due to receptor phosphorylation by an undetermined protein kinase. There is also an indication of increased P2X2/3 expression under neuropathic states.

P060123

Antagonistic Effects Of Bushen Decoction On Apoptotic PC12 Cells Induced By Glutamate Via Modulating Intracellular Ca^{2+} And Phosphorylation Of CaMKII

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Recently, we found that the serum of Bushen decoction (BS) in 20% concentration shows antagonistic effect on neurotoxicity induced by glutamate (Glu) in the PC12 cells. Here, we attributed this phenomenon to the calcium signal cascade modulated by BS. The model of apoptotic PC12 cells induced by Glu was erected. Flow Cytometry technique was employed to observe the variation of the intracellular calcium concentration ($[Ca^{2+}]i$). Western-blot assay was applied to detect the phosphorylation of CaMKII. The serum with BS in 20% concentration was discovered to be able to inhibit the increase of $[Ca^{2+}]i$ and the excessive phosphorylation of CaMKII during apoptosis of PC12 cells induced by Glu. Thus, we demonstrated that the mechanism of neuroprotective effect afforded by serum with BS might be related with inhibiting calcium overload and modulating phosphorylation of CaMKII.

Keywords: Bushen; Apoptosis; Calcium; CaMKII

Acknowledgements: This work was supported by National Natural Science Foundation of Shanxi Province (No. 19991091) and National Basic Research Program of China (No. 2004CB518906)

P060124

Castration of piglets under carbon dioxide anaesthesia

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Male piglets are world wide surgically castrated while conscious which is considered a welfare issue. The aim was to study castration of piglets under CO_2 anaesthesia with emphasis on welfare, recovery and anti-nociceptive effect of CO_2. Piglets were anaesthetised in a chamber pre-filled with a mixture of 30% O_2 and 70% CO_2.

Behaviour was observed until unconsciousness. After 1-2 min in the gaseous atmosphere the piglets were surgically castrated. Frozen sections from the lumbar spinal cord of the piglets were stained immunohistochemically for presence of neuronal Fos protein in dorsal horn neurons. Fos positive neurons were quantified stereologically. Unconsciousness appeared after 15 sec. Under introduction of anaesthesia some gasping appeared. Piglets recovered within 30-40 sec. After surgical castration of conscious piglets 14,140 neurons were Fos positive. Piglets castrated surgically after CO_2 anaesthesia for 1 or 2 min expressed only 1,152 or 503 Fos positive neurons, respectively. Thus, CO_2 anaesthesia completely inhibited castration-induced nociception and welfare was improved apart from gasping.

Key words: Castration, piglets, carbon dioxide, antinociception

P060125

EFFECTS OF THE ADENOSINERGIC NEUROMODULATORY SYSTEM ON ABSENCE EPILEPSY AND CEREBROVASCULAR PERMEABILITY

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Effects of adenosine (ADO) on the non-convulsive seizure activity have not been fully understood. For this, ADO agonists and antagonists were administered to WAG/Rij rats with genetic absence epilepsy, and their effects on epilepsy were evaluated with the number and duration of spike wave discharges (SWDs) in EEG. The activity of adenosinergic system on pentlenetetrazole (PTZ) induced convulsive seizures was also evaluated with the scoring of the seizure activity and examination of the cerebrovascular permeability changes. Administration of CA-DO to WAG/Rij rats via icv route caused an increase in the number and duration of SWDs. The ADO antagonists DPCPX and theophylline were caused decrease in the number and duration of SWDs. In the convulsive seizures, ADO increased the seizure latency. Treatment with ADO also significantly decreased the opening of blood-brain barrier (B-BB) during the seizure activity ($p < 0.05$). Our results indicate that adenosinergic system has anticonvulsive effect on convulsive seizures whereas it displays a proepileptic effect on nonconvulsive absence epilepsy. On the other hand, ADO antagonism facilitated convulsive seizure activity and caused increase in the B-BB permeability.

P060126

Separate roles for hippocampal β-adrenoceptors in memory processing.

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Noradrenaline modulates memory formation in the chick via adrenoceptor (AR) activation. Possible roles for the 3 β-ARs were investigated in the hippocampus. Chicks given weakly or strongly reinforced training on a single-trial bead discrimination task learn that a red bead is associated with a bitter taste. On test 2 hr after training, memory is shown by the tendency to avoid a red bead whilst continuing to peck at a blue one, whilst if memory is not consolidated, chicks peck at both beads equally. In vivo injections of selective β1, β2, and β3-AR agonists (RO363, zinterol, CL316243) or antagonists (CGP20712A, ICI115881, SR59230A) were made into the hippocampus at various times after the training trial. We have found differences in the times when memory is vulnerable to inhibition by selective β-AR antagonists or enhancement by selective agonists. Our data indicate a relationship between the β1-AR and long-term potentiation (LTP), while β2-ARs act during the second stage of LTP involving protein synthesis. In contrast, β3-ARs appear to have a role involving astrocytic metabolism. These studies establish important and specialised roles for β-AR subtypes in memory formation.

P060127

Calcium channel blockers potentiated hypnotic effect of pentobarbital through serotonergic system

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This study was undertaken to elucidate the mechanisms behind the interactions between barbiturates and Ca^{2+} channel blockers(CCB) on pentobarbital(PB)-induced hypnosis by using synergism with PB and the polysomnogram was recorded for analyzing sleep architecture. The results showed that bisbenzylisoquinoline alkaloid tetrandrine, dihydropyridine derivative nifedipine and other types of CCB, verapamil and diltiazem(DT) increased both the sleeping time in hypnotic dosage of PB(45 mg/kg, ip) treated mice and the rate of sleep onset in the subhypnotic dosage of PB(28 mg/kg, ip) treated mice in a dose-dependent manner, respectively, and these effects were significantly augmented by 5-HTP, the immediate precursor of 5-HT and antagonized by pretreatment of p-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase. DT, the most potent one used in this study, increased both total sleeping time and SWS, whereas decreased REM sleep in PB treated rats, and these effects were also potentiated by 5-HTP and antagonized by PCPA. These results suggested that the augmentative effect of CCB on PB-induced sleep may be influenced by serotonergic system.

Keywords: CCB, Pentobarbital, Serotonergic system, sleep

P060128

Antinociceptive Activity of Gabapentin in Mice

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Recently There were some reports about antinociceptive activity of gabapentin in addition to its anticonvulsant activity. In the present study we evaluated the central and peripheral antinociceptive activities of gabapentin and the role of serotonergic, nitrergic and opiodergic mechanism in its antinociceptive activity in mice.

Material and Methods: Gabapentin was injected intraperitoneally at 10, 30, 100 mg/kg doses to mice. Ciproheptadine (2 µg/kg), L-NAME (100 mg/kg), L-arginine (100 mg/kg) or naloxone (1 mg/kg) were injected intraperitoneally with 30 mg/kg gabapentin. Hot plate, tail flick and tail clip tests were used for the evaluation of central antinociceptive activity, and stretching test with acetic acide was used for the evaluation of peripheral antinociceptive activity.

Results: gabapentin and ciproheptadine had peripheral antinociceptive activities. Ciproheptadine decreased the peripheral antinocicptive activity of gabapentin. Naloxone did not change the central and peripheral antinociceptive activity of gabapentin. L-arginine decreased peripheral activity of gabapentin, while L-NAME increased central antinociceptive activity of gabapentin.

These results suggest that nitric oxide and serotonin may play a role in the central and peripheral antinociceptive activities of gabapentin but not opioidergic system.

Key Words: Gabapentin, antinociceptive

P060129

$A\beta_{25-35}$ induces synaptic dysfunction in organotypic hippocampal slice culture

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The memory loss of Alzheimer's disease might be due to the synaptic detects of damaged neurons in the hippocampus.

In this study, amyloid peptide $A\beta_{25-35}$ induced neuronal damage and change of presynaptic protein, using organotypic hippocampal slice culture was examined. In the pyramidal layer and dentate gyrus(DG) area, NeuN positive neurons are decreased and propidium iodide(PI) uptake, Fluoro-Jade B staining, and Annexin labeling are dramatically increased in a concentration-dependent manner. Expression of SNAP-25, the presynaptic protein, is severely reduced by $A\beta_{25-35}$ in the stratum radiatum of CA3 subfield and the molecular layer of DG, but that of synapsin, the presynaptic vesicular protein, is increased in the same area. These results suggest that $A\beta_{25-35}$ induced neuronal damage may partially relate to the synaptic dysfunctions.

Keywords: $A\beta_{25-35}$, synaptic dysfunction, neuronal death, organotypic hippocampal slice culture

This study was supported by grants from Korean Research Foundation [R04-2004-000-10019-0]

P060131

Smooth muscle contraction and relaxation by capsaicin via activation of vanilloid receptor TRPV1 and release of acetylchdine in mouse isolated colon and rectum

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Aim: We studied effects of capsaicin, a vanilloid receptor TRPV1 agonist, on smooth muscle tone in mouse isolated colon and rectum.

Methods: The rectum and distal, transverse and proximal colon were surgically isolated from male, ddY mice. The longitudinal change in smooth muscle tone was isotonically measured.

Results: In rectum and distal colon, capsaicin induced transient relaxation followed by transient contraction. Meanwhile, in transverse and proximal colon, only transient contraction was observed after the application of capsaicin. The reactivity to capsaicin in rectum and distal colon is more sensitive than that in transverse and proximal colon. Tetrodotoxin and the TRPV1 receptor antagonist iodo-resiniferatoxin almost abolished the capsaicin-induced transient relaxation and the transient contraction. Moreover, atropine markedly inhibited the transient contraction.

Conclusion: The present results suggest that TRPV1-expressed sensory nerves facilitate lower gastrointestinal motility through release of acetylcholine and/or other neurotransmitters.

Key word: capsaicin; sensory nerve; vanilloid receptor

P060132

Analgesic efficacy of CP 55940 in combination with diclofenac in rodents

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OBJECTIVE: To evaluate the possible enhancing effect of a non-selective agonist of cannabinoid receptors, CP 55940, to analgesia induced by NSAIDs, namely diclofenac. METHODS: Both substances were tested in mice in writhing test (using intraperitoneally administered acetic acid 0.7% 30 min. after s.c. administration of studied substances). Measurement of nociceptive response was started after another 30 minutes and lasted 20 minutes. In order to investigate analgesic efficacy of CP 55940 + diclofenac in rats, plantar test was used (measurement in 1, 3 and 6 h after s.c. administration of carrageenan in right hind paw). RESULTS: The combination of CP 55940 along with diclofenac was significantly more effective than placebo as well as than diclofenac, both 1 and 3 mg/kg ($P < 0.05$) in mice. The same combination provided analgesic efficacy in all measured intervals ($P < 0.05$), while both substances administered as monotherapy induced a low degree of analgesia only. CONCLUSION: CP 55940 has been shown to significantly increase the analgesia induced by diclofenac. Nevertheless, the treatment was accompanied by either sedation or agitation of animals.

Supported by: GAUK 74/2005/C/3.LF and VZ: MSM0021620816

P060133

Neuroprotective Effects of the Bidogically Active Components From Traditional Korean Medicine on the Brain Ischemia in Rats

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The purpose of the study is to observe the neuroprotective components of herbs which have been used to treat stroke in traditional Korean medicine. For global cerebral ischemia, male wister rats weighing 180 ± 10 g were used and common carotid arteries and vertebral arteries were occluded for 10 min. For focal cerebral ischemia, male SD rats weighing 300 ± 10 g were used and the right middle cerebral artery were occluded for 2 hrs. As the results, decursin (10 mg/kg) showed 36.46%, gomisin A, (30 mg/kg) showed 41.18% of neurorprotection in global cerebral ischemia. Nodakenin (30 mg/kg), wogonin (30 mg/kg) showed 61.33% and 42.67% of neuroprotection effect compared with control, respectively. Gomisin A showed the highest effect as 64.67% of neuroprotection in focal cerebral ischemia. In conclusion, it could be suggested that gomisin A in Schizandra chinensis, decursin and nodakenin in Angelica gigas, wogonin and baicalein in Scutellaria baicalensis are effective components for the treatment of stroke.

Key words: Scutellaria, Schizandra, Angelica, brain ischemia, stroke

Acknowledgement: This work was supported by grants from the Korea Food & Drug Administration (KFDA).

P060134

Static Magnetic Field Induced Analgesic Effect in Mice May Be Mediated by Opioid System

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The analgesic effect of magnetic fields in humans has widely been studied. The aim of the present work was to examine i) the effect of static magnetic field (SMF) on inflammatory visceral pain under whole-body exposure, ii) the possible mechanism of analgesic action induced by SMF. Method: pain reaction was elicited by 0.6% acetic acid injected intraperitoneally. The number of writhings was determined both in control group and in animals exposed to SMF. In order to analyse the mechanism of analgesic action opioid receptor antagonists were given sc. before the acetic acid. Results: SMF decreased the number of writhing during the 30 min observation period significantly (80 + 7 vs 37 + 4). Naloxone antagonised the SMF-induced analgesia, naltrindole (deltareceptor antagonist) also reduced it, but to less extent, nor-binaltorphimine (kappa-receptor antagonist) failed to affect the analgesic action. Conclusions: i) It was first demonstrated that SMF induces an opioid-mediated analgesia under experimental condition in mammals. ii) After determination of the optimized parameters of SMF, human studies can start.

Keywords: static magnetic field, analgesia, opioid receptor

The work was supported by ETT 389/2003

P060135

Inhibitory P2Y receptors and facilitatory P2X receptors modulate the release of neurotransmitters in the rat spinal cord

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In this study the modulation of [3H] noradrenaline (NA) and [3H] glutamate release by P2 receptors were investigated in rat spinal cord slices. ATP, ADP and 2-methylthioADP (2meSADP) decreased the electrical stimulation-evoked [3H] NA efflux with the following rank order of agonist potency: 2MeSADP > ADP > ATP.

The inhibitory effect of ATP was reversed by reactive blue 2 (RB2, 30 uM) and by 2-methylthioAMP (2-MeSAMP, 10 uM), and partly by MRS2179 (10 uM), but not by suramin (300 uM) and PPADS (30 uM). On the other hand, 2-methylthioATP (2-MeSATP, 10-300 uM), and ADP at a lower concentration range increased electrically evoked [3H] NA overflow. The facilitatory effect of 2-MeSATP was antagonized by PPADS and by NF449, (100 nM), but not by MRS2179. When the release of [3H] glutamate measured, ATP, 2-MeSATP, and 2-MeSADP all decreased electrically evoked tritium overflow, with the following rank order of agonist potency:

2MeSADP > ATP > 2-MeSATP. The effect of ATP was fully antagonised by suramin and by 2-MeSAMP, and partly by MRS2179, and PPADS.

In conclusion the release of NA and glutamate are subject to inhibitory modulation by P2Y12/13 receptors and facilitatory modulation by P2X1 receptors in the spinal cord.

P060136

Protection of GBE50 against excitatory and oxidative injury on cultured rat cerebral cortical neurons

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This study was performed to examine the protection of new Ginkgo biloba extracts (GBE50) and contained flavonoids and ginkgolides against excitatory injury and oxidative stress on cultured rat cerebral cortical neurons.

The neurons were checked by immunofluorescent methods. The drugs were added 30 min before the injury induced by oxidative stress (OH or H_2O_2) and excitatory damage (glutamate or NMDA). The neuronal survival was evaluated by the assays of methyl tetrazolium and lactate dehydrogenase. The results showed that the neuron viability decreased by glutamate or NMDA was improved by GBE50 in a dose-dependent manner. The flavonoids and ginkgolides showed protective effects on these cultured neurons in different extent. Oxidative stress by OH or H_2O_2 caused obvious injury. GBE50 and flavonoids produced dose-dependent protection against this oxidative damage. So GBE50, flavonoids and ginkgolides can protect the cultured rat cerebral cortical neurons against excitatory injury and oxidative stress in different extent.

Key Words: GBE50, cultured neurons, excitatory injury, oxidative stress.

Acknowledgement: This research was funded by "863" Project of Chinese government (No.2003AA2Z2032).

P060137

The action of bradykinin in rat cultured myenteric neurons is modulated by prostaglandin E_2 released from enteric glial cells

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To examine effects of bradykinin (BK) in the enteric nervous system (ENS), we investigated intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) and membrane potentials in response to BK in a primary culture of myenteric neurons isolated from neonatal rat. BK evoked a dose-dependent increase of $[Ca^{2+}]i$ that was abolished by a B2 receptor (B2R) but not a B1 receptor antagonist. Immunostaining indicated that B2R expressed in both neurons and glial cells. The BK-evoked $[Ca^{2+}]i$ increase was suppressed by cyclooxygenase (COX) inhibitors, and potentiated by prostaglandin E2 (PGE2). BK facilitated PGE2 release from cultured myenteric plexus cells. The increase of $[Ca^{2+}]i$ induced by BK in neurons was attenuated when myenteric plexus cells were cultured at low density and proliferation of glial cells was suppressed. BK evoked slow and sustained depolarization in neurons, which was sensitive to a COX inhibitor. These results suggest that BK activates B2R, resulting in $[Ca^{2+}]i$ increase and depolarization of enteric neurons, which were partly associated with PGE2 released from glial cells in response to BK and thus neuron-glial interaction play an important role in the functional relation of actions of BK in rat ENS.

P060138

Contribution of an autophagic mechanism in Kainic Acid-induced excitotoxicity in rat striatum

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AIM: To study the effects of an autophagy/lysosomal pathway in excitotoxicity mediated by Kainic Acid (KA) receptors. METHODS: Rat excitotoxic model was produced with stereotaxic administration of kainic acid into unilateral striatum. The neuroprotective effects of the autophagy inhibitor 3-methyladenine (3-MA) and the lysosomal cathepsin B inhibitor Z-FA-FMK were assessed with internucleosomal DNA fragmentation and Cresyl violet staining. Effects of 3-MA or Z-FA-FMK on KA-induced releasing of Cyto-C from mitochondria to cytoplasm, caspase-3 activation, Bcl-2 downregulation were detected with Western blot analysis. RESULTS: Pretreatment with 3-MA and Z-FA-FMK attenuated KA-induced internucleosomal DNA fragmentation and significantly reduced the striatal neuronal loss ($P < 0.01$, $n = 6$), inhibited KA-induced increases in cathepsin B activity ($P < 0.01$, $n = 6$), and inhibited KA-induced releasing of Cyto-C from mitochondria to cytoplasm, caspase-3 activation, and BcL-2 downregulation. CONCLUSION: Autophagy inhibitors and lysosome inhibitors have neuroprotective actions in against KA-induced apoptotic death of rat stritatal neurons by inhibiting autophagy/lysosome-mediated apoptotic signaling pathway.

P060139

The Ontogeny of NADPH-Diaphorase Neuron in Rat Striatal Development

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Ntric oxide synthase is localized in a subpopulation of striatal interneurons that stain selectively for NADPH-d. We studied the ontogeny of diaphorase-positive neurons in striatal sections from E20 to three weeks in rat. NADPH-dstaining was detected in embryological day 21. Over the next seven day in postnatal the number of neurons staining for NADPH-diaphorase increased rapidly. We have investigated the ontogeny of NADPH-d neurons in striatal neurons compared their development in fetal and neonatal rat brain. In particular, we looked for the earliest time of expression of NADPH-d; the increase in NADPH-d expression over time; the percentage of striatal neurons expressing NADPH-d; morphological features relating to somata, number and description of neurites, and neuritic branching; and neurchemical characteristics.

Keyword: striatum, NADPHdiaphorase, nitric oxide, synthase, ontogeny

Acknowledements: The authors wish to thank Ms. Mohammadzadeh for computerized analysis and Ms. Hosseini for typing and Mr Zohrehvand for technical assistance

P060140

Anticonvulsant activity of *Harpagophytum procumbens* DC [Pedaliaceae] secondary root aqueous extract in mice.

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In order to throw some light on the efficacy of *Harpagophytum procumbens* DC and provide pharmacological rationale for some of the folkloric, ethnomedical uses of the herb, the present study was undertaken to examine the anticonvulsant effect of *H. procumbens* secondary root aqueous extract (HPE) against pentylenetetrazole (PTZ)-, picrotoxin (PCT)-and bicuculline (BCL)-induced seizures in mice. Phenobarbitone (PBT) and diazepam (DZP) were used as reference anticonvulsant drugs for comparison. HPE (100-800 mg/kg i.p.), like PBT (20 mg/kg i.p.) and DZP (0.5 mg/kg i.p.), significantly delayed ($P < 0.05$-0.001) the onset and markedly reduced the duration of, and antagonized, PTZ-induced seizures. The plant's extract (100-800 mg/kg i.p.) also profoundly antagonized PCT-induced seizures, but only partially and weakly antagonized BCL-induced seizures. Moreover, HPE (100-800 mg/kg i.p.) depressed the central nervous system (CNS) of the mice used. Although the data obtained in the present study do not provide conclusive evidence, it would appear that HPE produces its anticonvulsant effect by enhancing GABAergic neurotransmission, and/or by facilitating GABAergic action in the brain.

Key Words: *Harpagophytum procumbens* secondary root; aqueous extract; anticonvulsant activity.

P060141

A novel synthetic squamosamide cydic analogue (compound FLZ) improves the rat brain mitochondrial dysfunction induced by Aβ25-35 in vitro

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Amyloid beta (Aβ) is thought to play a central role in the pathogenesis of Alzheimer's disease by probably directly leading to mitochondrial dysfunction. This investigation was to study the effect of compound FLZ on the dysfunction of rat brain mitochondria induced by Aβ25-35 in vitro. Mitochondria were incubated with aged Aβ25-35 for 30 min in the presence and absence of FLZ, the function of mitochondria was determined by biochemical and western-blot analysis. The results showed Aβ25-35 not only induced inhibition of the activities of α-ketoglutarate dehydrogenase, pyruvate dehydrogenase, ATPase, and respiratory chain complex IV, increased H_2O_2 and O_2^- production, and decreased the GSH level in mitochondria, but also induced the mitochondria swelling and cytochrome c release from the mitochondria. The addition of FLZ before Aβ25-35 significantly prevented the above toxic effects of Aβ25-35 on the mitochondria, indicating that FLZ protected against the mitochondria dysfunction induced by Aβ25-35.

Key Word: β-amyloid; Mitochondria dysfunction; Compound FLZ

This project was supported by the Ministry of Science and Technology of China (No G2000057010).

P060142

Triptolide inhibits COX-2 expression via NF-kappa B pathway in astrocytes

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Previous investigations have showed that triptolide possessed potent anti-inflammatory and immunosuppressive properties. In the present study, we examined the protective effects of triptolide on the inflammatory response induced by bacterial lipopolysaccharide (LPS) both in vivo and in vitro. Intrahippocampal injection of LPS (4μg) in rats significantly increased the immunoreactivity of glial fibrillary acid protein (GFAP) and cyclooxygenase-2 (COX-2) in the injected region, which was reduced by pretreatment with triptolide (10～50μg/kg) for 5 d. In the cultured human differentiated A172 astroglial cells, LPS (1 mg/L) increased the expression of COX-2 mRNA and protein, the production of prostaglandin E2 (PGE2) and the DNA binding activity of NF-kappa B, which were markedly attenuated by pretreatment with triptolide (0.2～5 μg/L) for 1 h. These results suggested that the protective effect of triptolide on neuroinflammation is mediated by decreasing COX-2 expression, at least partly, via the inhibition of NF-kappa B signaling pathway.

P060143

Improvement effects of 3,4-oxo-isopropylidene-shikimic acid on spatial learning ability on vascular demential rats

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Objective: To study the effects of 3,4-oxo-isopropylidene-shikimic acid (ISA) on spatial learning disorder in rats after left cortex infarction. Method: A focal lesion in the left sensor-motor cortex was induced photochemically using Rose Bengal as a photosensitive dye and cold light beam, then the rats were treated with ISA orally once a day. The cognitive effects of ISA were assessed in rats using the Morris water maze for spatial learning and memory. HE staining and Nissl staining were used to study its mechanism. Result: It was demonstrated that profound deficits in acquisition of this task were produced by unilateral lesions of the sensor-motor cortex. The neuronal morphology was damaged, and neuron loss was detected in the cerebral cortex of the model rats. ISA 100, 50, 25 mg/kg and Hyderdrine (0.6 mg/kg) could improve the learning and memory ability in model rats after administration for 30 days continuously, which was proven by shortened escaping latency and lessened initial angle in Morris water maze testing. ISA also improved the degeneration and necrosis of neuron. Conclusion: ISA improved learning and memory ability in vascular demential rats.

P060144

IRON-INDUCED RETINAL TOXICITY: MECHANISMS AND MANAGEMENT

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Siderosis bulbi caused by retained iron are vision-threatened. An investigation into its underlying mechanisms is crucial.

An iron particle/ $FeSO_4$ or an acrylate chip/ saline was intravitreously administered into one eye of the experimental/ control rat. Electroretinogram (ERG), measurement of reactive oxygen species/ glutamate, and Western blot were performed. The retinas were evaluated histopathologically.

The experimental siderosis caused a drastic ERG b-wave amplitude reduction, and an obvious stimulation in the glutamate release, the hydroxyl radicals formation, and the superoxide dismutase activity in retinal pigment epithelial (majority). This was supported by the Western blot result. There was also an obvious disorganization, and a wide-spreading ferric distribution in the whole retina. The retinal changes were ameliorated by certain ingredients of Chuan Xiong.

The results imply that the experimental siderosis stimulates oxidative stress, and excitotoxicity. This could explain why the toxic iron would further impair the retina, as shown by the ERG results. This is consistent with the pathological results. Importantly, the iron-induced retinal toxicity was protected by defined components.

P060145

The effect of CDK4 inhibitor to AML

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Mutationally activated tyrosine kinases provide a critical survival signal to cancer cells, thus, making such kinases and their downstream effectors attractive targets for cancer therapy. Chosen the receptor tyrosine kinase Flt3 that harbors an activating internal tandem duplication (ITD) in about 25% of AML patients. The use of a Flt3 inhibitor (THRX-165724, Theravance, Inc.) in two Flt3 ITD AML cell lines (MOLM13 and MV4-11) led to the inhibition of the INK4/CDK4,6/Rb/E2F pathway within three hours as reflected by the downregulation of D-cyclin gene expression followed by a decrease in D-cyclin protein. THRX-165724 had no effect on D-cyclin levels or Rb hyperphosphorylation in THP-1 and U937 cells, two AML cell lines that express wildtype Flt3. THRX-165724 did not affect the proliferation or survival of these two cell lines. We used PD 0332991, a highly selective CDK4,6 kinase inhibitor from Pfizer currently in phase I clinical trials for solid tumors.

KEY WORDS: CDK4 inhibitor, AML, FLT3 ITD, APOPTOTIC

P060146

THE PROTECTIV EFFECT OF GB ON CEREBRAL ISCHEMIA-REPERFUSION INJURY RATS

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We intend to explore the effect of GB(Ginkgobalide B) on cerebral ischemia-reperfusion injury and its possible mechanisms.

Rats were operated by 3h ischemia and 21h reperfusion, and iv. GB was given twice from lingua vein at the beginning of ischemia and 3 hours of reperfusion. Effects of GB on neurological defects, infarct size, and activity of SOD, GSH-PX, CAT, LDH, Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase and content of MDA, GSH, NO, LD in brain homogenate were observed. Results implicated GB 8, 4 mg/kg attenuated neurological defects, decreased infarct size. GB 8, 4 mg/kg inhibit the decrease of activity of SOD, CAT, GSH-PX, Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase in the cerebral ischemia-reperfusion rats brain homogenante. GB 8 mg/kg can increase the content of GSH. GB 8, 4, 2 mg/kg can decrease the content of MDA, NO and inhibit the increase of LDH activity. (Compared with vehicle $P < 0.05$). Summing up, GB prevented and treated experimental cerebral ischemia injury, decreased cerebral infarct size, improved neurological defects. The following might be its elements of cerebral protection: making energy metabolism better, antagonism to free radicals injury and acid toxication.

Key words: GB, ischemia-reperfusion

P060147

Neuroprotective effects of the novel compound FLZ on 1-methyl 4-phenylpyridiniumion (MPP^+)-induced neurotoxicity in SH-SY5Y cells

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Novel compound FLZ is a synthetic squamosamide cydic analogue. Previous study indicated FLZ-2A had strong antioxidant effects and might possess neuroprotective property. Therefore, the effects of FLZ on experimental Parkinson's disease (PD) cellular model were investigated. Aggregated α-synuclein is markedly included in Lewy bodies in brains of patients with PD and dementia with Lewy bodies. Release of Cytochrome c from the organellar fraction to the cytosolic fraction is required for activation of the Caspase 3-dependent cascade in apoptosis, and also for α-synuclein aggregation. In the present study, treatment of human neuroblastoma SH-SY5Y cells with 100 μM 1-methyl 4-phenylpyridinium (MPP^+) for 96 hrs induced Cytochrome c released from the organellar fraction to the cytosolic fraction, then the activation of Caspases 3, DNA fragmentation and the increase of the protein and gene expression levels of α-synuclein in the cells. Co-incubation with 0.1 μM and 1 μM FLZ inhibited the apoptosis and above-mentioned neurotoxicity induced by MPP^+. The significance of FLZ in the management of α-synuclein related neurodegenerative disorders was discussed.

Keywords: FLZ, α-synuclein, MPP+, Parkinson's disease

P060148

The alterations of γ-aminobutyric acid A receptor subunits and transporter mRNA expression after focal cerebral ischemia in rats

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By using reverse transcription polymerase chain reaction (RT-PCR) to determine the expression of α1、β2 subunits mRNA of GABA-A receptor and the GABA transpoter (GAT1) mRNA of cortex 7th day after cerebral ischemia, and 30 min before the brain ischemia was administration (i.p) MK-801 (3 mg/kg), LNAME (3 mg/kg) and diazepam (10 mg/kg) The results shown that the relative concentration of both α1 and β2 subunit of GABA in ischemia group were increased when compared with the control and sham group 7th day after cerebral ischemia, there was an significant difference ($p < 0.05$). GAT1 mRNA expression shown significant down-regulated in cortex area, compared with the sham group. ($p < 0.05$). After pretreatment with MK-801, diazepam and L-NAME compared with sham group, MK-801 and diazepam significant decreased the cortex α1 and β2 subunits mRNA expression in cerebral ischemia for 7 days. L-NAME have no significant effect on the two subunit mRNA expression.

Key words: cerebral ischemia; GABA-A mRNA; GAT1 mRNA

The project supported by National Natural Science Foundation of China No. 30171082

P060149

Neuroprotective action of prostaglandin A1 and its mechanisms involving NF-kB inhibition and PPARγ activation in rat models of focal cerebral ischemia

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In the present study, we investigate the neuroprotective effects of PGA1 and its mechanisms involving nuclear factor kappa B (NF-kB) inhibition and peroxisome proliferation-activated receptor γ (PPARγ) activation in rat models of focal cerebral ischemia. PGA1 16.5-66 nmol (icv) diminished infarction volume in a dosedependent manner ($P < 0.01$). Immunohistochemistry revealed that PGA1 significantly inhibited nuclear translocation of NF-kB in neurons in the ischemic cortex ($P < 0.01$). Western blot and RT-PCR analysis indicated that PGA1 could up-regulate the levels of NF-kB inhibitor protein IkBα, decrease phospho-IkB kinase α (pIKKα) protein levels, repress the expression of NF-kB target gene c-Myc mRNA ($P < 0.05$ or $P < 0.01$), and up-regulate the expression of PPARγ protein ($P < 0.05$, $P < 0.01$). The neuroprotective effect of PGA1 was reduced in PPARγ small interfering RNA (siRNA)-treated rats. The current findings provide the first evidence that PGA1 has neuroprotective activity on cerebral ischemic injury, and this effect may be related to blocking NF-kB signal transduction pathway and activating PPARγ. IKKα and PPARγ may be the target sites of PGA1.

Key words: PGA1; cerebral ischemia; NF-kB; PPARγ

P060150

Pharmacology of the electrical field-stimulated human longitudinal vas deferens

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A paucity of human data exists for neuronally stimulated vas deferens. Therefore, the aims were to establish and optimise an electrical field-stimulated (EFS) human longitudinal vas deferens preparation, and to investigate the functional activity of a range of standard compounds using tissue bath techniques. Phasic EFS responses were stable for up to 4 h and inhibited by 1 μM tetrodotoxin, guanethidine and prazosin. Responses were potentiated by noradrenaline, phenylephrine, clonidine, guanfacine, arginine vasopressin, oxytocin, atomoxetine and duloxetine (mean $pEC_{50} \pm$ s.e.mean values of 4.7 ± 0.1, 4.9 ± 0.1, 5.8 ± 0.1, 5.6 ± 0.1, 8.0 ± 0.1, 6.8 ± 0.1, 7.5 ± 0.8 and 6.7 ± 0.4, respectively (all n equal or greater than 3 donors)). Inhibition of EFS response was seen with UK14304, SNC-80, loperamide and NECA (mean $pEC_{50} \pm$ s.e.mean values of 7.7 ± 0.1, 6.8 ± 0.1, 7.5 ± 0.1 and 6.9 ± 0.2, respectively (all $n \geq 3$ donors)). Fluoxetine and U50488 (< 1 μM) were ineffective. These data demonstrate the potential use of human vas deferens as a translational pharmacology assay for investigating effects of compounds at native human GPCR's and noradrenergic transporters.

P060151

Nicotinic receptor activation increases [^{3}H] dopamine uptake and cell surface expression of dopamine transporters in rat prefrontal cortex

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The present study determined the effects of nicotine (NIC) on dopamine (DA) transporter (DAT) function and DAT trafficking in prefrontal cortex (PFC) and striatum after NIC (0.3 and 0.8 mg/kg, free base, s.c., 5-60 min post-injection) or saline. Mecamylamine (MEC; 1.5 mg/kg, s.c., 20 min prior to NIC or saline) inhibition of the effect of NIC was also determined. NIC at 0.8 mg/kg produced a 47% increase in maximal velocity (V_max) of synaptosomal [^{3}H] DA uptake in PFC at 15 and 30 min, compared to saline control. No differences in [^{3}H] WIN35,428 binding in PFC were found between NIC-treated and control groups. Biotinylation assays showed that NIC (0.8 mg/kg; 30 min) produced a 32% increase in DAT cell surface expression in PFC. In contrast, NIC (0.3 and 0.8 mg/kg) did not alter V_max for [^{3}H] DA uptake or DAT cell localization in striatum. MEC completely inhibited the NIC-induced increases in both V_max and cell surface DAT in PFC. These results suggest that the NIC-induced increase in DAT function and localization in PFC is nicotinic receptor mediated, and may play a role in NIC dependence.

Key words: nicotine, transporter, trafficking, prefrontal cortex.

Supported by DA 018372, RR15592 and NARSAD.

P060152

Activation of NF-kB and induction of c-Myc and p53 is associated with 6-hydroxydopamine-induced degeneration of dopaminergic neurons

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To evaluate contribution of NF-kB-dependent induction of cell cycle regulators to degeneration of dopaminergic (DA) neuron in animal models of Parkinson's disease, detailed time-course of DA neuron degeneration as well as changes in the expression of some apoptosis-related proteins were assayed by immunohistochemistry after unilateral infusion of 6-hydroxydopamine (6-OHDA) into rat med forebrain bundle. Degenerative processes of DA neuron began 12 h after 6-OHDA administration as evidenced by apositive silver staining and appearance of TUNEL-positive nuclei in SN. Tyrosine hydroxylase (TH) immunoreactivity decreased from 24 to 48 h and NF-kB was activated from 12 h after 6-OHDA treatment. The levels of c-Myc and p53 increased mainly in DA neurons as revealed by co-localization with TH immunoreactivity. The results suggest that administration of 6-OHDA to med forebrain bundle produces oxidative damage to DNA and activates NF-kB. 6-OHDA-induced rapid degeneration of DA neurons is accompanied by induction of c-Myc and p53. Thus NF-kB mediated apoptotic mechanisms may contribute to oxidative stress induced degeneration of DA neurons.

Key words: 6-hydroxydopamine; Parkinson's disease; NF-kB; P53

Supported by EAP 2004KJ194ZD and TAP 04023048.

P060153

Role of COXs on secondary damage after CNS injury. Is Ca chanel blockers or COX inhibitors more effective?

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Aim: Secondary damage after central nervous system (CNS) injury is driven in part by oxidative stress and CNS inflammation and is mediated by cyclooxygenases (COXs). The rapidly inducible COX2 isoform has been primarily linked to inflammatory processes, whereas expression of COX1 is confined to physiological functions. We research the localization of COX1-COX2 after traumatic brain injury (TBI) and the effect of 2 therapeutic agents that make COX inhibition or not.

Material and methods: 40 rabbit used in 4 groups for developing traumatic brain injury. Different cellular COX1-COX2 expression profiles were analyzed following TBI and compared effect of two therapeutic agents (nimedopine and indomethacine) on COX inhibition by using immunohistochemistry. Results: After TBI at the vessel endothelial, smooth muscle cells and CD68+ microglia/macrophages, COX1-COX2 protein expression related with injury increased.

Conclusion: The accumulation of COX1+ microglia/macrophages that were restricted to perilesional areas affected by acute inflammatory response points the role of COX1 in secondary injury and the COX1 expression must be a pharmacological target and COX2 must be take in hand in this situation.

P060154

(-) Clausenamide inhibit tau protein hyperphosphorylation and protect microtubule in diabetic mice

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The diabetic mouse (DM), induced by streptozotocin (200 mg/kg, i.p.), showed tau protein hyperphosphorylation and destruction of microtubules in hippocampal neurons. The present study is to detect the effects of (-) clausenamide (clau), a chiral compound, on inhibiting tau protein hyperphosphorylation and protecting microtubules using DM. (-) Clau was oral administration at doses of 7.5, 15, 30 mg/kg for 7 weeks in DM, then the behavior assays were performed, the microtubules in neurons of CA1 of hippocampus were detected and immunohistochemistry was used for various antibodies. The DM showed the expression of glycogen synthase kinase-3β and cyclin dependent protein kinase-5 increasing and protein phosphatase-1 decreasing, hyperphosphorylation of tau protein at Ser199/202 sites, destruction of microtubules and ability of learning and memory impaired. (-) Clau inhibited hyperphosphorylation of tau protein and ameliorated neuron lesion and the ability of learning and memory in DM. The results suggested that (-) clau is useful on treating some diseases which show hyperphosphorylation of tau protein and destruction of microtubules.

Key words: (-) clau; tau protein; hyperphosphorylation; microtubule

P060155

BCPT, A Novel Selective Monoamine Oxidase-A Inhibitor: Effect on Monoamine Metabolism in CUS Rat hippocampus

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A series of bioactive compounds from paecilomyces tenuipes has been previously designed and evaluated with the aim of finding the most potent and selective novel monoamine oxidase (MAO) inhibitors to be used in the therapy of neurological and affective disorders. Among them, BCPT, has been characterized in vitro as a potent, irreversible, and mechanism-based inhibitor of the MAO-A isoform with fluorimetricall. The ex vivo effect of BCPT on MAO activity in mouse brain was similar to that observed in vitro, showing more efficacy than in peripheral tissues. The in vivo effect of BCPT on amine metabolism also was evaluated after chronic treatment in chronic unpredictable stress (CUS) rats, the NE, DA, DOPAC, HVA, 5-HT and 5HIAA levels in the hippocampus were measured by high-performance liquid chromatography with electrochemical detection, the results showed that a decrease in the amine metabolites such as DOPAC, 5HIAA, and HVA confirmed MAO-A as the main responsible enzyme of DA, NA, and 5-HT metabolism, and between both MAO isoforms, MAO-A is the one responsible for monoamine metabolism in CUS rat hippocampus.

KEY WORDS paecilomyces; monoamine transmitters; MAO

P060156

Protective effect of triptolide on the TNF-alpha, IL-1 beta and NO production in BV2 cell

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Inflammatory response in the central nervous system mediated by activation of microglia is closely with the pathogenesis of parkinson disease. Tritpolide is an extracts of the traditional Chinese herb that has antiinflammatory effects. In this study, we investigated the inhibitory mechanisms of triptolide on microglia activation. The production of inflammatory mediators, such as tumor necrosis factor (TNF)-alpha and interleukin (IL)-1beta and nitride oxide (NO) was studied in thrombin-stimulated BV2 cells as a model of microglia activation. Triptolide significantly reduced TNF-alpha and IL-1beta and NO production as revealed by ELISA and Griess reaction, respectively. Also tritpolide reduced thrombin-induced mRNA expression of all three inflammatory factors. Moreover, thrombin could induce the activation of p38 MAPK in BV2 cell. The thrombin-induced production of NO was inhibited by the selective p38 MAPK inhibitor SB203580 and the activation of p38 MAPK was inhibited by triptolide. The results suggest that triptolide can inhibit the inflammatory factors in BV2 cell and its effect is mediated through the inhibition of p38 MAPK activation.

P060157

Inhibition of thrombin-induced microglial activation by triptolide protects dopaminergic neurons in the substantia nigra in vivo

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We examined the effects of triptolide on dopaminergic neurons degeneration induced by thrombin in vivo.

Seven days after thrombin injection in the rat substantia nigra (SN), tyrosine hydroxylase immunocytochemistry showed a significant loss of nigral dopaminergic neurons. This cell death was accompanied by localization of terminal deoxynucleotidyl transferase-mediated fluorecein UTP nick-end labeling (TUNEL) staining within dopaminergic neurons.

Intriguingly, triptolide could improve the survival rate of TH-ir neurons in the SNpc to 68% of the non-injected side. The observed neuroprotective effects were associated with the ability of triptolide to suppress the activation of microglia and subsequently the pro-inflammatory cytokine mRNA expression, including tumor necrosis factor TNF-alpha, interleukin-1b and inducible nitric oxide synthase from activated microglia. These results suggest triptolide can protect dopaminergic neurons against inflammatory challenge induced by thrombin.

P060158

Nimodipine Treatment to Assess a Modified Mouse Model of Intracerebral Hemorrhage

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One of the main limitations of intracerebral hemorrhage (ICH) research is lack of reproducible animal models.

ICH appears to be associated with a volume of edema and ischaemic injury surrounding the hematoma that may be reduced by nimodipine treatment. The present study was designed to produce a modified ICH model in mice based on the double-injection method initially developed by Dr. Belayev and accordingly performed to assess the pharmacological effects of nimodipine. ICH was induced by 30uL whole blood injection into the caudate nucleus. The changes for cortical blood flow (CBF) were studied by the technique of Laser Doppler Perfusion Measure (LDPM). Animals were rated on a behavioral test and sacrificed at 72 hours after ICH. The brain hematoma volume and edema were subsequently determined. ICH animals treated with nimodipine had marked improved CBF accompanied by the improvement of forelimb placing performance, though there was no marked difference in the hematoma volume, brain water content. In conclusion, the 30uL whole blood injection closely mimiced natural ischaemic events that occured in human massive ICH and confirmed the anti-ischaemia effect of nimodipine.

Keywords: ICH; nimodipine; mice

P060159

Experimental study on protection and mechanisms of TMP in acute spinal cord injury in Rats

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Methods: Fifty six adult rats were made as spinal cord contusion models at the T9 segment, which caused an acute and moderate injury and then equally divided into three groups at random. The functional recovery of the rats was evaluated using combined behavioral score(CBS) at 24,72,16, hours after injury. At every time after injury, also experiment was done on histology, TXB2 concentration in plasma by means of radioimmunoassay, the expression of ET-1 mRNA of injured spinal cord tissue by reverse transcriptase polymerase (RT-PCR). Results: In the group treated with TMP, the hind limb function of the injured animals recovered at different degrees compared with the simply injured group at the end of 168 hours($P < 0.05$). The results was closely similar with those morphologic findings. The concentration of TXB2 in plasma increased at 24 hours and then progressively improved till the 168 hours. The expression of ET-1 mRNA reached its climax at 24 hours. then, it decreased slowly to normal level at 168 hours. Conclusion: The treatment with TMP can alleviate the damage resulted from secondary injury and thus showed a promising future for treatment of SCI.

Key Words: TMP; SCI; TXB2; rats

P060160

Effects of corticosterone on cytosolic adenylate kinase in the rat hippocampal neurons cultured in vitro

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An increasing number of studies are revealing that prolonged exposure to elevated glucocorticoid levels has been associated with deficits in learning, memory and retrieval. However, the mechanisms involved in this detrimental effect are not well understood. In this work, 5 days after treated with 10^{-5} M corticosterone, cytosolic adenylate kinase (AK1) activity in the rat hippocampal neurons cultured in vitro was determined by the method of High Performance Liquid Chromatography. AK1 levels and expression were also investigated by using immunoblotting and semi-quantitative reverse transcriptase-polymerase chain reaction, respectively. The results showed that 10^{-5} M corticosterone could decrease AK1 activity and levels, as well as downregulate AK1 mRNA levels in contrast to 10^{-7} M corticosterone. These data suggested that exposure to elevated glucocorticoid levels might induce a decrease of AK1 activity by downregulating mRNA levels, indicating that a balance of adenylates at ATP-consuming and ATP-generating intracellular sites might be destroyed. Based on these results, we hypothesized that an abnormity of energy balance might be a mechanism by which corticosterone treatments influence memory.

Keywords: corticosterone, adenylate kinase, hippocampal neurons, energy balance

Acknowledgents: The study was supported by the following grants: Scientific Research and Development Program Tsinghua University (A2002162). Beijing Science and Technology Program (No. Z0004105040311). The Science and Technology Grant Tsinghua University (No. 03fd28).

P060162

Neuroprotective Effects Of Bushen Decoction Against Glutamate Induced Neurotoxicity In PC12 Cells

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The enhanced effect of Bushen Decoction (BS) on cultured PC12 cells proliferation and its antagonistic action on neurotoxicity induced by glutamate (Glu) were investigated with serum pharmacology method of the Chinese material medica (CMM) in vitro. The effect of BS on cultured PC12 cells activity and its antagonistic action on neurotoxicity induced by Glu was observed with MTT method. Flow Cytometry and Fluorescence microscope techniques were employed to observe the antagonistic effect of BS on PC12 cells early period apoptosis induced by Glu. We discovered that the serum with BS was able to enhance PC12 cells activity and exert antagonistic effect on Glu-induced neurotoxicity. Meanwhile, these beneficial effects produced by BS were found to be the strongest in 20% concentration of serum with BS. Moreover, it can inhibit apoptosis of PC12 cells induced by Glu which occurred in the early period. Thus, we demonstrated here that BS might exert a potential neuroprotective effect.

Keywords: Bushen decoction; Neuroprotective effect

Acknowledgements: This work was supported by National Natural Science Foundation of Shanxi Province (No. 19991091) and National Basic Research Program of China (No. 2004CB518906)

P060163

Blockade of cannabinoid CB1 receptor by AM251 inhibits cocaine's rewarding effects and cocaine-primed relapse by a DA-independent mechanism

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Previous studies demonstrate that blockade of CB1 receptors by SR141716A appears to have no effect on cocaine's rewarding effects. In the present study, we examined whether AM251, a novel CB1 receptor antagonist, inhibits cocaine reward and relapse, as assessed by cocaine self-administration (SA), brain stimulation reward (BSR) and cocaine-triggered relapse in rats. Systemic administration of AM251 (1-10 mg/kg) dose-dependently lowered the break-point for cocaine SA under a progressive ratio reinforcement schedule and dose-dependently inhibited cocaine-enhanced BSR and cocaine-triggered relapse. In vivo microdialysis demonstrated that cocaine priming significantly elevated extracellular dopamine (DA) and glutamate in the accumbens. AM251 blocked cocaine-induced increases in glutamate, but not in DA. AM251 alone dose-dependently elevated extracellular glutamate. Together, these data suggest that blockade of CB1 receptors by AM251 significantly inhibits cocaine's rewarding effects and cocaine-triggered relapse by a mechanism correlated to AM251-induced increases in glutamate, but not to a reduction in cocaine-induced increases in DA in the accumbens.

Key words: Cocaine, AM251, dopamine

P060164

THE SELECTIVE ADENOSINE A2A ANTAGONIST SCH58261 IS PROTECTIVE IN A MODEL OF FOCAL CEREBRAL ISCHEMIA IN THE RAT

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The A2A antagonist, SCH58261, was tested in a rat model of permanent focal ischemia induced by middle cerebral artery occlusion (MCAo). SCH58261 (0.01 mg/kg, i.p.) was administered 5 min, 6 h and 15 h after MCAo. In SCH58261-treated rats ($n = 14$) the contralateral turning behavior was definitely reduced with respect to vehicle-treated rats ($n = 13$) (number of rotations per hour, mean ± S.E.: 116.9 ± 34.6 vs 795.4 ± 170.6, $p < 0.0001$). 24 h after MCAo drug-treated rats showed significant improvement of the neurological score (mean ± S.E: 10.8 ± 0.4 vs 8.8 ± 0.5, $p < 0.001$) and reduction of the ischemic damage by 44% in the striatum ($p < 0.004$) and 24% in the cortex ($p < 0.02$). The phospho-p38 mitogen activated kinase (MAPK) was increased by 500% in the ischemic striatum of vehicle-treated rats ($n = 5$) and reduced by 70% in drug-treated rats ($n = 6$; $p < 0.01$). In the striatum and cortex, phospho-p38 immunopositive cells exhibited morphological features of activated microglia. Results demonstrate that treatment with an A2A antagonist is protective up to several hours after ischemia.

Key words: adenosine antagonism, focal ischemia, MAPK.

Acknowledgement: this work was supported by the Ente Cassa di Risparmio-Florence-Italy.

P060165

Antinociception of Ciproxifan in formalin test and its inhibition of intracellular translocation of nNOS in CNS

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AIM To investigate the antinociceptive effect of ciproxifan (CPF), an H3 receptor antagnist, in formalin test in mice and its inhibition of intracellular translocation of nNOS in the central neural system.

METHODES The antinociceptive effect of CPF was observed in the formalin test. The first phase (phase I) was recorded from 0 min to 10 min after the formalin injection. The second phase (phase II) was recorded from 15 min to 60 min after the formalin injection. After the formalin test, the intracellular translocation of neuronal nitric oxide synthase (nNOS) in brain and spinal cord was determined by immunohistology and Western blot.

RESULTS In formalin test, the subcutaneously injection of formalin into the paw evoked biphasic (phase I and phase II) licking behavior of the injected paw. The licking times of both phases were decreased by different doses of CPF. After formalin test, the results of immunohistology showed that the fluorescence of nNOS enhanced on neuron membrane after formalin stimulation. However, different doses of CPF weakened such enhanced fluorescence. In western blot, the nNOS protein belts of the membrane-associated fractions were increased after formalin stimulation and CPF could decrease these belts.

CONCLUSION CPF possesses antinociception in the formalin test. The results of immunohistology and western blot implicated that NO in brain and spinal was an important signal in formalin-induced algesia and nNOS could be activated and translocated from plasma-fraction to membrane-fraction in this algesic process. CPF could inhibit such intracellular translocation. CPF-induced antinociception might be related to the inhibition of the activation of nNOS resulting in the reduction of the levels of NO in brain and spinal cord.

P060166

Effect of β- Elemene on Human Glioma U87 Cells

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β- Elemene, isolated from the Chinese medicinal herb Rhizoma Zedoariae, was shown to exhibit antitumor activity. This study was designed to investigate the proliferation inhibitory effect of β- elemene on human glioma U87 cells. Viability of β- elemene- induced U87 cells was measured by MTT assay. Apoptotic cells with condensed or fragmented nuclei were visualized by AO/EB staining. β- Elemene induced U87 cell death dose- and time- dependently. The IC_{50} value ranged from 16.9 μg/ml to 42.5 μg/ml (6～24h). Cells treated with 40 μg/ml for 24h exhibited the apoptotic morphology and the reduction of cell volume. These results suggest that β- elemene showed a marked antiproliferative effect on human glioma U87 cells probably by inducing apoptosis in vitro.

Key word β- elemene; Glioma; proliferation; apoptosis

P060167

Effects of AST and AS- I on Antiapoptoticactivity and their machines in senescent rats treated by Hydrocortisone

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To explore the effect of Astragalosides (AST) and Astragals Saponin I (AS- I) on antiapoptoticactivity in thymocytes and cortex- hippocampus neurons and expression of p53 gene in senescent rats treated by hydrocortisone. Electron microscopy and agarose gel electrophoresis of DNA were used to observe the apoptosis of cells. Flow cytometry was used to measure the expression of p53 gene. The results showed that HC induced apoptosis of hippocampus neurons of senescent mice. The ultramicrostructure of hippocampus neurons showed characteristic chromatin condensing, under fragmentation and "apoptotic bodies". The apoptotic peaks were found by flow cytometry. Agarose gel electrophoresis of DNA from cultured thymocytes and hippocampus neurons treated with DEX revealed "Ladder" Patten. It was found that AST and AS- I preverted apoptosis of thymocytes and hippocampus induced by DEX in vitro. AST and AS- I inhibited apoptosis and protected injury of thymocytes and hippocampus neurons.

Key Words: AST, AS- I, Apoposis, P53, hydrocortisone

Acknowledgments: Supported by the NSF of Anhui Province (No. 00144414), the "Shiwu" TSF of Anhui Province (No. 01803016) and Department of Anhui Province Education (No:2005hbz18)

P060168

Effects of AST and AS- I on intracellular calcium concentration of cells in senescent rats treated by hydrocortisone

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To explore the effects of Astragalosides (AST) and Astragals Saponin I (AS- I) on intracellular calcium concentration ($[Ca^{2+}]i$) in senescent rats treated by hydrocortisone. The $[Ca^{2+}]i$ was measured by using double wavelength fluorescence spectrophotometer in thymocytes and hippocampus neurons intrasynaptosomes from mice. The results showed that $[Ca^{2+}]i$ in senescent thymus and hippocampus sitrasynaptosomes was significantly higher than $[Ca^{2+}]i$ of adult thymus and hippocamal intrasynaptosomes. The high level $[Ca^{2+}]i$ of hippocampus caused memory impairment in senescent mice. When treated with AST or AS- I, the $[Ca^{2+}]i$ in two kind cells decreased. Dexamamethasone (DEX), BayK8644, KCl and Glutamete (Glu) all elevated $[Ca^{2+}]i$ of thymocytes of neonate (7 days rats) and hippocampal neurons of fatal rats in vitro. When treated with AST or AS- I, the $[Ca^{2+}]i$ in cultured thymocytes and hippocampus neurons stimulated by DEX, BayK8644, KCl and Glu decreased.

Key Words: AST, AS- I, DEX, Glu, Glucocorticoid

Acknowledgments: Supported by the NSF of Anhui Province (No. 00144414), the "Shiwu" Technology Special Foundation of Anhui Province (No. 01803016) and Department of Anhui Province Education (No:2005hbz18)

P060169

Cognition Enhancing Effect of Liuwei Dihuang Pill on Deterioration of Learning and Memory induced by D- gal in Rats

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Objective: To probe the cognition enhancing effect of Liuwei Dihuang on deterioration of learning and memory induced by D- gal in rats, and to study the mechanism. Methods: Sub- acute aged animal model was replicated by administration of 75 mg/kg/d s.c. for 8 weeks. Meanwhile treating with different dose of Liuwei Dihuang pill for 8 weeks. The learning and memory ability was observed by Morris water maze. Activities of MAO and AchE in brain tissue were detected. Results: High and low dose Liuwei Dihuang pill can enhance learning and memory ability. It can also decrease activities of MAO and AchE in brain tissue. Conclusions: Liuwei Dihuang pill can improve the deterioration of learning and memory induced by D- gal in rats. The possible mechanism are regulating of central cholinergic nerve system and noradrenergic nerve system.

Key words: Liuwei Dihuang pill; D- gal; Learning and memory; Mechanism of action

P060170

Effects of exposure to the chronic mild stress on neurochemical and physidogical stress responses of rats

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The chronic mild stress (CMS) paradigm was developed in order to make a model of animals as symptom of depressive disorders. The purpose of this study was to investigate whether the effects of 5 weeks of CMS administration young adult male rats with respect to physiological and neurochemical indices of stress. In this study indcate a slower rate of weight gain in animals exposed to the chronic stressor regime. Also, CMS is elicited to hypertrophy of adrenal gland weight of the stressed (CMS) group. The sucrose intake test as a confirmation of behaviorally anhedonic status, was not changed between groups. In neurochemical analysis, the corticosterone levels were elevated in the CMS group relative to the normally housed control group. 5 weeks after the exposure to CMS paradigm c- fos immunoreactivity on PVN is increased in the CMS group. However, NADPH- diaphorase enzymatic activity on PVN is decreased in the CMS group at the same time. The effects of exposure to chronic stressor on physiological and neurochemical indices indicated that the administration of CMS can alter not only physiological stress responses but also neurochemical stress response triggering point in the rat brain.

Keywords: CMS, Rat, PVN, c- fos

Acknowledgement: This study was supported by a grant from the Wonkwang University Research Fund in 2005.

P060171

DL0108 prevents glutamate- induced apoptosis in SH- sy5y neuronal cells

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DL0108 is an important component of propolis, which has been investigated for its antioxidant, antibacterial and anti- inflammatory potential. To assess the protective effects of DL0108 on neurons, SH- SY5Y neuronal cells were treated for 12 h with glutamate (1 mM). Cell viability was determined by 3- (4, 5- dimethylthiazol- 2- yl)- 2, 5- diphenylte- trazolium bromide assay, and apoptosis was confirmed by cell morphology and DNA fragmentation. Cell morphology was evaluated with Hoechst33258/PI dye. Pretreatment with DL0108 (10^{-5}, 10^{-6}, 10^{-7} mol/L) increased cell viability dose- dependently, inhibited LDH release and attenuated apoptosis. Intracellular free $[Ca^{2+}]$ was increased after glutamate treatment. This increase was attenuated in cells pretreated with DL0108. Bax and bcl- 2 mRNA expression were also detected by RT- PCR analysis. Bax mRNA expression increased remarkablely following glutamate exposure and DL0108 pretreatment manifested a reduction effect. Bcl- 2 mRNA expression changes were not detected in groups with or without DL0108. Thus we concluded that DL0108 exerts its neuroprotective effects in glutamate injury model partly by decrease intracellular free $[Ca^{2+}]$ and bax/bcl- 2 ratio. DL0108 may be used as a neuroprotectart for treatment of acute brain injury and neurodegenerative diseases.

Key words: DL0108, glutamate, apoptosis, bcl- 2, bax

P060172

The expression of type I sodium channel α subunit was regulated up in spontaneously epileptic rat hippocampus

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OBJECTIVE Investigate mRNA and protein expression of type I voltage gated sodium channel α subunit in spontaneously epileptic rat (SER) and wild type control (WTC) hippocampus by control study. METHODS Total RNA was extracted from hippocampus after status epilepticus, and type I sodium channel α subunit mRNA expression were detected by RT-PCR. Type I sodium channel protein expression was detected by immunohistochemistry and immunofluoresence. Type I sodium channel α subunit mRNA of SER expressed significantly higher than that of wild type control in hippocampus, ($P < 0.01$). Type I sodium channel protein of SER increased significantly in hippocampus ($P < 0.01$). CONCLUSION The expression of type I sodium channel α subunit was regulated up in spontaneously epileptic rat hippocampus, which may be the reason of neron hyperexcitability or succeeding appearance after seizure.

Key words sodium channel; spontaneously epileptic rat; subunit; epilepsy

P060173

The expression of glutamate transporter GLAST in spontaneously epileptic rat brain hippocampus

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Objective Investigate the expression of glutamate transporters mRNA and protein in spontaneously epileptic rats (SER)、Tremor rats and Wistar rats by control study and explore further the role of GLAST in the occurrence of epilepsy. Methods the expression of mRNA were investigated in hippocampi by RT-PCR, the GLAST protein was investigated by immunohistochemistry. Results GLAST mRNA was lowered to normal level in Tremors, the protein of SER was lowered in dentate gyrus (DG) and CA3 of hippocampus. Conclusions Down-regulation of GLAST function was correlated with the occurrence of epilepsy; Gliosis may afect the occurrence of epilepsy through the role of glutamate transporters.

Key words: excitatory amino acid transporters; GLAST; mRNA; SER

P060174

Protective effects of Penehydidine Hydrochloride on transient forebrain ischemia reperfusion injury in gerbil

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Objective To study the protective effects and mechanism of Penehydidine Hydrochloride (PHC) on transient forebrain ischemia reperfusion injury in gerbils. Methods We performed Neurological function scores and calculate stroke index, then examined TXB_2 and 6-Keto-$PGF_{1\alpha}$ by radioimmunity. The content of intracellular free calcium in hippocampus were assayed by flow cytometry and the method of enzymology and patholistology were used respectively. Results The stroke index in PHC 0.24 reduced 27% than in Isc/R group and the pyramidal cell was damaged slightly after transient forebrain ischemia. PHC groups TXB2/6-Keto-PGF1α was obviously decreased. PHC could reduce the overload of $[Ca^{2+}]i$ and MDA content and increase the activity of SOD, GSH-PX, Na^+, K^+-ATPase of hippocampal neuron. Conclusions PHC has protective effects on ischemic brain injury, which is related to antagonistic effect on M_1 receptors and regulate TXA_2/PGI_2 equilibration and increase the oxygen free radical clearance of antioxidant in hippocampus.

Key words cerebral ischemia; muscarinic receptors; hippocampus

Acknowledgement Appreciate department of isotope and experimental center of affiliated hospital of Xuzhou Medical College.

P060175

DIFFERENT CENTRAL ACTION OF STREPTOZOTOCIN AND ALLOXAN ON COGNITION

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Strepotozotocin and alloxan are selective toxic for insulin producing/secreting cells, producing diabetes mellitus after peripheral, but not after central administration., with similar alterations of brain monoamine transmission found follo wing the both administrations. Recently, cognitive deficits have been found in streptozotocin-intracerebroventricularly (STZ-icv) treated rats, while cognition in alloxan-icv treated rats has not been investigated. By means of Morris Water Maze Swimming Test, we have compared memory and learning functions in STZ- and alloxan-icv treated rats, 3 months after the icv drug treatment. Contrary to the statistically significant decrement of these functions in STZ-icv rats, alloxan-icv treated rats had no significant cognitive deficits in comparison to the respective controls. Regardless their similar peripheral metabolic effects, and some similar central neurochemical effects, STZ- and alloxan-icv treatment demonstrate different influence on cognition, the latter being deprived of any effect.

Key words: streptozotocin; alloxan, intracerebroventricular; cognition

Acknowledgement: Supported by Croatian Ministry of Science, Education and Sport (0108253) and DAAD (A/04/20017)

P060176

A DOPAMINE AGONIST, PRAMIPEXOLE, AND COGNITIVE FUNCTIONS IN PARKINSON'S DISEASE

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PD has long been conceived to be mainly a motor disorder. In the last few decades it has been increasingly more recognized that many patients with PD will experience cognitive decline in the course of their illness. Dopamine agonists have shown beneficial therapeutic effects on motor symptoms in PD, but their influence on cognitive functions is still controversial. The aim of this study is to evaluate the influence of the dopamine agonist pramipexole on cognitive functions in PD patients already treated with levodopa.

The cognitive performance of 55 non-demented idopathic Parkinson's disease (PD) patients treated with levodopa alone or receiving dopamine agonist pramipexole as add on therapy to levodopa was evaluated in the present study during six month of treatment. Neuropsychological tests were administered two times. In the first assessment to differentiate test sensitive to cognitive changes typical for PD control group was also assessed. After six months of treatment PD patients were retested only with tests that differentiate them from control group. Compared to controls PD patients showed inferior performance on Stroop Interference test, Trail Making test, letter fluency and Hooper Visual Organization test. No statistically significant differences between two groups and first and second neuropsychological assessment was found. In conclusion: present findings indicate that pramipexole as add-on therapy to levodopa is safe in non-demented PD patients in terms of the effect on cognitive performance.

Key words: Parkinson's disease, cognitive functions, dopamine agonists, pramipexole

Acknowledgement: Supported by Croatian Ministry of Education, Science and Sport

P060178

Anticonvulsant effects of 3, 4 Dimethoxy toluene, the major constituent of Phoenix Dactylifera L. in mice

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The anticonvulsant effects of 3,4 Dimethoxy toluene (DMT), the major constituent of The Date palm (Phoenix Dactylifera L.) spathe, were investigated using pentylenetetrazole (PTZ), picrotoxin (Ptc), Nicotine (Nic) and maximal electroshock (MES)-induced seizure models. In PTZ-induced seizure, the intraperitoneally injection of DMT with a dose of 100 mg/kg, significantly delay the onset of seizures and produce 50% protective effect against mortality. In MES model, DMT showed complete inhibition of Toric hind-limb extension (THLE) and exhibited a complete protection against mortality. After mice were challenged with picrotoxin (12 mg/kg) DMT significantly delay the onset of convulsion and death. DMT exhibited complete protection against Nicotine (0.8 mg/kg) induced convulsion. These results indicate that DMT may have a promising anticonvulsant activity.

Key words: 3, 4 Dimethoxy toluene, anticonvulsant, Phoenix Dactylifera L.,

Acknowledgement: the author would like to thank King Faisal University for the support of this project.

P060179

CHOLESTASIS INDUCED NEPHROTOXICITY: THE ROLE OF ENDOGENOUS OPIOIDS

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The aim of this study was to investigate the role of endogenous opioids in cholestasis induced nephrotoxicity. 35 male rats were divided to 5 groups: group 1 (BDL rats received daily 20 mg/kg of naltrexone, S.C. for 7 days after BDL), group 2 (BDL rats received daily normal saline, S.C. for 7 days after BDL), group 3 (BDL rats), group 4 (sham), group 5 (rats with no intervention received a daily subcutaneous 20 mg/kg of naltrexone, S.C. for 7 days). 24 hour urine was collected to measure urinary N-acetyl-β-D-glucosaminidase (NAG). The kidneys were excised for light and electron microscopic studies. NAG activity in groups 2,3 (49.24 ±8.56 and 48.38 ±7.62 U/g creatinine) was significantly higher compared with group 1 (24.20 ±6.76 U/g creatinine) and groups 4,5 (28.32 ±7.58 and 25.24 ±7.01 U/g creatinine). NAG activity in groups 2, 4, 5 did not differ significantly from group 1. In light microscopy there were no significant changes between cortical portions of the kidneys. There were scattered enlargement, swelling and vacuolation of the medullary tubular cells in groups 2,3 compared with other groups. In electron microscopy there were swelling and enlargement of renal tubular cells, increase in number of lysosomes containing myeloid bodies and relative decrease in number of mitochondria especially in proximal tubules of groups 2,3 compared with other groups. There are significant changes in NAG activity and renal morphology of cholestatic rats compared with normal and cholestatic rats which received naltrexone. Cholestatic nephrotoxicity seems to be inhibited by naltrexone suggesting a role for endogenous opioids in inducing nephrotoxicity of cholestasis.

Keywords: Kidney, cholestasis, NAG, Opioids

P07. Cardiovascular Pharmacology - Antiarrhythmics

P070001

Predinical Screening Models for the Identification of Drug-induced QT Prolongation

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Introduction: Recent guidelines (ICHS7B) for the identification of drug-induced QT prolongation may have limitations. We analyzed 64 compounds tested in hERG and compared its effects in action potential duration (APD) studies in vitro and QTc in anaesthetized dogs.

Method and Results: 64 compounds were tested in hERG and 62,5% of these being positive and 37,5% negative. These 64 compounds were further examined for APD studies in either rabbit Purkinje fibers or isolated hearts. From the 40 hERG positive compounds, 62% were positive, 33% no effect and 5% shortening APD. In the group of 24 hERG negative compounds, 58% were inactive, 29% shortening and 13% prolongation of APD. 14 positive hERG positive compounds were further tested in dogs and only 29% positive, 64% no effect and 7% shortening QTc. From the 6 hERG negative compounds, 4 compounds were inactive, 1 compound prolonged and another one shortened QTc, respectively.

Conclusion: Our results indicate serious limitations in the use of only the hERG assay and/or the in vivo dog, as part of a predinical screening strategy of drug-induced QT prolongation.

Key words: drug, QT prolongation, APD, hERG

P070003

Ischemia impairs the association between connexin 43 and M3 subtype of acetylcholine muscarinic receptor (M3-mAChR) in ventricle myocytes

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We used Western blot analysis to examine the expression of connexin43 and M3-mAChR and their interaction in ventricular myocytes from control and the ischemic heart. We firstly showed that M3-mAChR was expressed in non-glycosylated and glycosylated forms. Immunostaining showed that connexin43 is closely associated with M3-mAChR in parts of cell membranes of myocytes. Immunoprecipitation of lysate of cardiac myocytes with M3-mAChR antibody pulled down a 44 kDa protein recognized by connexin 43 antibody. Ischemia specificly increased the expression of M3-mAChR in myocytes. On the other hand, ischemia decreased the expression of connexin43 in myocardium. We also examined the effect of ischemia on the interaction between M3-mAChR and connexin43. Ischemia suppressed the association of M3 with connexin43. Administration of choline before ischemia not only partially restored the expression of connexin43 but also attenuated the ischemia-induced suppression of the association between connexin43 and M3-mAChR. We conclude that connexin43 interacts with M3-mAChR and that ischemia specifically impairs the association between M3-mAChR and connexin43.

Key words: Gap junction channel, muscarinic receptor

P070004

Non-specific inhibitory effects of artemisinin on voltage-dependent ion channels in sensory neurons

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AIM: To investigate the effects of Artemisinin (Art) on voltage-gated ion channels in rat nodose sensory neurons (NSNs). METHODS: Whole-cell patch experiments were conducted on isolated NSNs of neonatal rats. Voltage-gated Na^+, K^+, and Ca^{2+} channels were recorded from NSNs. TEA, TTX, and CTX GVIA were selected as the references. RESULTS: (1) Total outward K^+ currents were recorded on C-type NSNs identified by AP waveform characters were blocked by Art 30-300 umol/L in a concentration-dependent manner, and Art-sensitive currents were similar to TEA (TEA 15 umol/L)-sensitive currents. (2) Also, in C-type NSNs identified by TTX 5 umol/L, Art 30-1000 umol inhibited both TTX-s and TTX-r Na^+ channels, and TTX-s Na^+ channels were more sensitive to Art. (3) N-type Ca^{2+} currents were knocked out completely with Art 1000 umol/L on board and this effect could be mimicked by 1 umol/L CTX GVIA. Art also blocked T-type Ca^{2+} channels. CONCLUSION: Data from this study showed that Art concentration-dependently and nonselectively inhibited all major ion channels expressed on NSNs.

KEY WORDS: nodose ganglia; sensory neuron; artemisinin; arrhythmia;

P070005

The potassium current abnormality induced by high homocysteine in human atrial myocytes

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BACKGROUND: A large body of evidence has indicated that high homocysteine portends an increased risk for human heart disease. However, the underlying cellular mechanism remains conjectural. It is well known that potassium channels play a critical role in the development of human heart diseases. So the aim of this study was to investigate acute direct effects of high homocysteine on potassium currents recorded in human atrial cells and to explore possible underlying mechanisms. METHODS: Human atrial myocytes were isolated from patient undergoing cardiac surgery with patients' consents, and the whole-cell patch clamp technique was used to record potassium currents in atrial cells of human heart in the absence and presence of high homocysteine. RESULTS: Homocysteine can significantly inhibit the transient outward and ultra-rapid delayed rectifier potassium currents and increase the inward rectifier potassium currents. CONCLUSIONS: The data presented in this study first revealed that the abnormality of potassium currents can be induced by high homocysteine in human atrial cells, which will be a new clue to explore mechanisms by which patients with high homocysteine was easy to suffer from heart diseases.

P070006

Study on the antiarrhythmic targets of flavonoids from Viscum coloratum

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To study the effects of flavonoids from Viscum coloratum (VCF) on inward rectifier K^+ current (IK1), transient outward K^+ current (Ito), delayed rectifier K^+ current (IK), L-type Ca^{2+} current (ICa-L), and action potential duration (APD) in isolated ventricular myocytes. Whole-cell patch-clamp was used to record IK1, IK, Ito, ICa-L and APD in single ventricular myocyte. In ventricular myocytes of rat, Ito was decreased from (26.64 ±6.67) pA/pF to (13.25 ±3.78) pA/pF at +60 mV and IK1 was decreased from (-26.23 ±7.52) PA/PF to (-18.11 ±5.89) pA/pF at -120 mV following VCF 250 ug/ml; In guinea pig, VCF had extended effect on APD in isolated ventricular myocytes of guinea pig. IK was increased from (8.27 ±2.40) pA/pF to (12.37 ±4.19) pA/pF at +70 mV and ICa-L was increased from (-6.89 ±1.76) pA/pF to (-9.39 ±2.84) pA/pF following VCF 250 ug/ml. It implies that VCF takes part in anti-myocardial ischemia and anti-arrhythmics partly due to the decreased of Ito, IK1 currents and increased of IK and L-type calcium currents. IK1, IK, Ito, ICa-L are the major targets of antiarrhythmic effect of VCF.

Key words: Viscum coloratum; APD; potassium channels

P070007

Effects of Ginkgolide B on Action Potential Duration and Ionic Channel Currents in Rat Ventricular Myocytes

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We investigated the effects of ginkgolide B(GB) on the action potential duration (APD) and ionic currents in rat ventricular myocytes, for purpose of exploring the possibility of GB to become a new anti-arrhythmic drug. We recorded the effects of 1 micromol/L GB on APD and most of the principal ion currents with patch clamp technique in rat ventricular myocytes which were acutely isolated by using collagenase II. The results are as following:1) GB shortened APD;2) GB inhibited the transient outward potassium currents, from(22.2 ±2.7) pA/pF to (18.6 ±0.5) pA/pF at +50 mV(n=5, P<0.05);3) GB inhibited the inward rectifier potassium currents at from -60 mV to -120 mV, the currents were from (-17.9 ±2.2) pA/pF to (-13.8 ±3.9) pA/pF at -120 mV(n=5, P<0.05); 4) GB decreased the L-type calcium currents at from −20 mV to +30 mV, the currents were from(-6.5 ±0.1) pA/pF to (-2.6 ±0.3) pA/pF at +10 mV(n=5, P<0.05). The results demonstrated that GB can affect several ionic channel targets, which are highly associated with the causing of arrhythmias, and finally shorten APD. It is confirmed that GB has the possibility of being developed as a new anti-arrhythmic drug in future.

Keywords: Ginkgolide B, ion channel, anti-arrhythmic drug

P070008

Cardiac MB Receptors Produces Cytoprotective Effects Against Ischemic Myocardial Injuries

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Aims: To explore the possible role of MB-mAChR in cytoprotection of myocardial infarction and further to detect its potential mechanisms. Methods: Studies were performed in a rat model of myocardial infarction and in isolated myocytes. The apoptosis in cardiomyocyte was detected by terminal deoxynucleotid transferase directed d-UTP nick and end labeling (TUNEL) assay and apoptosis-related proteins were measured by immunohistochemistry assay. $[Ca^{2+}]i$ in single cardiomyocyte was measured by confocal microscope. Results: Choline relieved myocardial injuries during ischemia or under oxidative stress, which was achieved by diminishing ventricular arrhythmias and protecting cardiomyocytes from apoptotic death. The beneficial effects of choline were reversed by the MB-selective antagonists but not by the M_2-selective antagonist. Choline/MB-mAChR activated antiapoptotic proteins Bcl-2, increased endogenous antioxidant reserve (SOD), and reduced proapoptotic proteins Fas and intracellular Ca^{2+} overload. Conclusion: Choline reduces ischemic myocardial injuries via stimulating the cardiac MB-mAChRs through modulating the expression of Bcl-2 and Fas.

KEY WORDS: MB-receptor; Apoptosis; Ischemia

P070009

Electrophysiological evidence of arsenic trioxide-induced prolongation of cardiac repolarization

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Arsenic trioxide (As_2O_3) has been found to be effective for relapsed or refractory acute promyelocytic leukemia (APL), but its clinical use is burdened by QT prolongation, torsade de pointes tachycardias (TdP), and sudden cardiac death. The aim of the present study was to elucidate the ionic mechanisms of As_2O_3-induced abnormalities of cardiac electrophysiology in guinea pig and Xenopus oocytes. Intravenous administration of As_2O_3 prolonged QT interval in a dose-and time-dependent manner in guinea pig hearts. By using whole-cell patch clamp technique and gene-clamp technique, we found that As_2O_3 significantly lengthened action potential duration (APD) measured at 50 and 90% of repolarization, enhanced L-type Ca^{2+} current (ICa-L), inhibited delayed rectifier K^+ current (IK) and inward rectifier K^+ current (IK1) in guinea pig ventricular myocytes, blocked HERG/IKr in Xenopus oocytes. As_2O_3 markedly disturbed the normal equilibrium of transmembrane currents (increasing ICa-L and suppressing IKr, IK1), and induced prolongation of APD, further degenerated into QT prolongation.

Keywords: arsenic trioxide; QT interval prolongation; L-type Ca^{2+} current; delayed rectifier K^+ current

P070010

Effects of aconitine on L-type calcium currents and cytosolic $[Ca^{2+}]i$ in rat ventricular myocytes

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Aim: To study the involvement of voltage-dependent calcium channel and subsequently changes of intracellular calcium concentration in aconitine-induced rat arrhythmias. Methods: Whole-cell patch-clamp techniques were used to record L-type calcium current (ICa-L). Intracellular $[Ca^{2+}]i$ was measured as fluorescent intensity (FI) by laser scanning confocal microscopy in isolated rat ventricular myocytes loaded with Fluo 3-AM. Results: Density of ICa-L in rat ventricular myocytes was increased significantly from 12.77 ±3.12 to 18.98 ± 3.89 pA/pF(n=10, p<0.01 from six rats) after exposure to aconitine 1 mmol L^{-1}. The time constant (τ) of ICa-L activation was not changed but that of inactivation showed a significant slower process after aconitine was administrated. The peak of $[Ca^{2+}]i$ elevation induced by KCl 60 mmol L^{-1} was unchanged, whereas the recovery process was slower than normal. Conclusion: Calcium channel is a potent target in aconitine-induced arrhythmia. And the long-phase sustaining state of higher intracellular free calcium concentration caused by aconitine may contribute to its arrhythmogenesis effect.

Key Words: arrhythmia; L-type calcium currents (ICa-L); aconitine; cytosolic $[Ca^{2+}]i$;

P070012

Study on the antiarrhythmic targets of matrine

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To investigate the antiarrhythmic targets of matrine on transmembrane ionic currents, whole-cell patch-clamp was used to record ionic currents in single ventricular cells of rat. In ventricular myocytes of rat, matrine 10 μmol/L prolonged APD50 from 82.80 ±26.23 ms to 107.8 ±32.69 ms (n=5, p<0.01), APD90 increased from 114.8 ±40.52 ms to 141.6 ±52.92 ms (n=5, p<0.01); Ik1 decreased from -19.33 ±5.61 pA/pF to -16.98 ±4.54 pA/pF at -120 mV (n=8, p<0.01); Ito decreased from 13.20 ±1.97 pA/pF to 12.21 ±3.03 pA/pF at +60 mV (n=8, p<0.01), 10 μmol/L matrine increased ICa-L from -8.56 ±2.92 pA/pF to -13.75 ±1.94 pA/pF at +10 mV (n=6, p<0.01). In a conclusion, Ik1、Ik、Ito、ICa-L are the major targets of antiarrhythmic effect of matrine.

P070013

Effects of Amiodarone and Quinidine on action potential and transmembrane currents in the presence of ouabain

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The study was designed to examine and assess effects of Amiodarone and Quinidine on action potential (AP) and transmembrane currents in the presence of Ouabain respectively in isolated guinea pig ventricular myocytes. Whole cell patch clamp was used to record currents in single ventricular myocytes obtained by enzymetic dissociation method. Ouabain was associated with prolongation of AP, decreases of Ik and Ik1, increase of ICa. Interestingly, in the presence of Amiodarone plus Ouabain, the APs shortened and recovered nearly to normal state while decreases of Ik, Ik1 and increase of ICa were alleviated. Quinidine impaired the increase of AP induced by Ouabain mostly. But its actions on ion currents were conflicting: ICa decreased; Ik and Ik1 changed into two directions in the presence of Ouabain, one was reduced continuously, other was increased. Increase of APD may be resulted from direct actions of Ouabain on ion channels. Amiodarone can correct the unbalanced ion channels to nearly normal state. Although Quinidine can recover the AP in some degree, but its discordant effects on potassium channels reflect individual variance in fact.

Key words Amiodarone, Quinidine, Ouabain, electrophysiology

P070014

Resveratrol, A Natural Ingredient of Grape Skin: Antiarrhythmic Efficacy and Ionic Mechanisms

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Resveratrol has been demonstrated to produce a variety of biological actions. Accumulating line of evidence supported the view that resveratrol may exert protective effect on the cardiovascular system. The aim of the study was to assess the

anti-arrhythmic profile as well as electrophysiological properties of resveratrol. We observe the antiarrhythmic effect of resveratrol on aconitine induced rat arrhythmia, ouabain induced guinea pig arrhythmia, and coronary ligation induced rat arrhythmia animal models. Resveratrol significantly and dose-dependently increased the doses of aconitine and ouabain required to induce the arrhythmia indexes. In coronary ligation induced rat arrhythmia model, resveratrol shortened duration of arrhythmia, decreased incidence of ventricular tachycardia and mortality. Electrophysiological experiment revealed that resveratrol could shorten APD through inhibiting of ICa and selective enhancement of Iks without an effect on Ikr.

P070015

Effects of Magnesium Taurate on Cesium Chloride-Induced Arrhythmias and Cardiac Electrophysiology in Rabbits

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Objectives: To study the effects of MTC on CsCl-induced arrhythmias, monophasic action potential (MAP) in rabbits and on functional refractory period (FRP), excitability of the isolated left atrium from rabbit. Methods: 1. The onset, duration and incidence of ventricular premature (VP), MAP and ECG were simultaneous recorded in CsCl-induced early after-depolarization(EAD) model. 2. Couple-stimulation was used to characterize the effects of MTC on FRP and excitability of left atrium. Results: 1. MTC could significantly prolong VP onset as compared with control. 2. The EAD amplitude was decreased by MTC as compared with the control group significantly ($p < 0.01$). 3. MTC could prolong FRP of isolated left atrium of rabbit compared with the control group significantly ($p < 0.01$). Conclusion: The Data showed that MTC had a significantly antiarrhythmic effect. It could reduce triggered action induced by EAD that might be one of the mechanisms of antiarrhythmic action. MTC could prolong FRP of left atrium but no effect on excitability in vitro.

P070016

Electrophysiological Characterization of a Novel Antiarrhythmic Agent-Sulcardine Sulfate

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Sulcardine sulfate (Sul), 4-methoxy-N-[3,5-bis(1-pyrrolidylmethyl)-4-hydroxybenzyl] benzenesulfonamide sulfate, is a new antiarrhythmic agent originated from herbal medicine Dichroa febrifuga. The Electrophysiological effects of Sul were investigated in animal hearts. ①Sul (10 mg/kg, iv) markedly prolonged the atrioventricular nodal conduction time, the His-Purkinje conduction time, and QRS duration, as evaluated with HBE and ECG II in rabbits. ② The effects of Sul on action potential (AP) were investigated in guinea-pig papillary muscle by means of standard microelectrodes. Sul produced a concentration-dependent decrease in the action potential amplitude and the maximum upstroke velocity of AP. ③The actions of Sul on cardiac ion channels were studied using patch clamp method in isolated guinea-pig and rat ventricular myocytes. Sul produced a concentration dependent reduction in I_{Na}, $I_{Ca,L}$ and I_{to1}. However, Sul did not affect the inward rectifier and the delayed rectifier K^+ currents (I_{K1} and I_K). In conclusion, the inhibitory activities on voltage-gated sodium, calcium and potassium channels by Sul contribute to its antiarrhythmic effect.

KEY WORDS: sulcardine sulfate; antiarrhythmic drug; voltage clamp

P070017

EFFECT OF 3-NITROPROPIONIC ACID ON ARRHYTHMIA AND BAX EXPRESSION IN ANESTHETIZED RAT HEART

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Objective: We have compared the effect of 3-Nitropropionate (3-NP), as a chemical preconditioner, and ischemic preconditioning in terms of bax expression during ischaemia-reperfusion injury in rat myocardium. Methods: 5 min regional ischemia (by the coronary ligation method) followed by 10 min reperfusion protocol were used to induce ischaemic preconditioning (IP) as a positive control in anesthetized rats; a time-matched non-preconditioning group served as control; and 3-NP (20 mg/kg, i.p.) was injected 3 hours before the surgical procedures in the third group. Rats from all groups were then subjected to 30 min ischemia-60 min reperfusion. During the experiments, hemodynamic parameters were recorded. The end of the experiments, hearts were removed and kept for the analysis of Bax expression (Western-blotting). Result: Arrhythmia and bax expression was markedly reduced in hearts preconditioned by ischemia or 3-NP. Conclusion: 3-NP was found as potent as IP to reduce bax expression.

Key words: 3-NP, chemical preconditioning, ischaemia, bax

Acknowledgement: This study was supported by Gazi University Scientific Projects Foundation Project code: 02/2004-24 and TUBITAK Project code: SBAG-AYD-477.

P070018

Effects of SEA0400, a novel sodium-calcium exchange inhibitor, on ouabain-induced arrhythmias in guinea pigs

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The cardiac sodium-calcium exchange (NCX) is one of the major regulators of intracellular Ca^{2+}. We investigated antiarrhythmic effects of SEA0400 (SEA) on ouabain-induced arrhythmias in guinea pigs.

In the whole animal arrhythmia model, we observed effects of SEA on the ouabain-induced arrhythmia using ECG recordings. In the isolated myocyte, we observed action potential configurations and oscillations due to calcium overload using the current clamp method. In the whole animal model, SEA at a dose range of 1-10 mg kg^{-1} (i.v., bolus) suppressed ouabain-induced arrhythmias dose-dependently. In isolated ventricular myocytes, SEA (0.1-3 μM) suppressed ouabain-induced oscillatory activity observed between action potentials. SEA (0.1-3 μM) also suppressed ouabain-induced NCX current (I_{NCX}) that is also called transient inward current (I_{Ti}). Our results indicate that both NCX and SR calcium channel ATPase (SERCA) are important and involved in arrhythmia and oscillatory activity induced by ouabain. The inhibition of these arrhythmias and oscillatory activity by SEA might result from the inhibition of NCX.

Na^+-Ca^{2+} exchange (NCX); SEA0400; oscillatory activity; current clamp

P070020

Therapeutic effects of Ginkgo biloba extract on levothyroxine induced hypertrophy with ischemia/reperfusion by improving oxidative stress in rats

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Objective: To characterize therapeutic effect of Ginkgo biloba extract (EGb 761) on arrhythmia in levothyroxine induced hypertrophy with ischemia/reperfusion. Methods: The rats were divided into five groups: normal, model, Propranolol (10 mg/kg.d x10d, ig), Egbh and Egbl (100, 50 mg/kg.d x10d, ig). The models were induced by levothyroxine (3 mg/kg.d x10d, s.c) and ligated on left coronary artery for 10 min and then reperfusion for 10 min in 11th day. Results: EGbh and Propranolol showed anti-hypertrophy and anti-arrhythmia effects and decreased the occurrence rates of arrhythmia significantly. In models, LV activities of glutathione peroxidase and SOD were reduced, while MDA and CK were elevated, Propranolol and GBEh attenuated them. Body weight was decreased and ventricular weight index was increased in models and ameliorated by Propranolol and GBEh significantly. Conclusion: GBE showed anti-arrhythmia effects by suppressing oxidative stress.

Key Words: arrhythmia; reperfusion; Ginkgo biloba extract; propranolol.

Supported by Scientific & Technical Program of Clinical Pharmacy from the Medical Sciences, Technology and Development Foundation of the Bureau of Health, Jiangsu Province (No P200406)

P070021

Ventricular Spiral Wave Formation Accompanied With Myocardial Ischemia-Reperfusion Injury In Dogs

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Objective To observe the relevance between ventricular spiral wave formation with myocardial ischemia reperfusion injury. Methods: Myocardial ischemia-reperfu-

sion injury was established in mongrel dogs by ligating left anterior descending coronary artery. Time table of ischemia - reperfusion was scheduled as ischemia 120 min, reperfusion 120 min, in which treatment was added at 60 min. The animals were divided into saline control, adenosine (100, 200 and 400 mg.kg^{-1}.min^{-1} and isosorbide dinitrate (ID) 1.5 mg.kg^{-1}.min^{-1}. ECG, epicardial ECG, infarct area by TTC staining was observed. Results: According to the order of treatment as above mentioned, ST - T elevation were 3.6 ±3.8, 3.1 ±3.2, 1.3 ±0.8, 0.9 ±1.1, 0.1 ±1.0 mV, Cardiac infarct weight were 5.45 ±4.04, 2.92 ±2.81, 0.48 ±1.00, 0.32 ±0.65 and 0.56 ±0.77g, respectively and the incidence rates of spiral waves and arrhythmias were both 40 % in saline group other than adenosine and isosorbide dinitrate groups. Conclusion Ventricular spiral wave formation accompanied with myocardial ischemia - reperfusion injury in dogs, which were prohibited by adenosine and isosorbide dinitrate.

Key Words: spiral wave Supported by National Natural Science Foundation China 30572194

P070022

Impact of general anaesthesia [GA] on systemic pharmacokinetics, haemodynamic and toxic effects of local anaesthetic agents [LAs]

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LA toxicity is a serious issue: it is often studied in anaesthetized subjects. We determined the impact of halothane GA on responses to LAs administered as simulated i.v. accidents. Cardiologic and pharmacokinetic responses of pre - prepared ewes (~50 Kg), conscious and with GA, were determined. Bupivacaine (B, 100 mg), levobupivacaine (L, 125 mg) or ropivacaine (R, 150 mg) were infused over 3 min; relevant controls were included. All LAs caused convulsions in conscious ewes and 3/11, 2/12 and 2/13 subjects with B, L and R had fatal arrhythmias; none died under GA. GA and LAs decreased left ventricular dP/dtmax; convulsions increased it. Mean min/max dP/dtmax were 66/233, 63/234 and 70/236 % of respective B, L & R pre - LA values in conscious ewes and 43/101, 30/104 and 35/99 % with GA. Commensurate effects were found on cardiac output and stroke volume. Blood B, L, and R concentrations were doubled with GA due to decreased clearance. GA produces data bias of effects and toxicity of LAs and probably of most other drugs. Pre - preparation of subjects is necessary to avoid this bias.

anaesthesia, cardiovascular
The Australian & New Zealand College of Anaesthetists is thanked for support.

P070023

The electrophysiological remodding of cardiac ventricular myocytes during the development of mouse cardiac hypertrophy and failure

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Heart failure is associated with a significant increase in the risk of lethal arrhythmias. We try to elucidate the cardiac electrophysiological remodeling and its molecular mechanism during the development of cardiac hypertrophy and failure. A mouse pressure over - loaded cardiac hypertrophy and failure model was established by aorta banding. Single myocytes were enzymatically isolated from endocardium of the free left ventricle wall. By using perforated patch - clamp we found that APD50 with the hypertrophied hearts was significantly prolonged and it was further increased in the failing hearts. However, APD90 maintained unchanged with the hypertrophied hearts and was significantly prolonged with the failing hearts. The recordings of voltagedependent K^+ currents by whole cell patch - clamp revealed a significant difference between hypertrophied and failing hearts. We conclude that cardiac ventricular myocytes with hypertrophied and failing hearts exhibit different property in electrical remodeling.

Key words: cardiac hypertrophy and failure; patch - clamp; action potential duration; voltage - dependent K^+ currents.
Supported by NCET - 04 - 0253, NSFC 30370571 and HeBSFC 200400628.

P070024

INVESTIGATION OF THE ANTIFIBRILLATORY DRUG INTERACTIONS BETWEEN LIDOCAINE AND CAPTOPRIL IN PERFUSED RABBIT HEARTS

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Recently, we have presented the antifibrillatory interactions between lidocaine and two antihypertensive drugs, propranolol (Almotrefi et al., 1999) and valsartan (Almotrefi & Arif, 2004). This abstract reports its interactions with captopril. Studies were carried out on hearts isolated from New Zealand white rabbits of either sex weighing 1 to 2 Kg. The method used has been described previously (Almotrefi & Baker, 1981). Perfusion with lidocaine produced significant, dose - dependent increase in VFT while perfusion with captopril did not cause any significant change. In addition, there was no significant difference in VFT with the combined infusion of 3.46 ol of lidocaine and 1 ol of captopril, in contrast to a synergistic antifibrillatory effect of the combined use of lidocaine and propranolol (Almotrefi et al., 1999). This suggests that captopril does not have antifibrillatory interactions with lidocaine, indicating its safety in combining with class 1 antiarrhythmic drugs.

antiarrhythmics, lidocaine, captopril. Almotrefi, AA & Baker, JBE (1981) Br. J. Pharmacol., 73, 373 - 377 Almotrefi, AA et al., (1999) Br. J. Pharmacol., 128, 55P Almotrefi, AA & Arif, M (2004) at: www.pa2online.org

P070025

Amiodarone plasma levels in patients

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Background: Amiodarone is an important antiarrhythmic agent, which possesses unique pharmacokinetic properties. Risk of potentially serious non - cardiac side effects increases substantially if amiodarone plasma levels exceed therapeutic range. Aim: Our aim was to evaluate the plasma levels of patients treated with amiodarone and to assess the proportion of patients with high risk of toxic side effects. Methods: Serum concentrations of amiodarone were determined by an HPLC - UV system (Gilson, Aspec) using amiodarone as an external standard. Results: Drug concentrations were analyzed in 571 patients (351 men, 220 women, mean age 66.4 years) receiving amiodarone. Therapeutic window has been reached in 217 patients (38 %), whereas 347 patients had plasma concentrations below 1 mg/l, and only 7 measurements (1.2 %) exceeded 2.5 mg/l. Conclusions: Amiodarone plasma levels in majority of patients receiving the drug do not reach recommended therapeutic range, but concentrations associated with high risk of toxicity are not frequent in clinical settings.

Key words: Amiodarone, HPLC, pharmacokinetics, therapeutic drug monitoring
Acknowledgment: Supported by a grant GAUK 19/C/2005

P070026

Antiarrhythmic effects of succinic acid (5 - epiandroene - 17 - one - 3β - ol) diester on QT interval[1]

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The pharmacological blockade of rapid delayed rectifier current (I_{kr}) led to long QT syndrome (LQTS). Butane acid - (5 - androsten - 17 - one - 3β - ol) - diester (A1998) had pharmacological effects on blocking ultrarapid delayed rectifier K^+ current (Ikur) expressed on Oocyte membrance by microinjected Kv1.5 mRNA. Anti - arrhythmic effects of A1998 on QT interval was tested by animal models. Guinea pigs were administered orally with A1998 solid dispersion for 5 days. During these days electrocardiograms were recorded. The results revealed that A1998 do nothing with the QT interval, PR interval and QRS duration. While Amiodarone (47.5 mg·kg^{-1}) significantly prolonged the QT and PR interval ($p < 0.05$). And Dofetilide (0.075 mg·kg^{-1}) had no influences on RR, PR and QT interval, but shortened the QRS duration. The results revealed that A1998 as a blocker of I_{kur} did not interfered QT interval, PR interval and QRS duration. In a word, A1998 has a prospect to be a new and safe drug as a class III anti - arrhythmic agent.

Keywords: I_{kur}; Butane acid - (5 - androsten - 17 - one - 3β - ol) - diester (A1998); QT interval
[1] Project supported by the National Ocean 863 Foundation of China, No. 2003AA620408; Supported Program of New Century Excellent Talent (No. NCET - 04 - 0808); Supported Program of Fok Ying Tung Education Foundation (No. 91036)

P070027

Abnormal expression of RyR2 and FKBP12.6 linked with sudden appearance of VF are regressed by puerarin in cardiomyopathic rats

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To investigate the effects of puerarin on arrhythmias of cardiomyopathic (CM) heart, SD rats were injected with L - thyroxin (0.3 mg/kg.d, sc) for 10 days to

induce CM and subjected to coronary artery ligation/reperfusion (L/R) to monitor incidence of VF. Puerarin (100 mg/kg.d, ig) was administered for 5 days. The expression of ryanodine receptor type 2 (RyR2) and FKBP12.6 (FK506 binding protein 12.6) in left ventricle (LV) were measured by PCR and Western Blot. We found the CM rats exhibited cardiac dysfunction and high incidence of VF after L/R (90% vs 20%, $P < 0.01$), as well as downregulation of mRNA and protein expression of FKBP12.6 and upregulation of RyR2 ($P < 0.01$ vs control). Puerarin suppressed the incidence of VF significantly (30%, $P < 0.05$ vs untreated), and restored the cardiac function and abnormal expression of FKBP12.6 and RyR2 ($P < 0.05$ or $P < 0.01$ vs untreated). These findings indicate that the high incidence of VF in the affected myocardium was relevant to the altered intracellular Ca^{2+} modulating system which can be regressed by puerarin.

Key words: Ventricular fibrillation; cardiomyopathy; Ca^{2+} signaling; Puerarin
This work was supported by NSFC (NO: 30572193).

P070028

Abrupt changes in expression of PKA, FKBP12.6 and ECE contribute to sudden appearance of VF on reperfusion in L-thyroxin induced cardiomyopathy in rat

Na Tao, Dai De-Zai*, Zhang Yuan, Dai Yin. yes

The channelopathy developed by L-thyroxin multi-doses does not exhibit VF until ischemia/reperfusion episode. It is hypothesized that the sudden appearance of VF on reperfusion is dependent on abrupt deterioration of expression of FKBP12.6 and SERCA2a by molecular events within 1-2 min and could be prevented by a Ca^{2+} channels blocker CPU86017. The rat cardiomyopathy(CM) induced by L-thyroxin and subjected to coronary artery ligation (CAL)/reperfusion to monitor incidence of VF. Calcium transients in the CMs showed a high diastolic $[Ca^{2+}]i$ which is due to calcium leak from the abnormal RyR2. The downregulation of the FKBP12.6 and SERCA2a was seen in CM before and after CAL, and a further abrupt depression on mRNA and protein expression was observed on reperfusion in association with VF. CPU86017 corrected almost all the abnormal events on reperfusion. In conclusion, abrupt down-regulation of FKBP12.6 and SERCA2a and up-regulation of PKA and ECE mRNA are likely involved in abrupt molecular events which promote the appearance of VF on reperfusion.

Key Words: cardiac arrhythmias; CPU86017; PKA; FKBP12.6;
Supported by project No: 30572193 &30230170 from NSFC

P070029

Effects of dipfluzine on experimental arrhythmias and mechanisms of action

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Methods Three arrhythmic models were used in the study. Laser scanning confocal microscope was used to observe intracellular free-Ca^{2+} concentration ($[Ca^{2+}]i$). Results In guinea pig model induced by ouabain, dipfluzine 20 mg/kg delayed the appearance of ventricular premature contraction(VP), ventricular tachycardia(VT), ventricular fibrillation(VF) and cardiac arrest(CA). Incidence of chloroform-induced VF in mice was reduced by dipfluzine 40 mg/kg. In the ischemia/reperfusion-induced arrhythmic model of rats, dipfluzine 20 mg/kg reduced the incidences of VT, VF and CA. The antiarrhythmic effect of dipfluzine was similar to that of verapamil but better than that of flunarizine. Dipfluzine decreased $[Ca^{2+}]i$ of the ventricular myocytes in a concentration-dependent manner. The elevation of $[Ca^{2+}]i$ evoked by high extracellular Ca^{2+} levels was attenuated by pretreatment or posttreatment with dipfluzine. Conclusion The results suggest that dipfluzine is an effective antiarrhythmic agent, and its mechanism is attributed to modulating intracellular Ca^{2+} homeostasis.

Key words dipfluzine, arrhythmia, intracellular calcium
Acknowledgement The project supported by National 863 Key Research Projects 2002AA2Z3132

P070030

The microarray expression analysis identified several key protein candidates as the potential mediators of total flavones of hoerospondias axillaries fructus on myocardial protection

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The total flavones isolated from choerospondias axiaris fructus (TFC) has showed a protection on myocardial ischemic injuries. However, the molecular basis of such protection remains unclear. The microarray analysis for the protein expression in myocardium provide a strong tool to explore the key protein candidates involved in the pathogenesis of ischemic injury. Surface enhanced laser desorption/ionization (SELDI) mass spectrometry with protein chip IMAC3, SAX2 and NP20 was used to compare the differentially expressed protein in TFC-treated and untreated ischemic myocardium in rats and the results were analysized with Proteinchip Software 3.0.2.. We identified seven differentially expressed proteins in TFC treated myocardium. These differential effects correlated with the expression of five downregulated proteins and two upregulated proteins, and four of them were discovered on the IMAC3 chip and one of them was discovered on the SAX2 chip. We suggest that the myocardial protection of TFC may be mediated by the differencial expression of these proteins which could be the key protein candidates for further investigation.

Key words: TFC; myocardial ischemia; proteome

P070031

The change of electrocardiogram in conscious mice during the development of pressure-overload cardiac hypertrophy and failure

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To elucidate the cardiac electrophysiological remodeling during the development of pressure-overload cardiac hypertrophy and failure by observing the changes in electrocardiogram (ECG) in conscious mice. The result revealed characteristic changes in the configuration of QRS and the J junction-S-T segment-T wave complexes at the different phases after aorta banded. Different phenotype of the aberrant repolarization may indcate there is different molecular mechanism involved in electrical remodeling in hypertrophied and failing hearts. $BaCl_2$ (25 mg/kg, iv) and adrenaline (200 mg/kg, iv) produced 90% incidence of ventricular arrhythmias in mice with failing hearts, but didn't induced any arrhythmia in mice with sham-operated and hypertrophied hearts. 4-Aminopyridine (4-AP) (2.5 mg/kg, ip), but not nimodipine (30 mg/kg, ip) prevented or abolished ventricular arrhythmias induced by $BaCl_2$ and adrenaline. The results suggest that 4-AP sensitive currents involve the high risk of ventricular arrhythmias in failing hearts.

Key words: electrocardiogram, cardiac hypertrophy and failure; arrhythmias; 4-aminopyridine.
Supported by NCET-04-0253, NSFC 30370571 and HeBSFC 200400628.

P08. Cardiovascular Pharmacology-Antihypertension Agents

P080001

Effect of rutaecarpine on blood pressure in the 2-kidney-1-clip hypertensive rats.

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Aim: To explore whether or not rutaecarpine can reduce systolic blood pressure (SBP) and reverse vascular remodeling. Method: Surgical procedures were performed under anesthesia induced by celiac injection of pertobarbital sodium. The left kidney artery was narrowed using one silver clip. One week after recovery from surgery, SBP was measured by tail-cuff method. The rats with SBP above 140 mmHg were adopted and divided into four groups, hypertensive rats and hypertensive rats with losartan (20 mg/kg) or rutaecarpine (20 mg/kg) or rutaecarpine (40 mg/kg) at the tenth weekend. The sham-operated rats underwent same procedures, but not clipped with silver clip. The mesentery artery and thoracic artery was sheared and preserved in 10% formalin, in order to obtain for morphological analysis. Results: After treatment with losartan or rutaecarpine, the SBP were significantly decreased compared with hypertensive rats ($p < 0.05$). In mesentery artery, the luminal diameter was significantly increased and the medium thickness was significantly decreased, compared with hypertensive rats. Conclusion: The rutaecarpine can reduce SBP and reverse vascular remodeling in the 2-kidney-1-clip hypertensive rats.

Key words: rutaecarpine, systolic blood pressure, vascular remodeling

P080002

PROSTACYCLIN: EDRF, EDHF and EDCF

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Prostacyclin (PGI2), the principal metabolite of arachidonic acid produced by cyclooxygenase in endothelial cells, was the first identified endothelium-derived relaxing factor (EDRF). It activates IP receptors on vascular smooth muscle cells and, in most arteries, produces relaxation. In some of those, PGI2 hyperpolarizes the smooth muscle cells by opening various populations of potassium channel and

the release of PGI_2 by the endothelial cells can contribute to the endothelium-dependent hyperpolarization (EDHF). Additionally, PGI_2 can stimulate TP receptors and evoke smooth muscle depolarization or/and spontaneous electrical activity. In the aorta of sportaneously hypertensive rats and aging Wistar Kyoto normotensive rats, the endotheliumdependent contractions elicited by acetylcholine involve the generation of reactive oxygen species, the activation of endothelial cyclooxygenase-1 and PGI-synthase, the release and diffusion of PGI_2 and subsequently the contraction of smooth muscle cells by the activation of TP receptors (EDCF). Therefore, PGI_2 is a Janus face prostaglandin, in the rule it protects the vascular wall, but in some instances it can contribute to endothelial dysfunction.

P080003

Endothelin-1 Expression in Vascular Adventitial Fibroblasts

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We hypothesized that aortic adventitial fibroblasts have the ability to produce ET-1, which may contribute to extra cellular matrix synthesis. Vascular adventitial fibroblasts were isolated from mouse aorta and incubated with various concentrations of angiotensin II (AngII). Prepro ET-1 and type I procollagen I mRNA levels were detected by RT-PCR. ET-1 peptide levels were measured by ELISA. Protein levels of procollagen I were detected by western blot. AngII induced a time- and concentration-dependent increase in prepro ET-1 mRNA levels ($n = 4$) and peptide ET-1 ($n = 6$). The AngII evoked increases in prepro ET-1 mRNA and ET-1 were blocked by losartan, an AT1-receptor antagonist but not PD123319, an AT_2-receptor antagonist. Moreover, AngII induced type I procollagen mRNA and protein expression was inhibited by BQ123, an ET_A-receptor inhibitor, but not BQ788, an ETB-receptor inhibitor, suggesting a significant role of adventitial ET-1 in regulation of extra cellular matrix synthesis. The results demonstrate that vascular adventitial fibroblasts are able to synthesize and release ET-1 in response to AngII.

P080004

Hypertension in the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS2)

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Background: Treatment of hypertension reduces cardiovascular events. There is a need to identify hypertension in the community. Method: 1944 subjects (901 men and 1043 women; age 52 ±12 yrs) of the Hong Kong Cardiovascular Risk Factor Prevalence Survey were recruited in 1995 - 6 and were followed up in 2000 - 4. The prevalence of hypertension in the cohort and the factors related to its development were determined Results: In 2000 - 4, the prevalence of hypertension was 23.5 % in men and 17.8 % in women. In those age ≥64 years, it was 55.3 ± 3.5 % in men and 50.6 ±3.7 % in women. In men <55 years, the prevalence of hypertension had increased since 1995 - 6. Among 1602 subjects normotensive at baseline, there were 258 cases of new hypertension after a median interval of 6.4 years. In multivariate analysis, age and baseline systolic blood pressure were significant predictors in both sexes. In men, BMI and plasma triglycerides were significant predictors, but in women, HDL was the predictor instead. Conclusions: Hypertension is common, especially in the elderly. As its development is related to metabolic factors, diet and exercise may prevent or delay its onset, or reduce the need for drug therapy.

P080005

Breviscapine exerts its vasordaxation effect on aortic artery ring via endothelial-dependent pathway

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Breviscapine is a flavonoid extracted from Erigeron breviscapus and have been confirmed to have both cardioprotective and neuroprotective effects. In this study we aim to explore the mechanism of its vasodilation effect by using aortic artery rings prepared from wistar rats. Breviscapine can dose-dependently relax norepinephrine precontracted endothelial intact aortic artery ring, but not that of endothelial denuded aortic artery ring. Breviscapine have no effect on KCL precontracted endothelial intact and endothelial denuded artery rings. The nitric oxide synthase inhibitor, L-NAME, can abolish the vasodilation effect of breviscapine. These results indicated that breviscapine can relax aortic artery ring via endothelial dependent nitric oxide pathway.

Key words: breviscapine; nitric oxide; vasodilation; artery ring

P080006

Effects of hypertension on contractile/diastolic function and calcium sensitivity in rat ventricular myocytes

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Aim To detect effects of hypertension on contractile / diastolic function and calcium sensitivity in rat ventricular myocytes. Methods The hypertensive rat model was prepared by partially ligating the left renal artery and removing the right kidney. Left ventricular myocytes were enzymatically isolated. The contraction and calcium transient of single myocyte from both normal and hypertensive rats were assessed in different extracellular calcium concentrations. Results Compared with cell from normal rats, the contractile and diastolic velocity of ventricular myocyte from hypertensive rat were increased significantly. But its intracellular calcium concentration and calcium kinetics were unchanged. The contractility of hypertensive rat myocytes increased more than that of normal rat myocytes at same extracellular calcium concentration. Conclusions The contractility of ventricular myocyte of hypertensive rats increased significantly, which may be only due to calcium sensitivity increase but not the intracellular calcium elevation.

Keywords: hypertensive rats; Contractile; Calcium transient; Calcium sensitivity

P080007

Antihypertrophic effect of ginsenoside Rg_1 on cardic myocytes and its mechanisms

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To study the potential inhibitory effect of ginsenoside Rg_1 (Rg_1) on myocardial hypertrophy, the cardiac myocyte hypertrophy model was indused by Ang II-0.1 μmol·L^{-1}, and the cell diameter, protein content and the expression of atrial natriuretic peptide (ANP) mRNA were used as hypertrophic parameters. For mechanism studies, the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), the nitric oxide (NO) content in culture medium and the expressions of ANP-, calcineurin (CaN)- and eNOS-mRNA were detected. The results showed that Rg_1 (12.5 - 50 μg·ml^{-1}) concentration-dependently reduced the increased cell diameter, protein content and the expression of ANP mRNA and increased the NO content and eNOS mRNA expression. Further more, Rg_1 could remarkably decrease the elevated $[Ca^{2+}]_i$ and CaN mRNA expression induced by Ang II. NG-nitro-Larginine ester could abolish the antihypertrophic effect of Rb_1. It is concluded Rg_1 inhibit the cardiomyocyte hypertrophy induced by Ang II, which may be related to its inhibitory effect on $[Ca^{2+}]_i$, promoting effect on NO formation, and involved in CaN signal pathway.

KEY WORD: angiotensin II; ginsenoside Rg_1; cardic myocyte hypertrophy; calcineurin

P080008

Vasodilative effect of YMII on rabbit aorta strips and its mechanisms

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To investigate the effect of YMII, a naftopidil ramification, on the vascular activities, we recorded the isotonic contraction of the thoracic aorta strips of rabbit and used the Fura-2/AM loaded vascular smooth muscle cells (VSMC) to observe the influences of YMII on the concentration-response curves for agonists and the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). The results showed that YMII could shift the concentration-response curve for noradrenaline (NA) to right in paralled manner and with a pA_2 value 8.06, contrary, it shifted the one for hight potassium (hight + K^+) to right in non-competitive manner and with a pD_2 value 5.02, and had no statistic influence on that for 5-HT. In Ca^{2+} free medium, YMII could inhibit the transient contraction induced NA (but no effect on that by caffeine) and the long-lasting one induced by addtion of Ca^{2+}. Further, it reduced significantly the $[Ca^{2+}]_i$ elevated by NA and hight K^+. It is suggested that YMII can relax the rabbit aorta strips, which may be attributed to its blocking effect on α-receptor, resulting in the inhibition of Ca^{2+}-influx and Ca^{2+}-release.

Key Words: YMII; vasodilative effect; rabbit aorta strips; intracellular free Ca^{2+}

P080009

The Cardiovascular Effects of Insulin-like Growth Factor-1 in the Nucleus Tractus Solitarii of Rats

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Insulin-like growth factor 1 (IGF-1) was a factor involved in arterial hypertension because of its effects on vascular tone. The aims of this study was to compare the cardiovascular effects of IGF-1 and the mechanisms of IGF-1 induced signaling pathway in the nucleus tractus solitarii (NTS) between SHR and WKY rats. The results indicated that microinjection of IGF-1 into the NTS of WKY and SHR produced depressor and bradycardic effects. Pretreatment with the PI3K inhibitor LY294002 significantly attenuated the responses evoked by microinjection of IGF-1 in both SHR and WKY. Moreover, mitogen-activated protein kinase kinase (p44/p42 MAPK) inhibitor PD98059 administration attenuated the cardiovascular effects of IGF-1 in WKY but had no effect in SHR. In conclusion, both IGF-1/PI3K and p44/p42 MAPK signal transduction pathways are involved in controlling central cardiovascular effects in WKY, whereas PI3K but not p44/p42 MAPK signaling pathway is involved in SHR.

Key words: IGF-1, NTS, blood pressure

Acknowledgement: This work was supported by grants from the National Science Council (NSC94-2320-B-075B-003) to Dr. Ching-Jiunn Tseng.

P080010

Cardiovascular Effects of alfa-Melanocyte-Stimulating Hormone in the Nucleus Tractus Solitarii of Rats

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α-melanocyte stimulating hormone (α-MSH) is an important regulator of food intake, metabolic rate, and inflammation. In the present study, we investigated the cardiovascular effects of α-MSH in the nucleus tractus solitarii (NTS) of spontaneously hypertensive rats (SHR). In SHR, microinjection of α-MSH (0.3-300 pmol) into the NTS produced dose-dependent depressor and bradycardic effects. The cardiovascular effects of α-MSH were abrogated by the antagonist of melanocortin receptor (MC3/4-R), SHU9119. Pretreatment with L-arginine, enhanced the duration of α-MSH-mediated hypotensive effects, whereas prior application of L-NAME significantly attenuated the effects of α-MSH. Pretreatment with inhibitor of iNOS, aminoguanidine, but not inhibitor of nNOS, 7-nitroindazole, attenuated the hypotensive effect of α-MSH. In summary, these results indicated α-MSH induced depressor and bradycardic effects in the NTS of SHR. The hypotensive mechanism of α-MSH was mediated via MC4-R and involved with iNOS activation in the NTS of SHR.

Key words: α-MSH, NTS, blood pressure

Acknowledgement: This work was supported by grants from the National Science Council (NSC94-2320-B-075B-002) to Dr. Ching-Jiunn Tseng.

P080011

Blood pressure variability is more important than blood pressure level in determination of cardiovascular damage in rats

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The importance of blood pressure variability (BPV) and blood pressure (BP) level in determination of cardiovascular damage was compared in two different rat models. In male sham-operated and sinoaortic denervated Wistar-Kyoto rats and spontaneously hypertensive rats (n = 34), BPV was more important than BP in cardiac damage, renal lesion and aortic hypertrophy. BPV and BP had independent effects, explaining 59% of the variation in these organ damages. In male F1 hybrids of Sprague-Dawley rats and spontaneously hypertensive rats (n = 44), the greater importance of BPV than BP was further demonstrated in left ventricular hypertrophy, glomerular damage and aortic hypertrophy. BPV and BP or BPV alone had independent effects, explaining 47% of the variation in these organ damages. It is concluded that BPV is a more critical determinant than BP level for cardiovascular damage in rats, strongly suggesting the significance of BPV control for cardiovascular protection. This wok was supported by the grants from the National Natural Science Foundation of China (30371649) and the Foundation for the National Excellent Doctoral Thesis Author (200369).

P080012

Effects of Magnesium Taurate Compound (MTC) on L-NNA-Induced Hypertension in Rats and NA, KCL-Induced Aorta Contraction in Rabbits

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Objectives: To study the effects of MTC on L-NNA-induced hypertension in rats and NA, KCl-induced aorta contraction in vitro in rabbits. Methods: 1. L-NNA was used to copy hypertensive model in rats. MAP, heart index (HI), ET-1 and CGRP of plasma were determined in low, middle and high doses of MTC, $MgSO_4$ and taurine groups. 2. Rabbit aorta strips were suspended in organ baths containing Krebs solution, and then isometric tension was measured in different status of NA, KCl with and without drugs. Results: 1. MAP of each MTC group was significantly decreased, compared with L-NNA. MTC-L and taurine can significantly inhibit ET-1, MTC-L and $MgSO_4$ can significantly increase CGRP ($P < 0.05$). 2. Each MTC group can inhibit the contractive action of aorta strip tension induced by NA, KCl and have a dose-dependence relationship. Conclusion: The data showed that MTC had an anti-hypertensive effect and significantly depressed the contractive action of aorta induced by NA and KCL.

P080013

Effects of the Angiotensin II Type 1 Receptor Antagonist Valsartan on the Expression of Superoxide Dismutase in Hypertensive Patients

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Oxidative stress induced vascular diseases has been known. Angiotensin (Ang) II is regarded as a pro-oxidant because it stimulate the production of free radicals (ROS). This study was to evaluate whether treatment with the Ang II receptor antagonist valsartan has an antioxidant effect in hypertensives. A placebo-controlled study was conducted in 48 hypertensives. Patients were followed every 4 weeks for 12 weeks after randomization to valsartan treated with 80-160 mg or placebo. The erythrocyte superoxide dismutase (SOD) activity and expression of SOD-mRNA in leucocytes (PMN) were measured. Valsartan showed inhibition of ROS in PMN from hypertensives. The erythrocyte SOD activity before treatment was over 2× higher in hypertensives. SOD activity decreased significantly after 12-weeks of treatment but not with placebo. The SOD-mRNA in the PMNs decreased over 3 months in the hypertensives receiving valsartan. Valsartan treatment resulted in a downregulation of SOD-mRNA and a reduction in SOD activity suggesting an antioxidant activity and reduction of ROS. These findings imply that valsartan may provide benefits to hypertensives beyond blood pressure reduction.

Keywords: Gene; ROS; SOD; Valsartan

P080014

CHRONOTHERAPY IN RESISTANT HYPERTENSION: IMPROVEMENT OF RENAL FUNCTION BY INCREASING THE DAY/NIGHT BLOOD PRESSURE (BP) RATIO.

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We have prospective evaluated the potential beneficial effects of chronotherapy on renal function in patients with resistant hypertension. We studied 213 patients who were receiving 3 antihypertensive drugs in a single morning dose. Patients were randomly assigned to one of two groups according to the modification in their treatment strategy: 1) Changing one of the drugs, but keeping all 3 in the morning. 2) The same approach but administering the new drug at bedtime. BP was measured for 48h at baseline and after 3 months of intervention. The diurnal/nocturnal BP ratio was slightly reduced with all drugs on awakening, but significantly increased in patients receiving one drug at bedtime ($P < 0.001$). The percent decrease from baseline in urinary albumin excretion (UAE) and increase in glomerular filtration rate (GFR) after treatment were significantly correlated with the increase in diurnal/nocturnal BP ratio ($P < 0.001$). Chronotherapy allows increasing the diurnal/nocturnal BP ratio towards a more dipper profile. This change in the circadian BP pattern is correlated with the improvement of renal function associated to reverting the high-risk non-dipper pattern into a dipper BP profile.

P080015

Effect of vascular endothelial growth factor (VEGF) on superoxide anion and endothelial function in streptozotocin (STZ)-induced diabetic rats

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The aim of this study was to determine if the ability of VEGF to preserve vasodilatory responses to acetylcholine (Ach) in the STZ-induced diabetic rats was related to superoxide anion generation. After induction of diabetes, changes in arterial pressure (BP) and superior mesenteric arterial (SMA) flow to i.v. infusions of Ach (0.1 - 12.5 mg/kg) were recorded in anesthetized rats treated with VEGF or iVEGF. In other rats, superoxide anion generation and endothelial nitric oxide synthase (eNOS) expression were determined in isolated aorta. The changes in BP and SMA conductance (SMAC) to Ach were attenuated in iVEGF treated STZ rats but not in non-diabetic SD rats. VEGF prevented the decrease in Ach-evoked responses observed in iVEGF treated STZ rats, and it prevented the dramatic increases in superoxide generation in these rats. Paradoxically, eNOS expression was enhanced in STZ rats and VEGF prevented these changes, findings that may be related to the "coupled/uncoupled" state of the enzyme. The results suggest that the preservation of Ach evoked responses by VEGF may be related to normalizing the oxidative stress environment of diabetic state. (Supported by HSFS and CIHR).

P080016

Adjuvant application of TLR4 agonist but not TLR2 agonist attenuates hypertension-induced cardiovascular hypertrophy and fibrosis in rats

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Immune system is important in development of cardiovascular hypertrophy and fibrosis. We aim to determine the roles of Toll-like receptors (TLRs) in cardiovascular hypertrophy and fibrosis induced by abdominal aortic constriction. Animals were intraperitoneally administered with or without TLR4 angonist LPS or TLR2 agonist PG-LPS, every three days for one week before modeling. Elevation of blood pressure led to a time-dependent reduction in expression of TLR4 in myocardial tissue. In contrast, expression of TLR4 was significantly elevated in LPS-pretreated rats but elevated blood pressure did not further increased in expression of TLR4. LPS but not PG-LPS significantly inhibited perivascular and interstitial fibrosis, attenuated hypertrophy of heart and aorta without affecting arterial pressure and heart rate. Pretreatment of animals with TLR4 but not TLR2 antagonist reversed LPS-induced cardiovascular protective effects. Also, LPS reduced expression of IL-10 and TGF-β in myocardial tissue. Our results suggest that TLR4 play a key role in reactive hypertrophy and fibrosis induced by elevation of arterial pressure.

Key words: hypertension, cardiac fibrosis, hypertrophy, TLR4

P080017

The role of tissue transglutaminase in arterial remodelling

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Resistance vessel inward remodelling occurs in essential hypertension, causing increased peripheral resistance. We have shown that tissue transglutaminase (tTG) mediates inward remodelling in vitro. In two series of experiments, we investigated whether also in vivo tTG is involved in inward remodelling and in reverting it. Cystamine (40 mg/kg/day) was used as an inhibitor of tTG. Second order mesenteric artery morphology was investigated with a pressure myograph. In the first series, constant infusion (osmotic minipump) of phenylephrine (1.44 mg/kg/day, n = 8) caused 16 % inward remodelling (lumen reduction) of the small arteries compared to vehicle infusion (n = 7, P < 0.01). The remodelling was inhibited by concomitant infusion with cystamine (n = 8) and vehicle (n = 7) for a week. In the second series, we showed that in rats which had been pretreated with phenylephrine (n = 8) one week of amlodipine infusion (6 mg/kg/day, n = 8) caused 24 % outward remodelling (i.e. reversion of inward remodelling. P < 0.001) and this was attenuated by concomitant infusion of cystamine (n = 8, P < 0.001). In conclusion, our results suggest that tissue transglutaminase is involved both in inward remodelling and in the reverting of it.

P080018

THE ANTIMIGRATORY EFFECT OF POTASSIUM DICLOFENAC WAS IMPAIRED BY AMLODIPINE IN SPONTANEUOSLY HYPERTENSIVE RATS (SHR).

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Recent studies demonstrated that both enalapril and losartan interfere with the effect of diclofenac on leukocyte behavior in spontaneously hypertensive rats (SHR). We studied if the same occurs with amlodipine. Male SHR were divided into four groups: vehicle, potassium diclofenac 1 mg/kg, amlodipine 10 mg/kg and diclofenac plus amlodipine, treated for 15 days (v.o.). The blood pressure (BP) was evaluated by indirect tail-cuff method; leukocyte rolling, adherence and migration were studied by intravital microscopy. Diclofenac did not change whereas amlodipine reduced the BP levels in SHR (by 18 %). Diclofenac did not interfere with the reducing effect of amlodipine. Diclofenac diminished leukocyte rolling, adherence and migration by 62, 66 and 79 %, respectively, whereas amlodipine only reduced leukocyte adherence (48 %) and migration (46 %). When both drugs were combined, diclofenac effect on adherence and migration, but not on rolling, was reduced (by 33 % and 27 %, respectively). In conclusion, similarly to enalapril and losartan, amlodipine interferes with the effect of diclofenac on leukocyte behavior in SHR.

Key words: Leukocyte, SHR, amlodipine, diclofenac.
Acknowledgement: FAPESP/PRONEX.

P080019

INVESTIGATION OF THE ANTIFIBRILLATORY DRUG INTERACTIONS BETWEEN LIDOCAINE AND CAPTOPRIL IN PERFUSED RABBIT HEARTS

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Recently, we have presented the antifibrillatory interactions between lidocaine and two antihypertensive drugs, propranolol (Almotrefi et al., 1999) and valsartan (Almotrefi & Arif, 2004). This abstract reports its interactions with captopril. Studies were carried out on hearts isolated from New Zealand white rabbits of either sex weighing 1 to 2 Kg. The method used has been described previously (Almotrefi & Baker, 1981). Perfusion with lidocaine produced significant, dose-dependent increase in VFT while perfusion with captopril did not cause any significant change. In addition, there was no significant difference in VFT with the combined infusion of 3.46 µmol of lidocaine and 1 µmol of captopril, in contrast to a synergistic antifibrillatory effect of the combined use of lidocaine and propranolol (Almotrefi et al., 1999). This suggests that captopril does not have antifibrillatory interactions with lidocaine, indicating its safety in combining with class 1 antiarrhythmic drugs.

antiarrhythmics, lidocaine, captopril.

P080020

NO RELEASE AND CALCIUM ENTRY BLOCKADE, NEW MECHANISMS OF ACTION OF METOPROLOL AND FOUR STRUCTURALLY RELATED ENANTIOMERS.

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Four structurally related enantiomers of metoprolol (with no beta blocking activity) relaxed rat aortic rings contracted by phenylephrine (an effect partially inhibited by L-NAME) and relaxed rat aortic rings depolarized by potassium (Melgar et al, 2004). The present work deals with a pharmacological characterization of metoprolol and the stereoisomers. The beta blocking action of these compounds was tested in the rat atria stimulated by isopropylarterenol, metoprolol was two orders of magnitude more potent than the the isomers. But, when tested for relaxation on aortic rings previously contracted by phenylephrine, they did it in a similar concentration. This effect was partially inhibited by L-NAME. When aortic rings were depolarized by potassium (80 mM), they contracted, but were relaxed by both metoprolol and the isomers at high, but similar concentrations. Depolarized aortic rings placed in a free calcium solution were contracted by increasing concentrations of calcium. Metoprolol and the isomers shifted the calcium concentration response curves to the right. These results suggests that a NO release and a calcium entry blockade may contribute to the antihypertensive effect of metoprolol.

Key words: metoprolol, calcium entry blockade, NO release, metoprolol stereoisomers.

P080021

LLShB Formula Inhibits ACE and Lowers BP in Anesthetized Spontaneously Hypertensive Rats

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The hypotensive proprieties of LLShB formula were assessed in anesthetized spontaneously hypertensive rats (SHR) in this study after chronic oral treatment. At Week 0, SHR with systolic arterial pressure (SAP) > 160 mmHg were selected and randomized into 4 groups (n = 10 each), receiving orally placebo, LLShB (90 or 270 mg/kg), or Hypotensive #0 Drug (H0D, 5 mg/kg) by gavage. BP was measured at Week 4 on a 16-channel physiograph (BIOPAC) under anesthesia with a sensor placing in arteria femoralis of the rats. Heart rate (HR), ECG, SAP, diastolic (DAP) and mean arterial pressure (MAP) were traced for 25 min. We found no changes in HR and ECG in all groups ($p > 0.05$). SAP, DAP & MAP were reduced with H0D ($p < 0.01$). At Week 4, dose-dependent reductions of SAP, DAP & MAP were seen with LLShB. Higher dose of LLShB at 270 mg/kg induced reductions of SAP, DAP & MAP ($p < 0.05$), but not at 90 mg/kg ($p > 0.05$). LLShB inhibited ACE activity in vitro by 6% & 23% at 100 and 500 ug/ml. Our data indicate that oral treatment with LLShB is effective in reducing BP in SHR in a dose-dependent fashion. LLShB appeared to show mild ACE inhibition.

P080022

ENALAPRIL RESTORES THE REDUCED BRADYKININ VASODILATION IN TYPE 2 DIABETES INCREASING B2 RECEPTOR PROTEIN

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In the present study we investigate the mechanism involved in the restoring effect of enalapril (E) on the reduced bradykinin (BK) vasodilation observed in type 2 diabetes. For this, diabetes was induced in 2 days old male Wistar rats by streptozotocin injection (150 mg/kg, i.p). After 14 weeks, diabetic rats (D) were treated with E (10 mg/kg/by gavage, for 21 days) and compared with untreated D and control rats (C). Using intravital microscopy, the increase in mesenteric arteriolar (12 - 25 um) diameter (in %) induced by BK (10 pmol), was compared in these rats. mRNA (by RT-PCR) and protein expression (by immunohistochemistry) of B1 and B2 kinin receptors were determined in whole mesenteric arteriolar bed. BK response reduced in D (C-7.02 ±0.20 and D-2.97 ±0.16 %) was restored by E treatment (D+ E- 6.11 ±0.22 %). There was no difference in mRNA and protein expression BK receptors between untreated D and C. E treatment increased B2 kinin protein expression without interfering with B1 receptor expression. We conclude that, in diabetic rats, enalapril-restoring effect on BK vasodilation might involve increase in B2 receptor protein.

Keywords: diabetes, bradykinin, enalapril.

Acknowledgements: FAPESP, PRONEX

P080023

Vasodilatory effect of glybenclamide on mouse mesenteric artery

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Sulphonylureas, such as glybenclamide (Gly) and gliclazide, are classical blockers of KATP channels. Vascular contractility changes induced by sulphonylureas were investigated using a wire myograph. The phenylephrine (PHE)-contracted resistance mesenteric artery (<100) were relaxed by Gly from C57 mice (EC_{50}, 36.0 μM; n = 10) and SD rats (EC_{50}, 0.47 μM; n = 8). Gliclazide had a similar vasodilatory effect in mice (EC_{50}, 17.7 μM; n = 3). Removal of endothelium (n = 7), pre-contraction with 100 mM $[K^+]_o$ (n = 7), or 25 mM $[K^+]_o$ + PHE (n = 8) significantly reduced the vasodilatory effect of Gly. Pre-contraction with PHE in the presence of either L-NAME (0.3 mM, n = 8) or $BaCl_2$ (0.1 mM, n = 8) also significantly reduced the vasodilator effect of Gly. However, pre-treatment with 4-AP (1 mM) + PHE (n = 8) or iberotoxin (0.1 μM) + PHE (n = 11) did not alter Gly-induced vasodilation (<100 μM). Pinacidil, a KATP channel opener, also induced a vasodilation (EC_{50}, 0.84 μM; n = 7). Discovery of this novel vasodilatory effect of sulphonylureas would be important for guiding further basic and clinical studies with the use of these compounds. (Supported by CIHR).

Key word: Glybenclamide, mesenteric artery, vasodilation, mice

P080024

Lacidipine Reduces High Blood Pressure and Cardiac Damage Induced by L-NAME in Rat: Effect on Leptin

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The study aims to explore the effect of NO synthase inhibition, using N-nitro-L-arginine methyl ester (L-NAME), on blood pressure, leptin, lipid and redox system in plasma and cardiac tissue, and to evaluate the effect of long-term prophylactic treatment with lacidipine (Lp). Hypertension was induced in rats by daily administration of L-NAME (50 mg/kg, po, 6 weeks). Rats were treated with Lp (3 and 6 mg/kg, po), starting 1 day after induction of hypertension and continued thereafter. A normotensive group was used for comparison. Long-term inhibition of NO synthesis produces rise of blood pressure, plasma leptin, cholesterol and triglycerides. Redox status of myocardial tissue was shifted to oxidative stress, but phospholipids were not altered. Lp normalized blood pressure and improved plasma lipid profile. Reduction in elevated leptin was observed with the high dose of Lp that also ameliorated cardiac oxidative stress. In conclusion, beside its antihypertensive effect long-term treatment with Lp has beneficial effect on plasma lipid profile and myocardial oxidative stress induced by NO synthesis inhibition. Its beneficial effect in reducing elevated plasma leptin warrants further study.

P080025

Protective action of a hydroalcoholic extract of a vinifera grape skin on experimental preeclampsia in rats.

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This study was designed to determine the protective effects of a vinifera grape skins extract (GSE, 200 mg/kg/day) in experimental preeclampsia induced by chronic inhibition of nitric oxide synthesis in pregnant rats. Blood pressure was measured with the tail cuff method on day 20 of pregnant control rats; pregnant rats treated with L-NAME, L-NAME plus GSE or GSE from day 13 to day 20 of pregnancy. Glucose was infused in anesthetized pregnant rats at day 20 and blood glucose and insulin was estimated at time zero, 15, 30, 45 and 60 minutes after beginning of glucose infusion. The number of fetus alive was also estimated at day 20 of pregnancy. Increase in arterial pressure, reduction of fetus alive at the end of pregnancy and increase in insulin resistance was observed in pregnant L-NAME rats but not in pregnant L-NAME plus GSE rats or in pregnant GSE rats. The present study demonstrated a protective effect of an extract obtained from skin of a vinifera grape in experimental preeclampsia since the deleterious effect induced by L-NAME that is, increased in stillbirth, hypertension and insulin resistance were significantly reduced by oral treatment with the extract.

P080026

ETB receptor activation increases blood pressure and sympathetic ganglionic O_2^- production in the presence of chlorisondamine

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In vivo endothelin type B (ETB) receptor activation induced an acute rise in mean arterial pressure (MAP) accompanied by increased superoxide (O_2^-) production in sympathetic ganglia. The goal of our present study was to determine if this elevated O_2^- anion concentration participates in the pathogenesis of ET-dependent hypertension by facilitating nicotinic neurotransmission through the ganglion. We used chlorisondamine (CHL) to block nicotinic input in autonomic ganglia. Sprague-Dawley rats were assigned to one of 3 treatments: 1) 2h infusion of the specific ETB receptor agonist sarafotoxin 6c (S6c), 2) CHL followed by 2h S6c, 3) CHL followed by 2h saline. MAP increased significantly following S6c and CHL-S6c treatment. To measure O_2^- levels, we removed ganglia following infusion and stained them with dihydroethidine (DHE). The DHE fluorescence intensities were significantly greater in both S6c and CHL-S6c rats compared to CHL saline infused rats. Our results show that hypertension and elevated O_2^- production following ETB receptor activation persist after ganglionic blockade, suggesting that ET-dependent hypertension may be impartial to preganglionic input.

Our research is supported by NIH P01 HL-70687

P080028

Scavenging Methylglyoxal Inhibited Hypertension Development in Spontaneously Hypertensive Rats

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Increased methylglyoxal (MG) and MG-induced advanced glycation endproducts (AGEs) have been shown in aorta of spontaneously hypertensive rats (SHR) but whether these changes are pathogenic for hypertension development in SHR is unknown. Chronic treatment of young SHR with aminoguanidine (AG), a scav-

enger for AGEs, significantly lowered blood pressure by 34 mmHg (n = 8, p < 0.05). Plasma and aortic MG levels, aortic levels of MG-induced AGEs (Nε-carboxyethyl-lysine and argpyramidine), and superoxide and peroxynitrate levels were significantly lowered after AG treatment. Reduced glutathione level was significantly increased by AG treatment in SHR aorta. Moreover, AG treatment reversed the morphological damage of vascular tissues in SHR, and increased acetylcholine-induced relaxart response of mesenteric arteries. In conclusion, MG and MG-induced AGEs contribute to the pathogenesis of hypertension by altering redox balance, causing vascular hypertrophic remodeling, and inducing endothelial dysfunction in SHR (Supported by CIHR & HSFC)

Key Words : methylglyoxal, advanced glycation endproducts, hypertension

P080029

Arginase II augments vasoconstriction: evidence from the arginase II knockout mouse

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Arginase II shares the substrate L-arginine with nitric oxide synthase (NOS) and is upregulated in hypertension. Using the wire myograph, we studied vasoactive responses in aorta isolated from arginase II knockout (KO) and c57bl/6J (WT) mice. Concentration response curves to phenylephrine (PE), noradrenaline (NA), acetylcholine (ACh), isoprenaline (Iso) and sodium nitroprusside (SNP) were constructed; some in the presence and absence of the NOS inhibitor; Nω-nitro-L-arginine (NOLA), others the βblocker, propranolol or Rho kinase inhibitor, Y-27632. Responses to NA, but not high K^+, were significantly reduced in KO aorta (n = 7-9, p < 0.05). Responses to neither ACh, Iso nor SNP differed. NOLA significantly blunted ACh and Iso relaxation and increased NA responses to a similar magnitude in both groups. NA and Iso responses post-propranolol were comparable. In contrast, Y-27632 abolished the difference in NA responses between WT and KO. Arginase II may influence blood pressure by increasing vasoconstriction via Rho kinase and not a βadrenergic or nitric oxide pathway.

This work is supported by an Australian NHMRC Program Grant. Ms Huynh is a recipient of the Monash Graduate Scholarship.

P080030

Enhancement of ACE2 and nitric oxide Levels by All-trans Retinoic Acid in SHR

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OBJECTIVE Angiotensin converting enzyme 2 (ACE2) can antagonize Ang II actions and potentiate NO release via Ang (1-7) and its receptor Mas. The aim of this study is to evaluate whether all-trans retinoic acid (atRA) regulates the ACE2 expression and Ang II/ NO balance in hypertension. METHODS Spontaneously hypertensive rats (SHR) and Wistar-Kyoto(WKY) rats were treated with atRA (10 or 20 $mg \cdot kg^{-1} \cdot day^{-1}$) given as daily intraperitoneal injection for one month. Real-time PCR and Western blot were performed to examine the mRNA and protein expression of ACE2, AT_1 receptor and endothelial NO synthetase (eNOS) in rats after atRA treatment, respectively. RESULTS Significant upregulations of ACE2 and eNOS expression were observed in heart in atRA-treated SHR (p < 0.05, respectively), accompanied by a reduction of AT_1 expression, an elevation of serum NO and a decrease of blood pressure (p < 0.05, respectively). However, in WKY rats, chronic atRA treatment had no effect on cardiac ACE2, AT_1 and eNOS expression, serum NO and blood pressure. CONCLUSION Increased ACE2 and eNOS expression by atRA contributes to a shift of Ang II / NO balance and reduced blood pressure in SHR. Thus, atRA may have potentially clinical value in the treatment of human essential hypertension.

Key Words : ACE2; nitric oxide; all-trans retinoic acid.

P080031

Caveolin-1 regulates static pressure-dependent activation extracellular signal-regulated kinase in vascular smooth muscle cells

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AIM: To investigate the effect of caveolin-1/ERK1/2 pathway on static pressure-dependent VSMCs proliferation. METHODS: Cultured VSMCs were respectively treated with 0, 120, 180 and 240 mmHg in a self manufactured pressure incubator for 24 hrs and then with 120 mmHg in different time. VSMCs proliferation was evaluated with cell counting and MTT assay. Western Blot was used to determine the expression of Caveolin-1 and phosphor-ERK1/2 (p-ERK1/2). RESULTS: VSMCs proliferation and ERK1/2 activation were significantly stimulated by static pressures of 120 mmHg and 180 mmHg with the peak at 120 mmHg. Static pressure of 240 mmHg had no effect on VSMC proliferation. Simultaneously, the expression trend of Caveolin-1 was opposite to that of p-ERK. We observed that VSMCs proliferation and p-ERK1/2 expression increased rapidly at the earlier stage(4 hrs), which followed by a steady state of VSMCs proliferation and a decline of p-ERK1/2. Furthermore, PD98059 prohibited static pressures-stimulated VSMCs proliferation and ERK1/2 activation. CONCLUSION: Static pressure stimulates vascular smooth muscle cell proliferation via the ERK1/2 pathway, which is regulated by caveolin-1.

P080032

Evaluation of the pharmacological effects of the new imidazolyl derivatives as calcium channel modulators

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Congestive Heart Failure has a broad prevalence and there is no definite medication for this disease. New 1,4-dihydropyridine derivatives, which are both able to decrease vascular tone and increase heart contractility, can be considered as helpful compounds for treatment of CHF. In this study, we evaluated the new derivatives of dihydropyridine which were synthesized to produce a dual cardioselective Ca^{2+} channel agonist/ vascular selective smooth muscle antagonist activity. The antagonist effects of these derivatives on the guinea-pig ileum, which has been contracted by KCl (40 mM), were examined. The agonist effects of these derivatives on the guinea-pig's left atrium, which has been stimulated by stimulator, were examined. The results revealed that all the examined derivatives have smaller effects on the ileum as compared to the nifedipine. Derivatives containing cyclopentanon ring on the 5th position of the dihydropyridine ring, were more effective. None of the examined derivatives had negative inotropic effects on the atrium, so can be useful in hypertensive patient in combination with CHF.

P080033

Synthesis and evaluation of calcium channel antagonist activity of some new imidazolyl 1,4-dihydropyridine analogues containing carbamoyl substitute

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The discovery that 1,4-dihydropyridine class of calcium channel antagonists inhibits Ca^{+2} influx represented major therapeutic advances in the treatment of cardiovascular disease such as hypertension, angina pectoris and other spastic smooth muscle disorders. Previous studies revealed that nitroimidazolyl group is bioisoester of nitrophenyl group in nifedipine analogues. In addition, it is clear that amidyl groups is bioisoester of the esteric groups, however, it is proposed that replacement of esteric group with amidyl one will be resulted in improvement of physicochemical properties.

In this study many unsymmetrical alkyl and aryl analogues of 5-(diethyl carbamoyl)-2,6-dimethyl-4-(1-methyl-5-nitro-1H-imidazole-2-yl)-1,4 dihydropyridine-3 carboxylate were prepared using modified Hantzsh reaction. In vitro calcium channel antagonist activities were determined by the use of high K^+ contraction in guinea-pig ileal longitudinal smooth muscle. They exhibited less in-vitro calcium channel antagonist activity (10^{-5} to 10^{-6} M range).

Key words: 1,4-Dihydropyridine, calcium channel antagonists

P080034

THE CARDIOVASCULAR ACTIONS OF ARCTIGENIN

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The present study was conducted to elucidate the cardiovascular actions of arctigenin (ARC). In Sprague-Dawley rats, ARC produced hypotension, bradycardia and blood flow increasing effects which significantly reversed by Nω-nitro-L-argi-

nine (L- NNA) and atropine. The hypotensive and blood flow increasing effects produced by ARC were significantly greater in SHR when compared with SD rats. In isolated Langendorff with retrograde perfusion rat hearts , ARC significantly increased coronary flow and ±dp/ dt that was partially affected by L- NNA and propranolol. In aortic rings precontracted with phenylephrine , ARC elicited a partially endothelium- dependent relaxation , which was completely inhibited by L- NNA. Additionally , ARC notably increased NO release in endothelial cells. In conclusion, these findings suggest that the plausible mechanisms of ARC in antihypertension could accout for simultaneous modulation of NO in association with muscarinic and beta- adrenergic receptors activation. An integrated mechanism of ARC had a beneficial effect on hypertensive animals.

P080035

Celiac ganglionectomy largely removes sympathetic nerves innervating mesenteric veins and arteries of rats

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The sympathetic nervous system, especially the components innervating the splanchnic region , is very important in regulating blood pressure , because the splanchnic vasculature accounts for a large portion of the capacitance function of the circulation. The celiac plexus contains most of the sympathetic neurons innervating the splanchnic organs. Celiac ganglionectomy (CGX) is a procedure where the celiac plexus is surgically removed , so it can be used to study the role of splanchnic sympathetic innervation in blood pressure regulation. This study was to validate the effects of CGX by examining the sympathetic nerves on mesenteric vessels via glyoxylic acid (GA) staining , and by measuring contraction of mesenteric vessels to electrical stimulation. GA staining showed that sympathetic nerves were largely eliminated 10 - 14 days after CGX, compared to those of SHAM rats. Electrical stimulation demonstrated that vessels of CGX rats responded minimally to electrical stimulation , whereas frequency- dependent constriction was observed in vessels of SHAM rats. These data indicate that CGX results in an effective sympathectomy of the splanchnic vascular bed.

Key word : celiac ganglionectomy , splanchnic vasculature

P080036

Antihypertensive and Vasodilator Effects of Ethandic Extract of Aralia elata in the Spontaneously Hypertensive Rats.

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The antihypertensive and vasodilator effects of ethanolic extract prepared from Aralia elata (AE) were assayed both in spontaneously hypertensive rats (SHR) and normotensive rats (NTR). SHR was subject to daily oral administration of AE (10 , 50 mg/ kg) for 12 weeks and segments of thoracic aorta used to assess vascular function. The systolic blood pressure (SBP) was significantly reduced on 2 weeks , and the lowered blood pressure was maintained during the entire period of administration. The weight index of heart , liver , kidney and brain were significantly reduced at higher concentration (50 mg/ kg) of AE-treated SHR than in vehicle-treated SHR. The vascular function was compared in aorta from each group. AE improved endothelium-dependent vasorelaxation but had no effect on endothelium-independent vasorelaxation. There no significant changes in NTR. In conclusion , these data demonstrate that AE reduces the elevated blood pressure and cardiac and renal hypertrophy in SHR. And AE is a good candidate for development as an antihypertensive agent.

Key words : hypertension , Aralia elata , spontaneously hypertensive rats , blood pressure

P080037

Studies on the antihypertensive effects of a standardized extract of Solanum indicum ssp. distichum

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Solanum distichum fruits have been used in African folk medicine as an antihypertensive , but no studies have been reported to assess this effect. An ethandic extract of the fruits has been standardized to contain not less than 0.2 % total glycoalkaloids and provided in a dry form. The antihypertensive action was tested in rats rendered hypertensive by the intraperitoneal injection of L- NAME twice daily for 1 week , when the rats developed a high blood pressure (measured non-invasively) accompanied by bradycardia. Simultaneous treatment of animals with L- NAME and the extract (orally) prevented development of hypertension but did not significantly affect the bradycardia. Starting treatment with the extract in doses of 1 - 100 mg/ kg orally for 1 week after the development of hypertension whilst continuing L- NAME administration , tended to normalize the systolic blood pressure. However , oral administration of the extract to normal rats for 4 weeks in doses up to 300 mg/ kg did not show any significant hypotensive effect. The present results show a definite blood pressure lowering effect of the extract in hypertensive but not in normotensive rats.

Keywords : Solanum distichum Hypertension L- NAME

P080038

Selective agonists reveal that alpha1A- and alpha1B-adrenoceptors contract tail artery of the young rat

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Multiple alpha1- adrenoceptors seem to contract rat tail artery. Alpha1A- predominates and either alpha1B- , alpha1D- , or alpha1L- is the second population. We characterized alpha1- adrenoceptors with selective agonists/ antagonists in tail artery of young Wistar rat. Tail artery was exposed to A61603 (N- [5 - (4 ,5 - dihydro - 1H- imidazol - 2 - yl) - 2 - hydroxy - 5 ,6 ,7 ,8 - tetrahydronaphthalen - 1 - yl] methanesulfonamide) or to phenylephrine (alpha1A- and alpha1- agonists) , and to prazosin (alpha1- antagonist) , the alpha1A- antagonists 5 - methylurapidil , RS 100329 (5- methyl - 3 - [3 - [4 - [2 - (2 ,2 ,2 ,- trifluoroethoxy) phenyl] - 1- piperazinyl] propyl] - 2 ,4 - (1H)- pyrimidinedione) , RS 17053 (N - [2(2- cyclopropylmethoxy) ethyl] - 5- chloro- a, a- dimethyl - 1H- indole - 3- ethylamine) , and the alpha1D- antagonist BMY 7378 (8 - [2 - [4 - (2- methoxyphenyl) - 1- piperazinyl] ethyl] - 8 - azaspiro[4.5] decane - 7 ,9 - dione) . A61603 showed 100 fold higher affinity over PHE. Prazosin , RS100329 , 5- MU, and RS17053 displaced agonists with high affinity indicating alpha1A- adrenoceptors while PHE activated alpha1B- adrenoceptors since BMY 7378 had low affinity.

Keywords : alpha1A/ B- adrenoceptors , rat tail artery
Conacyt grant 47481 , SNI , Fundacion Miguel Alemán , PAPIIT IN322005

P080039

Phenotypic importance of chromosome 17 in genetically hypertensive (LH) rats of the Lyon strain.

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Aims : LH rats associate hypertension , a metabolic syndrome and a marked proteinuria. A full genome scan showed that chromosome 17 contained Quantitative Trait Loci (QTLs) for all these abnormalities. In order to determine their influence we generated a consomic rat strain (LHBN17) in which the LH chromosome 17 has been fully substituted by a normotensive Brown Norway (BN) one. Methods : LHBN17 , LH and BN male rats were phenotyped. This included radio telemetric measurement of BP during normal and elevated salt intake as well as the determination of renal (creatinine clearance , proteinuria) and metabolic (plasma triglycerides and cholesterol) parameters. Results : LHBN17 compared to LH rats exhibited decreases in body weight , BP and its response to salt load. Their creatinine clearance was increased and proteinuria decreased. Plasma lipids were reduced. Except for triglycerides , chromosome 17 genes accounted only partially for the differences existing between LH and BN parents. In conclusion , the present work demonstrates that the chromosome 17 QTLs are of functional importance.

P080040

Discussion on the Chinese, American and European guidelines for the medicines in the treatment of hypertension

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Hypertension is a prevalent disease in China. In past ten years , hypertension prevalence and the number of patients increased progressively , and at present the hypertension prevalence in China is 18.8 % and 130 million people with hypertension nationwide as estimated. The choice of medicines is key point in the treatment of hypertension. There are hundreds of antihypertensive drugs in China , including Traditional Chinese Medicines (TCM) , chemical medcines and all kinds of compound preparations. The same drug often has many manufacturers and the price of it is great different. The choices of medicines for patients in China are often influenced by many factors , such as medicine imbursement , advertisement , and dissemination of the guideline for management of hypertension. Overall , the

awareness, treatment and control of hypertension in China are very low, and the situation of hypertension management in China is critical. In this article we compared the differences of the drug treatment in Chinese, American and European guidelines for the management of hypertension, and discussed the principles in choice of antihypertensive drugs.

Comparison of three guidelines: Chinese, American and European guidelines all mention that the specific drug classes may differ in some effect or in special groups of patients, so certain compelling indication requires certain antihypertensive drug classes. Three guidelines also emphasize the benefits obtained from the combination therapy and recommend the long-acting drugs or preparations with 24-h efficacy. The main differences of three guidelines exist in whether recommend the first-line drugs. Thiazide-type diuretics are recommended by American guideline (JNC7) in drug treatment for most patients with uncomplicated hypertension, either alone or combined with drugs of other classes. But the European guideline emphasizes identifying the first-line drugs is probably outdated and the major classes of antihypertensive agents (diuretics, βblockers, calcium antagonists, ACE inhibitors, angiotensin receptor antagonists) are suitable for the initiation and maintenance of therapy. Chinese guideline doesn't recommend the first-line drugs, but emphasizes the condition of patients should be considered in the choice of drugs. Traditional Chinese Medicines are invaluable resource of China, all kinds of antihypertensive TCM are widely used in the clinical, but owing to the deficiency of the high-quality evidence for TCM, the part of TCM is not included in the 2004 Chinese guideline for the management of hypertension.

Furthermore, another difference among three guidelines is the use of central acting drugs, such as reserpine, which is listed in JNC7, but not in Chinese and European guidelines. Reserpine is an old drug which has been used for many years in China. Owing to its adverse reactions in central nervous system, such as drowsy, depression and suicide tendency etc., and a variety of alternative drugs can be used in the clinical, nowadays the alone use of reserpine is very scarce. Reserpine has been deleted from the essential medicine list by WHO and China.

The choice of antihypertensive drugs: Lowering the patient's blood pressure can reduce cardiovascular risk, and the rational selection and use of drugs are important to reach the ideal control of blood pressure. The choice of antihypertensive drugs is determined by its efficacy and safety. When safety and efficacy are equal the lowest cost drug should be preferred. For the majority of patients without a compelling indication for another class of drug, a low dose of a thiazides diuretic should be considered as the first choice of therapy in China. Furthermore, although the same class of drugs often has similar mechanism of action, the difference of chemical and physical characteristics, the path of metabolisms and interactions may result in the different safety and efficacy in the same class of drugs. So the drug with high-quality clinical evidence should be preferred when selection in same class of drugs, the dose should be verified by randomized clinical trials (RCTs) to be safety and efficacy.

Conclusion: 2004 Chinese guideline for the management of hypertension is based on many scientific evidences, and its publication is very important for the prevention and cure of hypertension in China. Nowadays, we must strengthen the dissemination and implementation of guideline, promote the rational use of antihypertensive drugs and improve the control rate of hypertensive in China.

Key words: antihypertensive drugs, guidelines, first-line drug.

P080041

α_{1A}-Adrenoceptors control blood pressure in α_{1D}-adrenoceptors KO pithed mouse

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The pressor action of A61603 N-[5-(4,5-dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methanesulfonamide), 1A-adrenoceptor agonist and phenylephrine (PHE) in WT and in α_{1D} KO pithed mice, were evaluated. Male adult WT and KO mice were pithed, and diastolic blood pressure was recorded. A61603 evoked a similar maximal contraction in both WT and KO mice, it was two orders of magnitude more potent than phenylephrine (pD_2, 6.23 vs 4.30 and 6.30 vs 4.66 in KO and WT, respectively). PHE was a partial agonist in KO mice. RS100329 (5-methyl-3-[3-[4-[2-(2,2,2,-trifluoroethoxy) phenyl]-1-piperazinyl] propyl]-2,4-(1H)-pyrimidinedione), an α_{1A}-antagonist, right shifted the pD_2 for A61603 and decreased maximal effect in KO. It is very important to use selective antagonists to displace phenylephrine effect. Data show that α_{1A}-adrenoceptors are expressed in pithed mouse vasculature.

Key words: α_{1A}-adrenoceptors, pithed mouse, blood pressure, α_{1D}-KO
Conacyt grant 47481, Fundación Miguel Alemán and PAPIIT IN322205

P080042

NADPH OXIDASE MEDIATES ANG II-INDUCED ET-1 RELEASE IN ADVENTITIAL FIBROBLASTS

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We have recently reported that adventitial fibroblasts (AFB) of aorta are able to generate endothelin-1 (ET-1). This study demonstrated the physiological significance of the adventitial ET-1 expression, focusing on the effect on type I procollagen synthesis in mouse vascular AFB. Cultured AFB were incubated with angiotensin II (AngII, 10-7M), losartan (10-5M), an AT1-receptors antagonist, PD123319 (10-5M), an AT_2-receptors antagonist, BQ123 (10-6M), an ET_A receptors antagonist, and BQ788 (10-6M), an ET_B receptors antagonist. Messenger RNA levels of preproET-1 and type I preprocollagen were detected by relative RT-PCR. PreproET-1 and procollagen mRNA expressions were increased in cells treated with AngII. The increase in preproET-1 and procollagen mRNA levels attributed by AngII treatment was inhibited by both losartan and BQ123. These results demonstrate that ET-1 release is mediated by AT1-receptor and the adventitial ET-1 plays an important role in the regulation of collagen synthesis.

The work is supported by Canadian Institutes of Health Research

P080043

Spontaneous Contractions in the Aorta of the Spontaneously Hypertensive Rat

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To determine why quiescent aortae of spontaneously hypertensive rats (SHR) exhibit a potent spontaneous contraction after prolonged equilibration, the onset, amplitude, and duration of such contractions were quantified in aortic rings of 40-weeks-old SHR and Wistar-Kyoto (WKY) rats, under control conditions and after incubation with indomethacin (non-selective cyclooxygenase [COX] inhibitor), valeryl salicylate (COX1 selective), NS-398 (COX2 selective), S18886 (TP-receptor antagonist), BQ123 (ETA receptor antagonist) or L-NAME (NO synthase inhibitor). Spontaneous contractions occurred in aortas of SHR but not in those of WKY. They reached up to 50-60% of the maximal contraction to KCl, and were sustained (60 minutes or longer). Removal of the endothelium abolished the spontaneous contractions, as did indomethacin, valeryl salicylate and S18886. The contractions were reduced by NS-398 and BQ-123, but augmented by L-NAME. These data demonstrate spontaneous vasospasm in aortae of the aging SHR. Endothelin-1 and prostanoids (formed by endothelial COX1 and activating TP-receptors on vascular smooth muscle) contribute to the occurrence of spontaneous contractions, while basally released NO curtails them.

P080044

Primary care management of hypertension in patients considered 'at risk' of cardiovascular disease

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Objective: The aim of the study is to describe hypertension management in 55,000 Australians attending family practices. Methods: Practitioners identified subjects with at least one known risk factor and these were invited to participate. Assessment included measuring blood pressure, body weight, waist circumference, fasting total-, HDL- and LDL-cholesterol, triglyceride and glucose. Lifestyle behaviour and medication history were also ascertained. Results: Hypertensives (65.8%) had a mean age of 61.7 and blood pressure of 139/81 mmHg. 51% were female. 35% reported being physically active three or more time per week. Whilst 90.6% were receiving antihypertensive medication less than a third were achieving treatment targets. ACE inhibitors were the most frequently used medication. 45% of hypertensives were obese, 45% had impaired glucose tolerance, 11.5% were smokers and 28% were hypercholesterolaemic. 50% of these subjects were classified as having the metabolic syndrome. Conclusion: Strategies to manage overweight and obesity and to improve risk factor control in both treated and untreated hypertensive patients should be a major focus for general practice based research.

P080045

Differential Inflammatory Signal Transduction in VSMC from SHR and WHY

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The iNOS & COX-2 inflammatory genes were expressed in VSMC from SHR & WKY rats by IL-1β. The present study investigates differential activation of MAP kinase signal in SHR & WKY rats. iNOS & COX-2 mRNA/protein by IL-1β was determined by Real time-PCR/ Western blot in the absence and presence of PD98059, selective inhibitor of ERK pathways, SB203580, selective p38 MAPK inhibitor and JNKi, selective inhibitor of JNK inhibitor. The iNOS & COX-2 proteins/ mRNA in VSMC from 9 week-old WKY rats were higher than those from SHR. Phosphorylation of JNK was increased in SHR compared with WKY. IL-1β increased ERK phosphorylation in WKY rats and had a small effect in SHR. These results demonstrated that iNOS & COX-2 were reduced in VSMC from SHR, and IL-1β activates JNK in SHR but ERK MAP kinases in WKY rats that differential activation of these kinases may be important in altered inflammation in VSMC from SHR & WKY rats.

Keywards: SHR, WKY, IL-1β, inflammation

Acknowledgements: This study was supported by the Korea Science and Engineering Foundation (KOSEF) through the Agingassociated Vascular Disease Research Center at Yeungnam University (R13-2005-005-01003-0) (2006).

P080046

The Role of Vascular Na^+/Ca^{2+} Exchanger (NCX1) in Salt-Dependent Hypertension.

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Excessive salt intake is a major risk factor for hypertension. However, the molecular mechanisms underlying saltsensitive hypertension remain obscure. Recent our studies utilizing selective Na^+/Ca^{2+} exchanger (NCX) inhibitors and genetically engineered mice provide compelling evidence that salt-sensitive hypertension is triggered by Ca^{2+} entry through NCX1 in arterial smooth muscle (Iwamoto et al. Nat. Med. 10:1193-1199, 2004). SEA0400 dose-dependently lowered arterial blood pressure in salt-dependent hypertensive models, but not in other types of hypertensive rats. SEA0400 reverses ouabain-induced cytosolic Ca^{2+} elevation and vasoconstriction in small mesenteric arteries. Furthermore, heterozygous NCX1-deficient mice have low salt sensitivity, whereas transgenic mice that specifically express NCX1.3 in smooth muscle are hypersensitive to salt. Interestingly, chronic administration of ouabain produces more severe hypertension in transgenic mice than in wild-type mice. These findings have enabled us to explain how high salt intake leads to hypertension and further to describe the potential of vascular NCX1 as a therapeutic target for salt-sensitive hypertension.

P080047

NITRIC OXIDE IS INVOLVED IN THE MECHANISM FOR THE ANTIHYPERTENSION ACTION OF CARVEDILOL

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Carvedilol (Carv) is a unselective βadrenoceptor antagonist, that lowers blood pressure through a vasorelaxant effect mediated possibly by NO. We have shown that in the rat vas deferens (rvd) NO pathway potentiates the noradrenaline (NE)-release phenylephrine (Phe)-induced. We used this model of NE-release, in order to clarify the role of Carv on the production of NO. The NE released was measured in the presence of Carv and Carv + L-NAME (5 + 1μM). The effect of Carv on the contractile responses and on the NO production in the rvd, induced by Phe, was also studied. Carv (5, 20μM) failed to antagonize the effect of Phe on NE-release ($P < 0.05$, $n = 8$). In the presence of L-NAME, we observed a reduction on this release. In the contractile responses, Carv induced a concentration-dependent decrease of the vas deferens contractility (Emax: Carv 5 μM: 64.2 ±2.7 %; Carv 0.05μM: 97.4 ±3.7 % of control, $P < 0.05$, $n = 8$). Carv (20μM) increase NO production in vas deferens. The results obtained in the NE release and NO production protocols involve the Carv on the production of NO. These findings suggest that NO may be involved in the mechanism for the antihypertensive action of Carv.

Key words Carvedilol, NO, hypertension

P080048

Effect of Angiotensin on the Overflow of NPYir from the Perfused Mesenteric Arterial Bed of Spontaneously Hypertensive and Normotensive rats

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Angiotensin II (ANG) is known to enhance the nerve stimulation induced release of norepinephrine (NE) from sympathetic nerves in a variety of blood vessels(1). The aim of this study was to examine ANG effects on the release of the sympathetic cotransmitter NPY. ELISA was used to measure the overflow NPYir from the perfused arterial beds obtained from Sprague Dawley (SD) and Spontaneously Hypertensive Rats (SHR). Perfusion pressure was monitored on a grass recorder. ANG significantly enhanced the overflow of NPYir in both the resting and stimulated condition. The effect was significantly higher in preparations from 10-12 week old SHR compared to those from age matched SD controls. The effect was also greater in 10-12 week old SHR than in 4-6 week old SHR. Preparations obtained from 4-6 week old SHR show a marked increase in perfusion pressure when infused with ANG than those from 10-12 week SHR or SD controls. Therefore ANG can facilitate the release of NPY in a manner similar to NE.

Supported by USPHS-NIH-HLBLI 60260

P080049

CHIMERIC IGF-I RECEPTOR ANTISENSE TREATMENT REDUCES VASCULAR TARGET EXPRESSION

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We investigated the effects of a functional deficit in insulin-like growth factor-I (IGF-I) signaling in the normotensive and hypertensive rat cardiovascular system. We designed 2'-sugar modified novel chimeric antisense oligonucleotides, with a view to allowing nuclease resistance and high affinity, allowing us to use lower doses than those used by other groups. High doses of antisense used thus far in clinical trials have resulted in an unacceptable spectrum of adverse effects.

We have shown that our antisense reduces IGF-IR expression in resistance blood vessel walls by 67 + 2.5%, and that this results in significant functional changes in vivo. We observed greater than 50% reduction in maximal constrictor response to angiotensin II, and a significant reduction in aortic medial thickness after 2 weeks' IGF-IR AS treatment. Significant, specific in vivo antisense effects were observed using low doses, thus improving the therapeutic utility of these agents. These data suggest that the vasculature is a tissue particularly suited to antisense (and possibly siRNA) mediated reductions in target protein expression, and that therapeutic intervention aimed at vascular targets might well be an achievable goal.

P080050

Hemin induced Heme oxygenase-1 inhibits rat aortic vascular smooth musle cells proliferation under hypertension

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It has been suggested that Heme oxygenase (HO)-1, rate-limiting enzyme in the catabolism of heme to carbon monoxide, bilirubin and free iron, plays an important part in the regulation of cellular proliferation. We have examined the effect of the HO inducer Hemin on heme oxygenase-1 (HO-1) expression in rat aortic vascular smooth muscle cells (RAVSMC) and investigated the contribution of the heme oxygenase pathway in the control of RAVSMC proliferation. Incubation of RAVSMC with 1 μM Hemin resulted in a significant increase in HO-1 protein expression, as measured by Western blot. This effect was associated with a 50% decrease in IL-1β induced RAVSMC proliferation. Hemin inhibits proliferation of RAVSMC when cells are under oxidative stress such as inflammation or hypertension. The aiti-proliferative effect of the HO inducer was totally abolished by co-incubation of Hemin with tin protoporphyrin IX, a potent inhibitor of heme oxygenase. These results suggest that the heme oxygenase pathway is involved in RAVSMC proliferation under hypertension.

P080051

Local Haemodynamic Effects of Urotensin II in man in vivo

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Urotensin II (UII) is an 11 amino acid peptide found in the human cardiovascular system. Although animal studies suggest it is a potent vasoconstrictor, data in humans are conflicting. We have investigated the effect of UII in healthy volunteers (HV) and cardiovascular disease (CVD) patients.

Nine HV (27 ±2 yrs) and eight patients with CVD (63 ±5 yrs) received a brachial artery infusion of saline, followed by UII at 0.1, 1.0, 10, 100 and 300 pmol/min. Forearm blood flow (FBF) was measured every 5 min. Blood pressure and heart rate (HR) were assessed every 15 min. Data are means ±SEM, and FBF values are the ratio of flow in the infused vs control arm.

In HV, there was no change in FBF ratio (1.1 ±0.1 vs. 0.9 ±0.1, saline vs 300 pmol/min; p = 0.4), HR or mean pressure (MP). In contrast, although FBF ratio did not change (1.1 ±0.1 vs. 1.1 ±0.3, p = 0.8), there was a significant increase in HR (59 ±3 vs. 63 ±5 bts/min, p = 0.007) and MP (99 ±3 vs. 107 ±3 mmHg, p = 0.001) in the CVD subjects at 300 pmol/min.

UII has no vasoconstrictor effects in HV. However, in CVD subjects, UII has a modest vasopressor effect that may be mediated by an increase in cardiac output rather than peripheral vasoconstriction.

P080052

ENDOTHELIN B RECEPTORS DO NOT CONTRIBUTE TO THE REGULATION OF ARTERIAL STIFFNESS IN VIVO

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Endothelin-1 (ET-1), acting via endothelin A (ET_A) receptors, regulates arterial stiffness in vivo (1). However, the role of ET_B receptors is unknown. We investigated the role of ET_B receptors in the regulation of arterial stiffness. All studies were conducted in anaesthetized sheep, with the approval of the local Animal Care and Ethics Committee. Pulse wave velocity (PWV) was measured using a dual pressure-sensing catheter placed in the common iliac artery. In 5 sheep, the ET_B receptor agonist, sarafotoxin 6c (S6c, 7.5 pmol.min), was infused for 60 min and in 5 sheep, ET-1 (10 pmol.min) was infused for 60 min. In a further 5 sheep, the ET_B receptor antagonist, BQ-788 (1 nmol.min) was infused for 45 min, followed by saline for 30 min. Infusion of S6c did not alter PWV (change of 0 ±6%, mean ±STD, P = 0.8) whereas ET-1 significantly increased PWV by 11 ±3% (P < 0.01). BQ-788 did not alter PWV (change of -2 ±6%, P = 0.8). These results suggest that ET_B receptors do not regulate arterial stiffness, and confirm our previous findings in the ovine model that ET-1 acts predominantly via ET_A receptors to regulate arterial stiffness in vivo.

P080053

The individualities of different arteries to norepinephrine and acetylchdine in Spontaneous hypertensive rat-stroke prone strain

Wang fuwen[1*], Li jie[1], Hu zhili[1], Xie yanying[2].

The resting membrane potential (Em) of vascular smooth muscle (VSM) from different arteries in Spontaneous hypertensive rat-stroke prone strain (SHR-SP) and Wistar rats was compared, meantimes the individualities of VSM to norepinephrine (NE) and acetylcholine (ACh) were studied. The Em of VSM was recorded by using intracellular microelectrode. The Em of coronary artery, basilar artery and meddle cerebral artery from 12-week-old SHR-SP were (-42.40 ± 2.70) mv, (-45.39 ±3.9) mv, (-44.20 ±3.1) mv, and were higher 22%, 17%, 31% than that of Wistar rats, respectively. NE caused membrane depolarization of basilar artery and meddle cerebral artery, and had not influence on coronary artery. ACh induced membrane hyperpolarization of basilar artery and meddle cerebral artery, and depolarization of coronary artery. The effects of these agents revealed characteristics in dose-dependent manner. These results suggest the Em of different vessels in SHR-SP was higher and the reactivity to NE, ACh was significantly increased. The reactivity of different artery from the same animal have their own characteristics, named vascular individuality.

Key word SHR-SP; individuality; vascular smooth muscles; acetylcholine

P080054

Effects of intravenous urocortin on angiotensin-converting enzyme in rats

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We investigated the relationship between urocortin and the activity of angiotensin converting enzyme (ACE). The tissue ACE mRNA was determined by RT-PCR. Immunofluorescence studies were preformed to evaluate the effect of urocortin on ACE in cultured rat aortic endothelial cells (RAECs). Urocortin decreased the serum ACE level 1h after administration, whereas tissue ACE immunoreactivity and mRNA did not change. The prolonged administration of urocortin enhanced tissue ACE activity but the serum ACE level remained low. RTPCR analysis showed tissue ACE mRNA was elevated. Immunofluorescence studies demonstrated an increase of ACE intensity in RAECs exposed to urocortin for 72h. Astressin abolished the effects of urocortin. Extracellular signal-regulated kinase 1/2 (ERK1/2) pathway blocker, PD98059, markedly inhibited these effects, suggesting urocortin affects the activity of ACE through the ERK1/2 pathway. Thus, the changes of the ACE activity and its production of Ang II may play a role in the vasodilatory property of urocortin.

Keywords: Urocortin; ACE; Blood pressure

Acknowledgements: This work was supported by Natural Science Foundation of China (No. 30371646 and 30572185).

P080055

CHRONOTHERAPY WITH VALSARTAN-HCTZ COMBINATION IN HYPERTENSIVE PATIENTS: IMPROVED BLOOD PRESSURE REGULATION WITH BEDTIME ADMINISTRATION.

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This trial investigated the administration time-dependent efficacy of valsartan-HCTZ combination. We studied 82 hypertensive patients (21 men), 52.2 ±12.6 years of age, first randomly assigned to receive valsartan (160 mg/day) either upon awakening or at bedtime for 12 weeks. HCTZ (12.5 mg/day) was then added to valsartan for another 12 weeks. Blood pressure (BP) was measured for 48h at each visit. The significant BP reduction after valsartan monotherapy ($P < 0.001$) was slightly but not significantly larger after morning dosing. The day/night ratio was unchanged after valsartan on awakening, but significantly increased with bedtime dosing ($P < 0.001$). Combination therapy resulted in a significant added efficacy, comparable between treatment-times. The day/night ratio remained unchanged after morning treatment and was further increased when the combination was administered at bedtime ($P < 0.001$). In patients not properly controlled with valsartan alone, the addition of 12.5 mg/day HCTZ efficiently reduces BP for the 24h independently of dosing time. Bedtime administration may be preferred for increased efficacy during nocturnal resting hours and the potential associated reduction in cardiovascular risk.

P080056

AMBULATORY BLOOD PRESSURE IN THE PREDICTION OF CARDIOVASCULAR EVENTS AND EFFECTS OF CHRONOTHERAPY: THE MAPEC STUDY.

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The MAPEC study was designed to investigate whether normalizing the circadian blood pressure (BP) profile towards a more dipper pattern reduces cardiovascular risk. This prospective study investigates 2643 subjects (1328 men), 51.9 ±14.1 years of age. At inclusion and yearly thereafter, BP was measured at 20-min intervals from 07:00 to 23:00h and at 30-min intervals at night for 48 hours. The median time of follow up so far was 3.2 years. Based on the baseline BP profile, cardiovascular morbidity was similar for extreme-dippers (1.23 events/100 patient-years) and dippers (1.14), but significantly higher for non-dippers (2.81) and mainly for risers (8.70). When morbidity was analyzed on the basis of the BP profile closer to the event, results indicate a diminished morbidity in extreme-dippers (0.38) and dippers (0.89), and an increased morbidty in non-dippers (3.23) and risers (10.70). The probability of event-free survival is markedly correlated with the day/night BP ratio. Most important, results suggest that increasing this ratio towards a more dipper pattern by Chronotherapy decreases cardiovascular risk, while decreasing the day/night BP ratio is associated with increased morbidity and mortality.

P080057

CHRONOTHERAPY WITH TORASEMIDE IN HYPERTENSIVE PATIENTS: INCREASED EFFICACY AND THERAPEUTIC COVERAGE WITH BEDTIME ADMINISTRATION.

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This trial investigated the administration time-dependent efficacy of torasemide. We studied 90 hypertensive patients (39 men), 51.9 ±12.8 years of age, randomly assigned to receive torasemide (5 mg/d) either upon awakening or at bedtime. Blood pressure (BP) was measured for 48h before and after 6 weeks of therapy. Efficacy of torasemide was higher after bedtime dosing as compared to the administration of the drug on awakening ($P < 0.004$). The time-response curves indicate a full 24h therapeutic coverage only when torasemide was administered at bedtime. The percentage of patients with controlled BP was significantly higher after bedtime treatment (61 versus 23%, $P < 0.001$). The 24h urinary secretion of sodium and potassium remained unchanged after treatment for both groups. A single dose of 5 mg/d torasemide is effective for BP reduction during the 24h only after bedtime administration. The differences in efficacy and therapeutic coverage, without significant increase in natriuresis nor in secondary effects, as a function of the circadian time of treatment with torasemide here documented should be taken into account when prescribing this loop diuretic for treatment of patients with essential hypertension.

P080058

CORRELATION BETWEEN PLASMA FIBRINOGEN AND THE DIMINISHED DIURNAL/NOCTURNAL BLOOD PRESSURE RATIO IN HYPERTENSIVE PATIENTS.

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Fibrinogen is a significant marker of the potential risk of myocardial infarction and stroke. We have investigated the possible correlation between fibrinogen and ambulatory (ABPM) blood pressure (BP) in hypertensive patients. We studied 3430 hypertensive patients (1632 men), 52.7 ±14.5 years of age. BP was measured for 48h. Blood samples were obtained in the early morning after nocturnal fasting, on the first day of ABPM. Fibrinogen is characterized by a highly significant negative correlation with the day/night systolic BP ratio ($r = -0.150$; $P < 0.001$), as well as by positive correlations with the nocturnal means of systolic BP and pulse pressure. Extreme-dippers showed the lowest average fibrinogen (297.9 mg/dl), followed by dippers (300.6 mg/dl), non-dippers (313.8 mg/dl), and risers (334.9 mg/dl; $P < 0.001$ between groups corrected by age). Plasma fibrinogen is markedly elevated in relation to the progressive loss in day/night BP regulation. Results indicate that, apart from the day/night ratio, the nocturnal mean values of systolic BP and pulse pressure may be the most relevant ABPM characteristics for cardiovascular risk assessment.

P080059

CHRONOTHERAPY INCREASES BLOOD PRESSURE (BP) CONTROL AND REDUCES THE PREVALENCE OF NON-DIPPING IN PATIENTS WITH ESSENTIAL HYPERTENSION.

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We investigated the impact of the time of therapy on the circadian pattern of BP in hypertensive patients. We studied 4930 patients (2359 men), 52.8 ±13.5 years of age. Among them, 1811 were untreated, 1869 were receiving all their drugs on awakening, 443 were taken all drugs at bedtime, and 807 were treated with drugs on awakening and at bedtime. BP was measured for 48h. Among untreated patients, 42.9% were non-dippers. In patients treated with all drugs on awakening, the percentage of non-dippers was increased to 59.1% ($P < 0.001$). This prevalence was reduced to 45.8% in patients treated at both times, and even further to a lowest 34.5% in patients taken all drugs at bedtime ($P < 0.001$). The percentage of patients with controlled BP increased from 39.5% with all drugs on awakening to 52.4% with all drugs at bedtime ($P < 0.001$). Antihypertensive therapy, mostly given exclusively on awakening, significantly modifies the circadian pattern of BP towards a progressive diminished day/night BP ratio with increasing number of drugs. Chronotherapy allows reducing the prevalence of an altered non-dipper BP profile, associated with an increased cardiovascular risk, while also markedly increasing BP control.

P080060

CHRONOTHERAPY WITH SPIRAPRIL IN HYPERTENSIVE PATIENTS: CHANGES IN THE DAY/NIGHT BLOOD PRESSURE RATIO AS A FUNCTION OF THE TIME OF ADMINISTRATION.

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This trial investigated the administration time-dependent efficacy of spirapril. We studied 100 hypertensive patients (42 men), 45.0 ±13.9 years of age, randomly assigned to receive spiraptil (6 mg/d) either upon awakening or at bedtime. Blood pressure (BP) was measured for 48h before and after 12 weeks of therapy. Efficacy of spirapril was slightly higher after morning dosing as compared to the administration of the drug at bedtime ($P = 0.004$). Morning administration of spirapril was more effective than bedtime dosing in reducing the diurnal mean of BP ($P < 0.001$), but significantly less effective in controlling nocturnal BP ($P < 0.001$). Accordingly, the day/night ratio was reduced after spirapril on awakening and significantly increased towards a more dipper pattern after bedtime dosing ($P < 0.001$). Circadian time of treatment with spirapril has a significant effect of the day/night BP ratio, modifying the BP profile towards a more non-dipper pattern after morning dosing. These administration-time dependent effects should be taken into consideration when prescribing this ACE-inhibitor, as a function of the baseline BP profile of each individual hypertensive patient.

P080061

ADMINISTRATION TIME-DEPENDENT EFFECTS OF NEBIVOLOL ON THE DIURNAL/NOCTURNAL BLOOD PRESSURE (BP) RATIO IN HYPERTENSIVE PATIENTS.

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This trial investigated the administration time-dependent antihypertensive effects of nebivolol. We studied 173 hypertensive patients (68 men), 45.3 ±12.1 years of age, randomly assigned to receive nebivolol (5 mg/d) either on awakening or at bedtime. BP was measured for 48h before and after 8 weeks of treatment. The efficacy of nebivolol was fully comparable independently of dosing time. There was a significant reduction in diurnal/nocturnal BP ratio when nebivolol was administered after awakening ($P < 0.001$), but not after bedtime dosing ($P > 0.055$). The prevalence of non-dipping was doubled after morning dosing ($P < 0.001$) and remained unchanged after bedtime dosing with nebivolol. Results indicate that nebivolol efficiently reduces BP throughout the 24h independently of the circadian time of administration. The diurnal/nocturnal BP ratio was significantly reduced towards a more non-dipping pattern only after morning dosing. Results suggest that dosing time with nebivolol should be chosen at bedtime, without any loss in efficacy as compared to the usual morning administration, but avoiding the reduction in diurnal/nocturnal BP ratio that seems to be associated to higher cardiovascular risk.

P080062

CHRONOTHERAPY WITH NIFEDIPINE GITS IN HYPERTENSIVE PATIENTS: IMPROVEMENT OF SAFETY PROFILE WITH BEDTIME ADMINISTRATION.

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This trial investigated the administration time-dependent efficacy, safety, and regulation of the circadian blood pressure (BP) of nifedipine GITS. We studied 130 hypertensive patients (62 men), 52.3 ±10.9 years of age, randomly assigned to receive nifedipine GITS (30 mg/d) either on awakening or at bedtime. BP was measured for 48h before and after 8 weeks of treatment. The BP reduction after 2 months of therapy was similar at both treatment times ($P > 0.349$). Efficacy was slightly but not significantly higher after bedtime dosing on the nocturnal mean of BP. The day/night BP ratio was slightly reduced after morning treatment, but increased after bedtime dosing. Most important, bedtime administration of nifedipine GITS significantly reduced the incidence of edema from 18.8 to 1.6% and the total number of secondary effects from 20.3 to 4.7% as compared to morning dosing ($P = 0.009$). The added efficacy on nocturnal BP, the slight increase in day/night ratio, and, most important, the markedly improved safety profile of bedtime as compared to morning administration of nifedipine GITS, all indicate that this CCB should preferably be administered at bedtime in patients with essential hypertension.

P080063

ADMINISTRATION TIME-DEPENDENT EFFECTS OF ANTIHYPERTENSIVE TREATMENT IN PATIENTS WITH RESISTANT HYPERTENSION.

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This trial evaluated the impact on the circadian pattern of blood pressure (BP) of modifying the time of treatment without increasing the number of drugs in patients with resistant hypertension. We studied 250 patients who were receiving 3 antihypertensive drugs in a single morning dose. Patients were randomly assigned to one of two groups according to the modification in their treatment strategy: 1) Changing one of the drugs, but keeping all 3 in the morning. 2) The same approach but administering the new drug at bedtime. BP was measured for 48h at baseline and after 3 months of intervention. There was no effect on BP when all 3 drugs were taken on awakening. The baseline prevalence of non-dipping (21%) was unchanged after treatment (14%). The BP reduction was statistically significant with one drug at bedtime. This effect was markedly larger in the nocturnal than in the diurnal mean of BP. Thus, while only 16% of the patients in this group were dipper at baseline, 57% were dipper after therapy. Results indicate that, in resistant hypertension, time of treatment may be more important for BP control and for the proper modeling of the circadian BP pattern than just changing the drug combination.

P080064

Improvement in blood pressure and cardiac hypertrophy with a low-dose combination Indapamide and Telmisartan in spontaneously hypertensive rats

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Objects: To explore a new compound preparation for anti-hypertension more effective and safely.

Methods: Experiments were conducted on male spontaneously hypertensive rats (SHR). At 14 weeks of age with weight of 300 gram, 24 SHR, whose tail arterial SBP is more than 140 mmHg, were randomly divided into 3 groups: (1) An untreated group; (2) Low-low dose combination: treated with a low dose of indapamide(0.06 mg/kg/day) and low dose of telmisartan (3.57 mg/kg/day); (3) High dose telmisartan monotherapy. All SHR had been treated with drugs by gavage for successive 4 weeks. SBP was measured once before the trial and 4 times. Cardiac function was assessed by isolated Langendorff heart perfusion. The following parameters were examined: body weight (BW), heart weight (HW), left ventricular weight (LVW) and left ventricular end-diastolic pressure (LVEDP).

Results: After 4 weeks of treatment, both high doses of telmisartan monotherapy and combination of low dose of indapamide and telmisartan significant decrease the SBP ($p < 0.01$), and there was no significant difference between these two treatments. Compared with the control group, the ratio of HW/BW and LVW/BW, LVEDP were all decreased in Low-dose combination group ($p < 0.05$).

Conclusion: Combination of low dose of indapamide and telmisartan significant decrease the SBP of SHR, and has superior efficacy on reducing left ventricular hypertrophy.

Key words: hypertension, indapamide, telmisartan

Acknowledgement: This work was supported by grants from Guangdong Provincial Natural Science Foundation for Research Team (015015)

P09. Cardiovascular Pharmacology - Drugs Used in Heart failure

P090001

The antiarteriosclerosis mechanisms of Scallop skirt-glycosaminoglycan

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In order to investigate the antiarteriosclerosis mechanisms of scallop skirt-glycosaminoglycan(SS-GAG), RT-PCR analysis, immunohistochemical staining, enzymatic method and flow cytometry so on were employed. SS-GAG can inhibit the formation of foam cells derived from macrophage(U937) and vascular smooth muscles cells(VSMCs), and decrease the overexpression of some cytokines (TNFα, IL-6 and IL-8) and intracellular Ca^{2+} level. Furthermore, SS-GAG up-regulated the mRNA and protein expression of low density lipoprotein(LDL) receptors and scavenger receptor class B, type I(SR-BI), a well characterized high density lipoprotein(HDL) receptor in macrophage(RAW264.7). It also down-regulated the overexpression of scavenger receptor CD36 induced by Ox-LDL lesion, restrained the oxidative modification of RAW264.7 to LDL. SS-GAG can modulate lipoprotein metabolic disorders and the expression of some lipoprotein receptors. At the same time, SS-GAG inhibited excessive proliferation of VSMCs and protected endothelial cells from oxidation damage. The above is maybe the antiarteriosclerosis mechanisms of SS-GAG.

Keywords: SS-GAG, arteriosclerosis, macrophage, VSMCs, endothelial cells

P090002

The electrophysiological remodeling of cardiac ventricular myocytes during the development of mouse cardiac hypertrophy and failure

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Heart failure is associated with a significant increase in the risk of lethal arrhythmias. We try to elucidate the cardiac electrophysiological remodeling and its molecular mechanism during the development of cardiac hypertrophy and failure. A mouse pressure over-loaded cardiac hypertrophy and failure model was established by aorta banding. Single myocytes were enzymatically isolated from endocardium of the free left ventricle wall. By using perforated patch-clamp we found that APD_{50} with the hypertrophied hearts was significantly prolonged and it was further increased in the failing hearts. However, APD_{90} maintained unchanged with the hypertrophied hearts and was significantly prolonged with the failing hearts. The recordings of voltage-dependent K^+ currents by whole cell patch-clamp revealed a significant difference between hypertrophied and failing hearts. We conclude that cardiac ventricular myocytes with hypertrophied and failing hearts exhibit different property in electrical remodeling.

Key words: cardiac hypertrophy and failure; patch-clamp; action potential duration; voltage-dependent K^+ currents

Supported by NCET-04-0253, NSFC 30370571 and HeBSFC 200400628.

P090003

The aquaretic effect of the selective ORL-1 receptor agonist ZP120 is vasopressin-dependent.

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ZP120 produces a marked aquaresis by suppression of renal AQP expression, but it is unclear if this effect is dependent on circulating vasopressin (AVP) levels. To examine the relationship between AVP and the aquaretic response to ZP120, urine flow rate was measured before and during i.v. infusion with ZP120 (1 nmol/kg/min) or vehicle (saline) during conditions with low, normal and high circulating AVP levels in conscious rats (n = 77). Design: 1) AVP release was suppressed by maximal water loading (WL; 15 ml/h) induced by i.v. infusion of hypotonic fluid; 2) AVP levels were clamped at a supra-physiological level by i.v. infusion of AVP (30 pg/min/kg); and 3) the aquaretic response to ZP120 was compared between AVP-deficient Brattleboro rats and Long Evans rats. Results: Our data demonstrated that the aquaretic response to ZP120 was abolished during maximal WL, during AVP clamp, and in Brattleboro rats. Conclusion: These data suggest that ZP120 exerts its aquaretic effect through a vasopressin-dependent mechanism. The lack of effect in AVP-deficient rats suggests that ZP120 inhibits AVP release.

P090004

Anti-inflammatory Effects of Methotrexate Improves Cardiac Remodelling and Function in a Rat Model of Dilated Cardiomyopathy

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We investigated the effects of methotrexate (MTX) on cytokine production and indices of ventricular remodeling in dilated cardiomyopathy. Lewis rats were randomly allocated to a myocin-induced dilated cardiomyopathy (DCM) group receiving placebo (n = 10), a DCM group receiving MTX (MTX group; 0.1 mg/kg/d for 30d; n = 10) or healthy control group (n = 10). Samples were analysed for cytokine levels, collagen volume fraction (CVF) and perivascular collagen area (PVCA). Cardiac function was measured by echocardiography. TNFα, IL-6 and IL-10 levels were higher ($P < 0.05$) in DCM than in healthy controls. MTX reduced plasma levels of TNFα and IL-6, but increased IL-10 levels ($P < 0.01$) in DCM animals. Collagen I/III ratio, CVF and PVCA were lower in the MTX than DCM group (2.59 ±0.25 vs 4.22 ±0.54, 2.93 ±1.11 vs 23.33 ±4.43 and 7.27 ±2.41 vs 13.74 ±4.29 respectively; $P < 0.01$). Left ventricu-

lar end-diastolic dimension was reduced (6.06 ±0.37 vs 6.46 ±0.28 mm; P< 0.05), and ejection fraction increased (84.77% ±3.60% vs 62.73% ± 10.11%; P< 0.01) by MTX compared to DCM. These data indicate that MTX modulates inflammatory responses, which may reverse remodeling and improve heart function in DCM.

Key words: cytokine, methotrexate, DCM

P090005

The Study on Pharmacological Effect of Daidzein

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Objective To study the pharmacological effect of Daidzein(DD) Methods The antiarrhythmia methods, Study the effect of DD on ventricular fibrillation and arrhythmia. Study the effect of DD on dose-response curve of KCl, $CaCl_2$, contraction of thoracic aorta strips induced by NE. Study the effect of DD on resisting hypoxia, on cerebral ischemia mice, on the myocardial consumption of oxygen induced by ISO. Study the effect of DD on the constriction of gall bladder of guinea pig induced by Ach, Hs, K^+ and cumulative Ca^{2+} Results DD can prevent ventricular fibrillation induced by chloroform in mice and arrhythmia induced by aconitine in rats, can antagonize the arrhythmia induced by Adr, can prevent ventricular fibrillation induced by $CaCl_2$ in rats. DD can make the dose-response curve of thoracic aorta strips induced by NE, KCl, $CaCl_2$ shifted right. DD could prolong the living time of above methods mice. DD has antagonized effect on the gall bladder constriction induced by Ach, Hs, excessive K^+, cumulative Ca^{2+}. Conclusion DD has protective effect on arrhythmia, has effect on resisting hypoxia, has antagonized effect on the gall bladder constriction.

Key words: Daidzein, arrhythmia, resist hypoxia, gall bladder constriction

P090006

Adrenoceptor Blockade Alters Plasma Gelatinase Activity in Patients with Heart Failure and MMP-9 Promoter Activity in a Human Cell Line (ECV304)

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We investigated the effects of adrenoceptor blockade on matrix metalloproteinase (MMP) activity in heart failure (HF) patients, and the role of adrenoceptors in modulating MMP-9 promoter activity. Patients received standard therapy or standard therapy plus carvedilol (CVD). MMP activity and tissue inhibitor (TIMP-1) expression were measured by zymography and immunoblotting. MMP-9 promoter activity was assessed in ECV304 transfected cells following exposure to isoprenaline (ISO) or phenylephrine (PE) and their inhibitors. CVD attenuated proMMP-9 activity (44.0 ±4.9 vs 60.8 ±6.7 AU) and reduced the MMP-9:TIMP-1 ratio (P< 0.05) compared to the non βblocker group. ISO caused an increase in MMP-9 promoter activity (80.6 ±14.8 fold; P< 0.001), which was blocked by propranolol. PE also increased promoter activity, (23 ±3.7 fold; P< 0.05), but was resistant to prazosin. These data indicate that CVD tips the degradative balance to a less degradative phenotype in HF patients, which may reduce remodeling as a direct consequence of a β but not αadrenoceptor-mediated reduction in MMP-9 transcription.

Key words: MMP, heart failure, adrenoceptor

P090007

Oral antibodies to AT1 angiotensin II receptor-a novel option in the treatment of chronic heart failure

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The treatment of chronic heart failure (CHF)-a major cause of lethality-is an unmet medical need. To develop a novel therapeutic for CHF, we performed preclinical and clinical research of a drug candidate containing antibodies to C-terminal fragment of AT1 angiotensin II receptor (ultra-low doses for oral administration). Animal studies in 2 strains of hypertensive rats revealed the new drug's hypotensive effect was comparable to that of losartan, but the former's positive influence on the heart was more pronounced. Phase II clinical trials of the new drug as 6-month add-on therapy of CHF were run as randomised placebo-controlled and involved 60 CHF patients. For 6 months, they received Cardos or placebo (6 oral tablets/day) added to standard therapy (at least ACE inhibitor and beta-blocker). Cardos doubled the efficacy of standard therapy for CHF: the number of patients with improved functional class increased from 23.3% to 46.6%; 6-min walking ability increased by 10.8% (4.7% on placebo), left ventricular ejection fraction increased by 6.5% (1.7% on placebo); the drug was well tolerated. Cardos holds promise to considerably enhance treatment modalities for CHF.

P090008

Effects of clebopride on HERG channel currents expressed in CHO cells and action potential of rabbit Purkinje fiber

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Inhibition of the potassium current IKr and QT prolongation has been known to be associated with drug-induced torsades de pointes arrhythmias (TdP) and sudden cardiac death. We investigated the cardiac electrophysiological effects of clebopride, a class of antidopaminergic gastrointestinal prokinetic reported to prolong the QT interval. by using conventional microelectrode recording techniques in isolated rabbit Purkinje fiber and whole-cell patch clamp techniques in human ether-à-go-go related gene (hERG)-stably transfected CHO cells. Clebopride at 10 μM significantly decreased resting membrane potential, Vmax of phase 0 depolarization (p< 0.05) and significantly prolonged action potential duration at 90% repolarization (APD90) (p< 0.01) whereas action potential duration at 50% repolarization (APD50) was not prolonged. For IhERG, the IC_{50} value was 0.16 ± 0.02 μM. The effect of clebopride on action potential is low relatively as that of hERG channel. That may be why clebopride affects inward ion channels. Therefore, further studies that include inward ion channels such as sodium, calcium integrating hERG assay data will be necessary to predict the torsadogenic risk of clebopride in humans.

P090009

Cardioprotective effects of mineralocorticoid receptor antagonists in the rat heart

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Mineralocorticoid receptor (MR) antagonists reduce mortality in patients with heart failure on top of ACE inhibition. To investigate the underlying mechanism, we compared the actions of aldosterone and angiotensin (Ang) II in the rat heart, and we investigated the effects of the MR antagonists spironolactone and eplerenone in rat Langendorff hearts that were exposed to 45 min coronary artery occlusion + 3 hours of reperfusion. Under normal conditions, Ang II and aldosterone increased left ventricular pressure (LVP) and decreased coronary flow. Neither spironolactone nor eplerenone blocked these effects, suggesting they do not involve MR. During ischemia and reperfusion, spironolactone and eplerenone reduced infarct size (from 68.2% to 45.3% and 53.4%; P< 0.05), and increased LVP recovery (from 44.2% to 52.5 and 60.5%; P< 0.05), whereas aldosterone did not affect these parameters. In conclusion, spironolactone and eplerenone improve the condition of the heart following ischemia and reperfusion. This does not relate to interference with the MR-independent effects of aldosterone on inotropy and vasoconstriction.

Key words: aldosterone, left ventricular pressure, coronary flow, ischemia and reperfusion

P090011

PHARMACOLOGICAL ACTIVITY AGAINST CHAGAS DISEASE OF DERIVATIVES OF 2-[(o-R1) PHENYL]-3-[(o-R2)-IMINEPHENYL]-INDOLE.

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T. Cruzi is the causative of Chagas disease, in South and Central America. The chronic phase occurs several years after infection, with cardiac pathologies. In the research of the therapy of Chagas disease we synthesized seven new derivatives of 2-[(o-R1) phenyl]-3-[(o-R2)-iminephenyl]-indole. The bioassays were using blood of infected Swiss Albino mice of T. Cruzi. The infected blood was used at 1500 forms/mL. The positive control was gentian violet at concentration of 250 micrograms/mL. The indole was dissolved in DMSO at 125, 62 and 31 micrograms/mL. The bioassays were in Trypomastigotes in triplicate at 4 °C and the percent of lyses was determinated after 24 hours. The compound 3 (R1 = H; R2 = Br) have been major lyses as Benznidazole and Nitrofurtimox; against

the NINOA and INC - 5 strain of T. Cruzi and the compound 4 (R1 = R2 = CH3 -) only with NINOA strain of T. Cruzi. Conclusion : The compounds 3 and 4 are more active to smaller concentration that the chemotherapeutic agent Benznidazole and Nitrofurtimox used against Chagas disease.

KEY WORD: Iminephenyl-indole activity Chagas disease.

Acknowledgment : To the support by project DGAPA-UNAM PAPIIT IN225503.

P090012

PHARMACOLOGICAL ACTIVITY AGAINST CHAGAS DISEASE OF NEW DERIVATIVES OF 2 - [(o - ; p- R2) PHENYL] - 1 - [(5 - R1-THIOFURAN - 2-YL) METHYLEN] - HYDRAZONE.

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In the research the therapy of Chagas disease, we synthesized ten new derivatives of 2 - [(o- ; p- R2) phenyl] - 1 - [(5 - R1-thiofuran - 2-yl) methylen] - hydrazone. The bioassays were in Epymastigotes culture of CL-Brener strain of T. Cruzi. The drugs in ethylic alcohol were added at the cell at concentrations : 333 ; 166 ; 16 ; 1.0 and 0.1 micrograms/ mL, in triplicate at 4 °C and the lyses was determinated after 24 hours. The compounds 4b (R1 = H; R2 = p- F) ; 5b (R1 = H; R2 = H) ; have been major lyses that the Benznidazole and Nitrofurtimox at 166 and 16 micrograms/ mL. The second bioassays we used blood infected of Swiss Albino mice, (1500 forms/ mL) against Trypomastigotes of the INC - 5 and NINOA strain of T. Cruzi. The positive control was gentian violet at 100 micrograms/ mL. The compounds with major lyses of Trypomastigotes were 1a (R1 = NO_2 ; R2 = p- Br) , 2a (R1 = NO_2 ; R2 = p- Cl) , 5a (R1 = NO_2 ; R2 = H) , 4b (R1 = H; R2 = o- F) and 5b (R1 = R2 = H) at 125 ; 62 ; 31 and 15 micrograms/ mL that the Benznidazole and Nitrofurtimox. Conclusion : Five of the compounds are more active that Benznidazole and Nitrofurtimox.

KEY WORD: Thiofuran - 2-yl- hydrazone activity Chagas disease.

Acknowledgment : To the support by project DGAPA-UNAM PAPIIT IN225503.

P090013

The anti-apoptosis effect of enantiomers of carvedilol to cardiomyocyte

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OBJECTIVE: Carvedilol as a racemic drug, the detail role of its enantiomers plays in its effect to heart protection is not known very well. The aim of this study is to investigate the anti-apoptotic effect of R(+) and S(-) enantiomers of carvedilol to cardiac myocyte and their sereoselectivity difference between these two enantiomers. METHODS: The myocardial cell line H9C2 - 1 was applied in this study. Cell was induced to apoptosis by isopropylarterenol (ISO) for 12h, R (+) carvedilol or S(-) carvedilol was added as the treatment with 2μM, 10μM dose. Apoptotic cell was identified by Hoechst33258, and for the determination of apoptosis ratio Annexin V-FITC/PI double-staining assay was applied with flow cytometer. RESULTS: ISO induced a largely amount of H9C2 - 1 cells to apoptosis ($p < 0.01$) , while R(+) carvedilol and S(-) carvedilol could all decreased the apoptotic ratio markedly ($p < 0.01$). The late stage and total apoptotic ratio of 2μM R(+) carvedilol + ISO was lower than 2μM S(-) carvedilol + ISO group($p < 0.05$) , but there is no significant difference between 10μM group. CONCLUSION: This study shows that R(+) carvedilol and S(-) carvedilol can all effectively inhibit the apoptosis of H9C2 - 1 cardiac myoctyte induced by ISO, and stereoselectivity difference of this activity does exist between this two enantiomers.

KEY WORDS: Carvedilol, Enantiomer, cardiomyocyte.

ACKNOWLEDGMENTS: This work was supported by GDNSF (015015, 2004B30601009, Y02084).

P090014

Beta1-Adrenoceptor (AR) Arg389Gly polymorphisms affect responses to carvedilol in patients with idiopathic dilated cardiomyopathy

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Objectives : Beta-Blockers have become widespread and standard therapy for heart failure patients, however considerable variability exists in the improvement in left ventricular function with their use. We hypothesized that polymorphisms of Beta1- and Beta2-ARs may contribute to variable responses of Beta-blockers. Methods : PCR-RFLP was used to genotype the Beta1-AR loci in 118 patients with non-ischaemic cardiomyopathy and chronic heart failure treated with carvedilol. Baseline echocardiography was obtained retrospectively and repeated after stabilization of the maximally tolerated carvedilol dose for one year. Results : To date, we have genotyped at amino acid 389 of the Beta1-AR in heart failure patients. The prevalence of the three genotypes was Arg Arg 52 %, Arg Gly 42 %, and Gly Gly 6 %. The preliminary results suggested that patients with the Arg389 Arg genotype had a greater mean improvement of ejection fraction compared with Gly389 carriers (Arg Arg 18.5 SE 1.7 % vs Arg Gly 12.6 SE 1.7 % vs Gly Gly 5.5 SE 3.2 %, P = 0.0048). Conclusion : These data could demonstrate an influence of the Beta1-AR genotype on the response to carvedilol in this group of patients with non-ischemic cardiomyopathy.

P090015

Beneficial effect of pentoxifylline on dystrophic progression of mdx mice

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Pentoxifylline (PTX; 50 mg/ kg daily i. p. for 4 - 8 weeks) , an aspecific phosphodiesterase inhibitor with anti-inflammatory, anti-ischemic and anti-fibrotic activities, was administered to exercised mdx mice, a model for Duchenne muscular dystrophy. In vivo, the treatment contrasted the exercise-induced decrease in forelimb muscle force. The ex vivo analysis included multidisciplinary biophysical, biochemical and histological approaches. PTX-treated limb muscle fibers had a restored calcium homeostasis. In fact the voltage threshold for contraction was returned to the control values ; in parallel both the resting cytosolic calcium and the activity of calcium permeable TRP-like channels were markedly reduced. The treatment also fully counteracted the impairment of chloride channel conductance in diaphragm fibers. PTX-treated diaphragm and hind limb muscles showed a decrease in both non-muscle area and pro-fibrotic cytokine TGF-beta. In parallel the regenerating area was increased and the plasma level of creatine kinase reduced. The wide mechanism of action and a direct effect on the structures handling calcium may account for the beneficial effects of PTX in muscular dystrophy (Telethon GGP05130).

P090016

Concomitant Use of Carvedilol to Angiotensin Converting Enzyme Inhibitor Therapy in Patients with Left Ventricular Systolic Dysfunction

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Objective : To study the effect of carvedilol as concomitant to ACEI therapy in patients with left ventricular systolic dysfunction. Methods : This retrospective study included patients with left ventricular systolic dysfunction (LVD-left ventricular ejection fraction (LVEF) less than 35 %) and undergoing surgical revascularization. Group-I patients received carvedilol and angiotensin converting enzyme inhibitor (ACEI) following surgery while group-II patients received ACEI but no b-adrenoceptor blocker. Functional status and 2D echocardiography and color Doppler characteristics before and after drug treatment were compared. Results : LVEF was significantly ($P < 0.05$) improved in carvedilol receiving group with corresponding greater functional status improvement. There was a comparable reduction in LV diameters and mitral valve regurgitation in both the groups. Mortality rate up to 12 months of treatment was 2.15 % in group-I and 7.14 % in group-II. Conclusions : Carvedilol, as concomitant to ACEI therapy, improves greatly cardiac function, functional status and overall mortality rate even in high-risk patients. However, its short-term administration does not produce significant effect on LV remodeling.

P090017

Comparative study of different extracts from Scutellaria baicalensis root protect against Coxsackiecirus B3 minduced cellular infection

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OBJECTIVE: To explore antiviral effect of crude and refined extracts from

Scutellaria baicalensis root METHODS: Microcell culture performed and cell activity determined with CPE and MTT. RESULTS Both groups have significant effect in protecting viral infected cells, no significant difference in prophylactic rate between the two groups. At the concentration of 1/2 TD0, the protecting rate of crude extract of scutellaria group is higher than refined extract($P < 0.05$). By HPLC assay the baicalin in crude extract is 26.91 mg/ml, and 38.26 mg/ml in refined group. At TD0 concentration, the contents in the two groups are similar, 0.042 mg/ml and 0.048 mg/ml, respectively. CONCLUSION: Refined Scutellaria baicalensis root extract shows no significant direct deactivating action on virus, while ingredients other than refined extract in crude extract may have this function. Both refined and crude extracts have obvious therapeutic effects, probable active ingredients may be major components in refined extract and some other components like flavones in crude extract.

KEY WORDS: Scutellaria baicalensis, coxsackievirus B3 m

Supported by Natural Scientifc Foundation, China, No. 30371832

P090018

Effects of carvedilol on parasympathetic nerve system in adriamycin-induced rat failing heart

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Carvedilol may improve the prognosis of heart failure(HF) better than selective β-blockers. Not all of its effects, however, can be explained by direct actions on the sympathetic nervous system. This study was therefore performed to investigate the possible alterations of parasympathetic nerve system in different regions of the adriamycin-induced failing rat heart, and the potential effects of carvedilol on this system. Karnovsky-Roots staining combined with point counting methods, and immunochemical streptavidin-biotin complex staining and image analysis were used to test the distribution of cholinergic nerves and the expression of muscarinic cholinergic (M_2) receptors, respectively. Our results show that the cholinergic nerve system was downregulated in the failing heart group, while the density of M_2 receptors was increased in the carvedilol 3- and 10- mg/kg body weight groups, especially in the endocardial tissues of the left-ventricular free wall. It is concluded that upregulation of M_2 receptors may be one of the potential mechanisms by which carvedilol exert its action on HF.

Key words carvedilol; M_2 receptors; cholinergic nerves; heart failure.

Acknowledgements This work was supported by the National Natural Science Foundation of China (No. 30470633, 30270554).

P090019

Pharmacological Evidence for the Involvement of Central and Peripheral Opioid Receptors in the Cardioprotective Effects of Fentanyl

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We investigated the involvement of central versus peripheral opioid receptors (OR) in the effects of fentanyl (FENT) in a model of myocardial ischemia/reperfusion (I/R) injury associated to pharmacologically-induced sympathetic overactivity through intracerebroventricular (icv) injection of L-glutamate in anesthetized rabbits submitted to 35 min of coronary occlusion followed by 120 min of reperfusion. Rabbits received naloxone HCl icv. or naloxone methiodide iv, a quaternary compound that does not cross the blood-brain barrier, 5 min before FENT treatment (5 or 50 μl/kg, iv). Infarct area was reduced only by FENT 50 (from 51 ±2 to 24 ±2 %). This protective effect was abolished by peripheral (42 ±4 %) but not central OR blockade (32 ±3 %). The number of premature ventricular complexes (PVCs) during the ischemic period (54 ± 3) was reduced by FENT 50 (19 ± 7), an effect blunted by central (40 ±3) but not peripheral (18 ±7) blockade of OR. Mortality rate (50 %) and incidence of ventricular tachycardia (55 %) were completely abolished by FENT 50. It is concluded that fentanyl presents cardioprotective effects mainly characterized by central antiarrhythmic and peripheral antiischemic actions.

P090020

Protective effects of preconditioning and postconditioning of ACh and Ado on contractility of isolated rat ventricular myocytes

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Protective effects of adenosine(Ado) and acetylcholine(ACh)-induced ischemia preconditioning and postconditioning were investigated on the contractility of isolated rat ventricular myocytes. A video-based edge-detection system was used to monitor single ventricular myocytes contraction. Ado and ACh were administrated for 6 min before ischemia as preconditioning, or 15 min after ischemia as postconditioning. Ado and ACh receptor antagonists and mitoKATP inhibitor were used to analyze pathways underlying the effects on pre and postconditioning. Results: (1) The contractility of ischemic heart cells was improved by both Ado and ACh during preconditioning, as well as postconditioning. (2) Observed effects of Ado and ACh were missing in the presence of Ado A_1 receptor and ACh M_2 receptor antagonists, respectively. (3) Ado and ACh-induced pre and postconditioning were also blocked by mitoKATP antagonist. Our results show that both Ado and ACh protect the contractility of ischemic heart cells during preconditions and postconditioning. The postconditioning of Ado and ACh is relative to the A_1 and M_2 receptor, respectively. MitoKATP are implicated in the postconditioning of both ACh and Ado.

Key words: adenosine; acetylcholine; A_1 receptors; M_2 receptors

Acknowledgements: This work was supported by grants from the National Natural Science Foundation of China (No. 30470633, 30270554).

P090021

Effects of lipoteichoic acid induced delayed preconditioning ischemia/reperfusion injury in spontaneous hypertensive rat

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Myocardial ischemia-reperfusion injury (IRI) model was induced. Pretreated with To investigate the role of lipoteichoic acid (LTA) induced delayed preconditioning on ischemia by LAD ligation for 30 min, followed by 60 min of reperfusion in spontaneous hypertensive rats. Pretreated with LTA (1 mg/kg, ip) 24 h before the experiment significantly reduced CK-MB and LDH release in coronary effluent, and obviously decreased myocardial apoptosis in left ventricle and simultaneously increased the expression of Bcl-2 protein and decreased the expression of Bax protein at the end of reperfusion. In addition, LTA pretreated increased the expression of iNOS and eNOS mRNA and protein and decreased the expression of HIF-1α mRNA and protein in left ventricle at the end of reperfusion. The protective effects were abrogated by pretreatment of the rats with aminoguanidine, The expression of iNOS mRNA (real time RT-PCR) in hearts also increased after LTA pretreatment for 4 h, 8h, and 18 h. It suggests that enhanced NO production by iNOS is obligatorily required to mediate the protection of LTA preconditioning and as an effector of the protection in SHR heart.

KEY WORDS Lipoteichoic acid; Preconditioning; Reperfusion injury; SHR

P090022

Angiotensin AT_2 receptors are expressed in $CD8^+$ T cells and mediate IL-10 production following myocardial infarction

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Less is known about the AT_2 receptor in this condtion. Less is known about angiotensin AT_2 receptors in acute ischemia-induced heart injury. We aimed here to elucidate the role of AT_2 receptors in response to acute myocardial infarction. The regulation of AT_2 receptors on cardiac cellular level were first analyzed in rats with acute myocardial infarction by immunofluorescence labelling. Increased AT_2 receptor immunostaining was detected in clusters of small lymphocyte-like cells accumulating in the interstitial space of both infarcted and non-infarcted myocardium with abundance in the peri-infarct zone. Multiple immunofluorescence staining revealed that cardiac AT_2 receptors were preferentially upregulated in a fraction of $CD8^+$ T cells which were also characterized by IL-10 but not Fas-ligand expression. Furthermore, we could provide evidence that cardiac AT_2 receptors mediated IL-10 expression and reduced cardiac injury. Moreover, in a subset of $CD8^+$ T cells ($CD45RA^-CD27^+$) isolated from human peripheral blood, we could show that stimulation of AT_2 receptors engendered IL-10 production.

Our studies demonstrate for the first time that AT_2 receptors mediate IL-10 production in $CD8^+$ T cells, which may contribute to cardioprotective effects of AT_2 receptors following ischemic cardiac injury. These findings reveal an undescribed role of AT_2 receptors in modulating adaptive immune response.

P090023

Protection of Oxyphenamone on Myocardium against Ischemia-reperfusion Injury

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Objective To investigate the protective effects of oxyphenamone(oxy), a calcium sensitizer, on myocardium against ischemia-reperfusion injury (I-R). Methods The regional I-R was established by ligaton of the left anterior descending coronary artery (LAD) followed by reperfusion (10/15 min in rats, 30/60 min in cats) and the global I-R in rat hearts was created by stopping the perfusion (40 min) followed by reperfusion (30 min). Results Administration of oxy (infusion 1～10 μmol L^{-1}, iv 0.1～8 mg kg^{-1}) ameliorated the ventricular arrthymia, antagonized the changes in myocardial CPK, LDH, MDA, SOD, GSH, GSH-px, ATP, PCr and mitochondrial [Ca^{2+}], improved cardiac hemodynamics and preserved the integrity of myocardial ultrastructure dose-dependently.

Conclusion Oxy could protect myocardium against I-R remarkably.

Key words Oxyphenamone; Myocardial ischemia-reperfusion

P090024

The Gisenoside Rh2 Could Increase Contraction Force of Isolated Toad Heart

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Objective: To study the effects of Gisenoside Rh2 on myocardial contraction action in vitro. Methods: Ginsenoside Rh2, a purified dammarane-type tetracyclic triterpenoid soponin, was prepared from total saponins of the leaf and stem of Panax Ginseng and P. notginsen by alkaline degradation. The Straub perfusion heart model of toad was used to observe the effects of Gisenoside Rh2 on myocardial contraction force and cardiac rate. Results: the myocardial contraction force increased at the concentration of 50, 100, 200 mg/L, and the cardiac frequency didn't show markedly change in those concentrations. Conclusions: The Gisenoside Rh2 could increase contraction force of isolated toad heart, and there is no significant effect on the heart rate in vitro.

Key words: Gisenoside Rh2, heart.

Acknowledgement: Thanks for the Shandong Engineering Research Center of Natural Drug to provide the Ginsenoside Rh2.

P090025

Degradation of transcription factor NFAT5 is induced by doxorubicin exposure in cultured cardiomyocytes

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Although recent evidences support that nuclear factor-activated T-cell (NFAT) 5, as also known tonicity response element binding protein (TonEBP), is responsible for diverse cellular responses, the role of NFAT5 in the heart has been elucidated. We examined the effect of doxorubicin (Dox), which leads to cardiac toxicity, on NFAT5 activity in cultured cardiomyocytes. Luciferase assay revealed that Dox treatment (0.3 micro M) for 24 h caused decreases in the transcriptional activity of NFAT5. Western or Northern blot analyses showed that Dox remarkably reduced the expression of NFAT5 protein in cardiomyocytes, while NFAT5 mRNA was barely downregulated, respectively. Further, treatment with proteasome inhibitor MG-132 prevented Dox-induced degradation of NFAT5. In cardiomyocytes cultured under serum-depleted condition, selective NFAT5 inhibition by adenovirus vector encoding dominant-negative NFAT5 increased CPK leakage and decreased cell viability, as assessed by MTS assay, compared with cardiomyocytes expressing beta-gal. Thus we conclude that NFAT5 is degraded by Dox exposure in cardiomyocytes, which may result in cardiac toxicity.

Key words: cardiomyocyte, NFAT5, doxorubicin, cell survival

P090026

MELANOCORTINS PROTECT AGAINST MYOCARDIAL ISCHEMIA/REPERFUSION INJURY THROUGH THE ACTIVATION OF AN EFFERENT CHOLINERGIC PATHWAY

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A vagus nerve-mediated, brain cholinergic protective mechanism, is operative in circulatory shock. We investigated, therefore, role and functional mechanism of such vagal efferent pathway(s) in an model of ischemic heart disease. Anesthetized rats were subjected to transient coronary artery occlusion (5 min) followed by reperfusion: occurrence of ventricular tachycardia (VT), ventricular fibrillation (VF), and lethality, were recorded up to the 5th min after reperfusion. Electrical stimulation of efferent vagal fibres (5 V, 2 ms, 1-9 Hz, for the whole period of ischemia/reperfusion) reduced the high incidence of VT, VF and lethality, the increase in free radical blood levels and left ventricle histological alterations. Treatment with some melanocortin peptides (162 nmol/kg i.v. or 16.2 nmol/kg i.c.v.) produced the same protective effects of electrical stimulation, and with the same muscarinic receptor-dependent mechanism, seemingly through brain activation (mediated by melanocortin MC_3 receptors) of such efferent vagal pathway. These findings could provide the potential for a novel approach to management of ischemic heart disease.

Key words: myocardial ischemia, vagus nerve, melanocortins.

P090027

Effects of N-n-butyl haloperidol iodide on myocardial Ischemia/reperfusion injury and Egr-1 expression in rats[1]

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Aim: The present study was designed to investigate the relationship between the protective effects of N-n-butyl haloperidol iodide (F_2) on myocardial ischemia/reperfusion (I/R) injury and the expression of Egr-1.

Methods: The models in vivo and in vitro were established. Plasma creatine kinase, creatine kinase MB isoenzyme and lactate dehydrogenase were measured to assess the degree of injury of myocardial tissue. Ultrastructure was detected to assess the degree of injury of cultured cardiomyocytes. Egr-1 mRNA and protein expressions were observed by RT-PCR, immunohistochemistry and immunocytochemistry.

Results: I/R caused the leakage of myocardial enzymes in rats. Hypoxia/re-oxygenation (H/Re) caused ultrastructural damages in cultured myocytes. F_2 can inhibit above damage changes induced by I/R or H/Re. Meanwhile, I/R or H/Re induced strong expressions of Egr-1 mRNA and protein in myocardial tissue and cultured cardiomyocytes, which were inhibited by F_2.

Conclusion: The protective effect of F_2 on I/R- or H/Re-induced myocardial injury may be partly mediated by inhibiting Egr-1 expression

Keywords: N-n-butyl haloperidol iodide; Myocardial ischemia/reperfusion injury; Egr-1 expression

[1]Project supported by the National Natural Science Foundation of China (No 30472028)

P090028

PROTECTIVE EFFECTS OF N-n-BUTYL HALOPERIDOL IODIDE ON MYOCARDIAL ISCHEMIA-REPERFUSION INJURY IN RATS

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To test the cardioprotective efficacy of N-n-butyl haloperidol iodide (F_2), a novel compound derived from haloperidol, the myocardial ischemia-reperfusion injury models were studied. In these models, rats were subjected to 60 min of ischemia induced by ligation of the left coronary artery, followed by 60 min of reperfusion. Different doses of F_2 were intravenously injected before the onset of ischemia. The changes of hemodynamics were recorded by means of cardiac catheterization with continuous ECG recordings during the experiment. After reperfusion, The infarct area and the area-at-risk were calculated. The results show that the administration of F_2 could ameliorate the hemodynamics of myocardial ischemia-reperfusion injure in a dose-dependent manner. In F_2 group, ECG recovered faster and infarct size was smaller than control group. We conclude that F_2 could exert an apparently protective effect against myocardial ischemia-reperfusion injury.

Key words: myocardial ischemia-reperfusion injury; hemodynamics; myocardial infarct size

This work was supported by the grants from the National Natural Science Foundation of China (No. 30070304)

P090029

Cardioprotective effects of Nitric Oxide-Aspirin in myocardial ischemia-reperfusioned rats

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In this study, the cardioprotective effect of the NO-Aspirin, the nitro-derivative of aspirin, was compared with that of aspirin in a rat model of myocardial ischemia/reperfusion. Rats were given aspirin or NO-Aspirin orally for 7 consecutive days prior to 25 min of myocardial ischemia followed by 48 hour of reperfusion (M/R). Treatment groups included vehicle (Tween 80), aspirin (30 mg/kg/day)

and NO-Aspirin (56 mg/kg/day). NO-Aspirin, compared to aspirin, displayed remarkable cardioprotection in rats subjected to M/R as was evidence of mortality rate and infarct size. Mortality rate for vehicle, aspirin and NO-Aspirin groups were 34.8%, 27.3% and 18.2%, respectively. Infarction size for the vehicle group was 44.5% (±2.7%) of the left ventricle (LV). For the aspirin and NO-Aspirin groups, infarction size was 36.7% (±1.8%) and 22.9% (±4.3%) of the LV, respectively. NO-Aspirin treated groups showed increased activities of superoxide dismutase (SOD) compared to the vehicle group. NO-Aspirin could downregulate iNOS, COX-2 but upregulate VEGF genes expression after M/R. Rats administered with N^G-nitro-L-arginine methyl ester (L-NAME, 20 mg/kg) demonstrated aggravated myocardial damage in terms of mortality and infarct size, the exacerbation were attenuated by co-administered with NO-Aspirin. The beneficial effects of NO-Aspirin appeared to derive in large part from the NO moiety, which elicits late preconditioning, decreases oxidative stress and modulates gene expression of iNOS, VEGF and COX2, results in reducing the extent of myocardial injury following ischemia and reperfusion.

P090030

Geranylgeranylacetone protects rat striatum neurons against heat injury via induction of Hsp70[a]

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To evaluate the protective effect of geranylgeranylacetone (GGA) against heat injury to rat striatum neurons. Primary cultivated striatum neurons were pretreated with GGA for 24 h, and then heat-treated at 43°C for 1h. Cell viability was detected by the release of lactose dehydrogenase (LDH). Membrane surface ultrastructure of neurons was investigated by atomic force microscopy. Hsp70 expression in neurons was determined by RT-PCR. Furthermore, we investigated the effects of quercetin, a inhibitor of Hsp70 synthesis, on the viability and Hsp70 expression in heat-treated neurons after GGA treatment. GGA pretreatment significantly attenuated the release of LDH and prevented the damage of membrane surface ultrastructure. A significant increase of Hsp70 was found in GGA-treated neurons. Furthermore, quercetin pretreatment eliminated the protective effect of GGA and inhibited GGA-induced Hsp70 expression. GGA protects striatum neurons against heat injury and this protection is dependent on the Hsp70 synthesis.

geranylgeranylacetone; Heat shock proteins; heat injury; striatum

[a]Supported by grant from National Natural Science Foundation of China (#30500185)

P090032

Effect of Hemorrheology on Coronary Artery Ligated Beagle Dog with HQSM

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Objective To study effect of Huangqi Shenmai active part (HQSM) on hemorrheology in coronary artery ligated Beagle dog. Method Beagle dogs were randomly divided into six groups: model control, HQSM(low, moderate, high), Diltiazem Hydrochloride Tablets and Huangqi Shengmai Yin, 7~8 Beagle dogs in each group. Right external jugular vein was separated after anesthetized Beagle dog opened chest. Acute myocardial ischemia model was established by ligating the left anterior descending branches of coronary artery. Drug was administrated into duodenum. Blood was obtained immediately before ligation and at 0, 30, 60, 90, 120, 180 min respectively after therapy which anticoagulated with heparin. Hemorrheology indexes such as WBV, PV, ESR, Hct, WBRV, EAI, EDI, ERI, EEI and ECG II were observed. Result HQSM can improve PV and descend increasing of ESR, EAI apparently, but show no visible influence on other hemorrheology indexes. Diltiazem Hydrochloride Tablets and Huangqi Shengmai Yin showed no effect on indexes of hemorrheology compared with model control group. Conclusion HQSM can ameliorate the hemorrheology in Beagle dog induced acute ischemic myocardium by ligating coronary artery at some degree.

[Fund]: Lv Guiyuan, Maga-projects of Science Research for the 10th Five-Year Plan

P090033

Effect of HQSM on Hemodynamics of Beagle Dog with Acute Ischemic Myocardium

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Objective To observe influences of Huangqi Shenmai active part (HQSM) on hemodynamics in Beagle dog induced acute ischemic myocardium by ligating coronary artery. Method Beagle dogs were divided randomly into six groups: model control, HQSM(low, moderate and high), Diltiazem Hydrochloride Tablets and Huangqi Shengmai Yin, there were 7~8 Beagle dogs in each group. Left anterior descending branches of coronary artery was ligated to make acute myocardial ischemia in anesthetized open-chest Beagle dog. The drug was administrated by duodenum. The relative parameters of hemodynamics and ECG II were monitored immediately at the follow corresponding time points such as before ligation and at 0, 30, 60, 90, 120, 180 min respectively after therapy. Result HQSM can decrease LVEDP and increase $-dp/dt_{min}$ compared with model control group; Huangqi Shengmai Yin showed no effect on parameters of hemodynamics except decreasing LVEDP compared with model control group. Conclusion HQSM can improve the diastolic function of left ventricular on acute myocardial ischemia in Beagle dog, and its effect is better than Huangqi Shengmai Yin.

[Fund]: Lv Guiyuan, Maga-projects of Science Research for the 10th Five-Year Plan

P090034

Protective Effect of HQSM on Acute Ischemic Myocardium of Beagle Dog

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Objective To study protective effect of Huangqi Shengmai active part (HQSM) in Beagle dog induced acute ischemic myocardium by ligating coronary artery. Method Beagle dogs were randomly allocated to six groups: model control, HQSM(low, moderate and high), Diltiazem Hydrochloride Tablets and Huangqi Shengmai Yin, there were 7~8 dogs in each group. Acute myocardial ischemia model was established by ligating the left anterior descending branches of coronary artery in anesthetized open-chest dog. Drug was administrated into duodenum. Ischemic severity and range which measured by epicardial electrocardiogram recorded before ligation and at 0, 30, 60, 90, 120, 180 min respectively after therapy. Myocardial infracted area were calculated at 180 min after therapy. Result Moderate and high dosage of HQSM can decrease myocardial ischemia degrees, limit myocardial ischemia ranges, reduce the ischemia zone compared with model control group. Huangqi Shengmai Yin can decrease the myocardial ischemia range at 90 min after therapy, but showed no effect on other indexes. Conclusion HQSM have preventive effect on acute myocardial ischemia in Beagle dog and the effect is better than Huangqi Shengmai Yin.

[Fund]: Lv Guiyuan, Maga-projects of Science Research for the 10th Five-Year Plan

P090035

Protective Effect of Total Flavones of Rhododendra on ischemic myocardial injury in rabbits

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This study was to investigate the effect of total flavones of rhododendra (TFR) on ischemic myocardial injury in rabbits. Rabbit ischemic myocardial injury was induced by occluding the anterior descending of the left artery (LAD). The ECG was recorded; the plasam creatine kinase (CK), nitric oxide (NO) and endothelin-1 (ET-1) levels were measured using spectrophotometry, Griess method and radioimmunoassay, respectively. The myocardial ischemic size and infarction size were determined by dual staining with Evan's blue and Nitroblue tetrazolium reduction test (N-BT). A typical ECG S-T segment elevation and an increase of plasam CK activity were seen 6 and 24 hours after the induction of ischemia. These changes were inhibited in rabbits treated with either TFR (30, 60 mg/kg) or EGB for 7 days, indicating a protective effect of TFR on ischemic myocardial injury. The myocardial ischemic size and infarction size were 40.7±3.6% and 36.8±3.6% respectively in control group, while TFR (60 mg/kg) pretreatment for 7 days significantly reduced both myocardial ischemic size (32.40±5.38%, $P<0.05$) and infarction size (28.7±5.8%, $P<0.05$). In addition, occluding of LAD resulted in an increase of ET-1 and a decrease of NO levels in the plasam, effects that were inhibited by TFR treatment, suggesting a possible mechanism that may be related to the protective effect of TFR against myocardial ishchemic injury.

Keywords: Total Flavones of Rhododendra (TFR); ischemic myocardial injury;

myocardial infarction; creatine kinase (CK); nitric oxide (NO); endothelin (ET)

P090036

Recombinant human erythropoietin enhances myocardial angiogenesis and attenuates detrimental cardiac remodeling in mice post myocardial infarction

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We aimed to investigate the effects of recombinant human erythropoietin (EPO) on myocardial angiogenesis and cardiac remodeling during development of heart failure post myocardial infarction (MI). Adult male mice (C57BL6) were subjected to MI and randomly assigned to EPO and vehicle treatment groups (n=30 per group). EPO was administered 2,500 U/kg (i.v.) immediately after MI, followed by 1,000 U/kg (i.v.) every 2 days in the 1st week, and twice a week for 3 weeks afterwards. Four weeks after MI, hemodynamic measurements were determined using a Millar tip-transducer catheter. Myocardial capillary density and myocyte cell size were measured morphometrically. EPO treatment increased LV +dP/dt while LV volume and LV wall thickness were decreased compared to vehicle group ($P<0.05$). Myocardial capillary density at the infarct border zone was increased by 77% in EPO treatment compared to vehicle group ($P<0.05$). Cross-sectional area of myocytes was decreased by 32% in EPO treatment compared to vehicle group ($P<0.05$). In conclusion, recombinant human EPO enhances myocardial angiogenesis and attenuates detrimental cardiac remodeling post-MI in mice.

Key words: Erythropoietin, heart failure

P090037

Geranylgeranylation is necessary in Na^+/Ca^{2+} exchanger mRNA increase by lisophosphatidylcholine in H9c2 cells.

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Cardiac Na^+/Ca^{2+} exchanger (NCX1) expression levels change under various pathophysiological conditions. However, its mechanism is unknown. We previously showed that fluvastatin (Flv), an HMG-CoA reductase inhibitor, decreased NCX1 mRNA and protein by inhibiting a small G-protein, RhoB in H9c2 cardiomyoblasts (2005). Conversely, lisophosphatidylcholine (LPC) increased NCX1 mRNA and protein by activating RhoB. RhoB requires isoprenylation for its activation with either farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP). Here, we investigated which isoprenoid is involved in the effect of LPC. Treatment of H9c2 cells with Flv for 24 hours decreased NCX1 mRNA by 40%. Addition of GGPP or FPP to Flv restored NCX1 mRNA to control level. No difference was observed between GGPP and FPP. When LPC was applied with Flv, NCX1 mRNA remained decreased. However, when LPC and GGPP, but not FPP, were applied simultaneously, NCX1 mRNA increased to a level significantly higher than the control. Furthermore, a GG-transferase inhibitor, but not F-transferase inhibitor, inhibited the effect of LPC. We conclude that geranylgeranylation but not farnesylation of RhoB is invdlved in the effect of LPC on NCX1.

Key words: H9c2 cells, Na^+/Ca^{2+} exchanger, small-G proteins, isoprenoids

P090038

Enhanced Apoptosis and Myocardial Injuries in Metallothionein-Null Mice by Doxorubicin Treatment

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Cardiotoxicity is the major limiting factor for the application of doxorubicin (DOX) in cancer chemotherapy. Metallothionein (MT) is a low-molecular-weight and sulfur-rich protein. Recent studies have indicated that many MT inducers protect against DOX-induced cardiotoxicity but the mechanisms remain unclear. This study was aimed to investigate the effects of DOX in wild-type (MT+/+) and MT-null (MT-/-) mice heart. MT+/+ mice and MT-/- mice were received respectively single administration of DOX (15 mg/kg, i.p.) or equal volume of saline, and were killed on the 4th day after the injection. Obvious injuries were caused by DOX in MT+/+ mice heart including elevated serum creatine kinase, morphological changes as examined by light microscopy and electron microscopy, lipid peroxidation, protein oxidation, apoptosis as detected by TUNEL test and caspase-3 activation. All of these DOX-induced toxic responses were significantly enhanced in MT-/- mice heart. These results demonstrate that MT null mice were more sensitive to DOX-induced myocardiol injuries and apoptosis in vivo.

Key Words Doxorubicin, Cardiotoxicity, Metallothionein, Apoptosis
Supported by China Natural Science Foundation grant 30572281

P090039

Protective effects of paeoniflorin on myocardial ischemia injury in rats

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Objective To observe effects of paeoniflorin (PF), isolated from a traditional Chinese herb Paeoniae Radix, on myocardial ischemia injury in rats. Methods The rat model of acute myocardial ischemia was reproduced by ligating the left coronary artery at the anterior descending branch. After 6 h, SOD, MDA and CK in serum were determined. ST-segment elevation in ECG, the myocardial infarct size and the morphological changes were also observed. Results In the vehicle group, the content of MDA, the activity of CK, the infarction size and ST-segment increased greatly, whereas the activity of SOD decreased obviously, compared with the sham group. PF 60 mg/kg and PF 120 mg/kg decreased the activity of CK, the size of infarction, myocardial ischemia degree(∑-ST) and myocardial ischemia area(N-ST); meanwhile, the activity of SOD increased remarkably. Moreover, PF 60 mg/kg and PF 120 mg/kg could reduce myocardial necrosis and leukocyte infiltration significantly. Conclusion PF could effectively relieve ischemic injury in rats with acute myocardial ischemia.

Key words: paeoniflorin, myocardial ischemia injury, myocardial protection

P090040

Effects of lipoteichoic acid induced delayed preconditioning on cardioplegic arrest/reperfusion injury in donor heart of rat

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To investigate the role of endogenic nitric oxide in lipoteichoic acid (LTA) induced delayed preconditioning on reperfusion injury after cardioplegic arrest by the langendorff method in rats. Pretreated with LTA (1 mg/kg, ip) 24 h before the experiment significantly improved the recovery of cardiac function with a significant increase in CF, LVDP, +dp/dtmax, -dp/dtmax at 30 min and 60 min of reperfusion, reduced CK-MB and LDH release in coronary effluent, and obviously decreased myocardial apoptosis in left ventricle at the end of reperfusion. In addition, LTA pretreated raised the concentrations of NO in coronary effluent, and increased the expression of iNOS mRNA in left ventricle at the end of reperfusion. The protective effects were abrogated by pretreatment of the rats with L-NAME, while pretreatment with L-NAME alone did not significantly affect any of the parameters investigated. It suggests that LTA could induce the delayed cardioprotection associated with improvement cardiac function and reduction of myocardial necrosis and apoptosis. Enhanced endogenic NO production by iNOS is obligatorily required to mediate the protection of LTA preconditioning.

[KEY WORDS] Lipoteichoic acid; Preconditioning; Reperfusion injury; Organ preservation

P090041

Pharmacotheraputics and Blood Concentrations of Digoxin in Patients with Heart Failure

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Objective: To study the pharmacotheraputics and to evaluate relationship between serum digoxin concentrations (SDC) and clinical effections in patients with heart failure. Methods: SDC was determined by the chemical luminary enzyme immunoassay The clinical data of 372 inpatients were analyzed. The effective therapupeutic serum concentration ranges from 0.5ng/ml to 2.0ng/ml. Results: Totally 372 patients were analyzed, among which 227 patients (61.02%) were within the effctive theraputic concentration, 35 patients (9.41%) were above 2.0ng/ml and another 110 patients (29.56%) were below 0.5ng/ml. Conclusion: Great individual difference exists in the serum concentration digoxin, and the causes are various. therefore, the serum concentration monitoring plays an important role in the administration of digoxin in clinical practice.

key words: digoxin; heart falure; pharmacotherapy; blood concentration

P10. Cardiovascular Pharmacology - Lipid Lowing Agents

P100001

The effect of Daming capsule on the mRNA expression of M receptors different isoforms on hyperlidemic rat's cardiac muscle

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Objective To interpret the molecular mechanism of Daming capsule's decreasing blood lipid effect on hyperlidemic rats by studying the quantitative expressions of all subtypes of M receptor mRNA on the myocardial. Methods A hyperlidemic rat model was performed firstly, total RNA of the myocardial was extracted using Trizol method. To investigate the difference between M receptors, M_2、M_3、M_4、M_5 receptors expression in groups of hyperlipemia, normal and drug were examined using RT-PCR technology. Results The expression of M_2、M_4、M_5 receptor decreased, but the expression of M_3 receptor increased during hyperlipemia, the differences have statistical significance. The mRNA level of M_3 isoform increased, M_2 isoform decreased after giving Daming capsule comparing with hyperlipemia group, the differences have statistical significance. However, the differences of M_4 and M_5 mRNA have no statistical significance. Conclusion The increase expression of M_3 receptor is one of the mechanisms that Daming capsule decreases the blood lipid.

Key words: Daming capsule; M receptor; Hyperlidemic; RT-PCR

P100002

Atorvastatin might inhibit myocardial hypertrophy in vitro via up-regulating PPARs expression

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The study was to investigate the effects of atorvastatin on AngII-induced myocardial hypertrophy and the PPARs expression in vitro. The AngII-induced hypertrophic myocardial cells (MC) were treated with atorvastatin (10, 1, 0.1, 0 micro mol/L) and then evaluated by detecting the surface area, ^{3}H-leucine incorporation and atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), matrix metalloproteinase 9 (MMP9), MMP2, interleukin-1beta (IL-1beta) and PPAR-alpha, beta and gamma subtypes of MC. Also, PPARs protein was imaged by immunofluorescence staining in MC. It was showed that atorvatatin exerts down-regulative effects on the surface area, ^{3}H-leucine incorporation and mRNA expression of ANP, BNP, MMP9, MMP2 and IL-1beta of hypertrophic MC. Meanwhile, atorvastatin could reverse the decreases of three PPAR subtypes in mRNA and protein levels. The results demonstrate that atorvastatin inhibits cardiac hypertrophy in vitro and PPARs might be involved in it.

Key words: atorvastatin; myocardial hypertrophy; PPAR

P100003

Dealing with Dyslipidemia. A cross-sectional study on the usage of statins in hospital in Jakarta-Indonesia.

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A preliminary study on the pattern of the use of statins from May-July 2004 in three hospitals in Jakarta was done to see the responder rate of the patients. The inclusion criteria are outpatients diagnosed with dyslipidemia treated with statins either as first choice or add-on therapy, and other lipid-lowering drugs. And 243 cases (127 male and 116 female) were recorded. The average age of both group of patients are 56 ± 12 yrs (male) and 55 ± 12 yrs (female). Atorvastatin is the most used statins as first choice and as add-on (38.1%, and 1.6%, respectively), followed by rosuvastatin (20.5%), fluvastatin (11.5%), pravastatin (10.7%); whereas lovastatin (0.4%) and simvastatin (3.7%) were least prescribed. Patients' TC levels were reduced significantly (Δ±14%, $p < 0.0001$), whereas Tg levels were not significantly reduced (Δ±8%). Around 46% cases (72 out of 243 cases) met the NCEP ATP III goals on TC level. The most combinations used, with respect to atorvastatin, are fenofibrate (11.3%), pravastatin (9.3%), rosuvastatin (3%), and gemfibrozil (2%). Whereas, regarding rosuvastatin, were gemfibrozil (9%), ciprofibrate (5%), and atorvastatin (3%). Large numbers of patients were not often checked up or low in compliance. Conclusion, statins usage in some hospitals in Jakarta has been inappropriate, due to the various factors, such as, prescribers, patient's aspects, and the national health system. Therefore, to observe the efficacy of statins in clinical setting, a large scale of the alike study should be conducted.

Keywords: statins; efficacy; hyperchdesterolemia; drug combination

P100004

Heterologous expression of lipoprotein-associated phosphdipase A2 in different expression systems

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Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a key enzyme involved in atherosclerosis. We herein examined the feasibility of expressing and purifying recombinant Lp-PLA2 in different heterologous expression systems. We found that recombinant Lp-PLA2 was expressed at high levels and exhibited strong enzyme activity in insect cell-baculovirus expression system, and that the functional enzyme could also be produced in P. pastoris. The inclusion of a Kozak sequence significantly increased the expression level of recombinant Lp-PLA2 in insect cells, but had little effect on the expression of recombinant Lp-PLA2 in P. pastoris and E. coli. P. pastoris-produced Lp-PLA2 could be purified rapidly and conveniently through a one-step procedure, while baculovirus-produced Lp-PLA2 could be efficiently purified through a two-step procedure. This ability to readily produce and purify recombinant Lp-PLA2 will facilitate further studies on this enzyme.

Keywords: Lipoprotein-associated phospholipase A2; Atherosclerosis; Cloning; Expression

Acknowledgements: We are grateful to Prof. Xingzu Zhu, Dr. Weiyu Zhang, and Dr. Wanchun Sun for their helpful advice and technical support.

P100005

Protective Effect of Human Urotensin II (hUII) on Myocardial Ischemia and Reperfusion Injuries in Rats

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Objective To study the effects of hUII on myocardial ischemia and reperfusion injuries in rats. METHODS Rat myocardial ischemia was induced by isoprenaline (Iso, 8 mg/kg) injected subcutaneously, the changes of ECG and myocardial CK, LDH and NO were detected. The myocardial ischemia-reperfusion model was induced by ligating of the anterior descending branch of left coronary artery 30 minutes and reperfusion 60 minutes, the ECG and the infarct size (IS) of myocardium were detected, the serum CK, LDH, NOS, NO were examined. RESULTS On the rat myocardial ischemia model, 300, 1000 and 3000 pmol/kg hUII significantly attenuated the raise of ΣST segment in ECG, reduced CK and LDH, increased NO. On the model of rat myocardial ischemiareperfusion, 300, 1000 and 3000 pmol/kg hUII significantly and dose-dependently attenuated the raise of ΣST segment and IS of myocardium, hUII (1000 and 3000 pmol/kg) markedly inhibited the increases of CK and LDH activities and the decreases of NOS activity and NO content. CONCLUSION HUII has significant protective effect on rat myocardial ischemia and reperfusion injuries via increasing of NO production.

Key words: human urotensin II, myocardium, ischemia, reperfusion

P100006

Effects of S-allylcysteine on nitric oxide production and antioxidant enzyme activities in hyperlipidemic rats.

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Garlic has been used as a traditional medicine for prevention and treatment of cardiovascular diseases. We investigated the effects of its major component, S-allylcysteine (SAC), on nitric oxide (NO) production and antioxidant enzyme activities in hyperlipidemic rats. The present study demonstrates that SAC inhibited NO production in serum, liver and kidney through the suppression i NOS activities in hyperlipidemic rats in a dosedependant manner. SAC also inhibited NO production by LPS treatment in serum, liver and kidney through the suppression of T-NOS and i NOS activities in normal rats. SAC increased the activities of superoxide dis-

mutase (SOD) and the level of glutathione (GSH) , and decreased the level of malondialdehyde (MDA) in serum, liver and kidney of hyperlipidemic rat. Furthermore, SAC increased vitamin C concentration while decreased arginine concentration in serum of hyperlipidemic rat. These data suggested that SAC inhibited the NO production by reducing iNOS activity and arginine concentration and exhibited antioxidant activity, which may play a pharmacologically important role in protection from oxidative injury and pathogenesis of atherosclerosis.

P100007

The Effects of Caveolin - 1 on Cholesterol Efflux of Lipid-loaded Cells Derived from Vascular Smooth Muscle Cell

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Aim To investigated the effects of caveolin - 1 on cholesterol efflux in vascular smooth muscle cells (VSMCs) induced by ox-LDL. Methods HPLC and tritium label analysis were employed to measure the cellular cholesterol ester(CE) , total cholesterol(TC) and cholesterol efflux respectively. Oil Red O dyeing was performed to observe the cellular lipid droplets. Western blot were used to show the caveolin - 1 expression. Results ox-LDL (75 mg/ L) treatment decreased the caveolin - 1 expression of VSMCs with the peak at 48 h. When the caveolin - 1 expression declined to 50 %, cholesterol efflux decreased 65 %. Oil Red O dyeing showed a significant increase of lipid droplets. Antisense caveolin - 1 oligonucleotides treatment increased further cholesterol accumulation in cells. On contrast, caveolin - 1 over-expression by transfecting the pcl-neo-cav - 1 plasmid into VSMCs improved markedly the cellular lipid load. and the content of TC declined by 50 %. Furthermore, when pcl-neo-cav - 1 △ plasmid lacking cholesterol binding domain was transfected into VSMCs, the cellular cholesterol accumulation increased by 2 folds. Conclusion Caveolin - 1 mediated the cholesterol efflux of VSMCs induced by ox-LDL.

P100008

Effects of Panax Notoginseng total saponins on Serum Lipid Concentrations in Triton WR1339 - Treated Rats

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Using Triton WR 1339 - treated rats, we explore the possibility of Panax Notoginseng total saponins (PNS) on suppression of increases in serum lipid concentrations. At 24 hr after Triton WR1339 injection, the total cholesterol and triglyceride, LDL- C and HDL- C concentrations in the PNS group underwent statistically significant decreases (17.8 %, 18.0 %, 32.6 % and - 31.5 % respectively) compared with those in the Triton-treated group. These data indcated PNS can be good at regulating serum lipid concentrations, especially can statistically up-regulate HDL - C, so it is benefit for CHD.

Key words: Panax Notoginseng total saponins (PNS) ; Triton WR1339; total triglyceride (TG) ; total cholesterol (TC) , low density lipoprotein cholesterol (LDL- C) , high density lipoprotein cholesterol (HDL- C)

P100009

Hypolipidemic effects of polymers extracted from culture broth, mycelia, and fruiting bodies of Auricularia auricula-judae in dietary-induced hyperlipidemic rats

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Hypolipidemic effects of polymers extracted from culture broth (CP) , mycelia (MP) , and fruiting bodies (FP) of Auricularia auricula-judae was investigated in dietary-induced hyperlipidemic rats. The animals were administrated with polymers at the level of 100 mg/ kg body weight daily for 4 weeks. Hypolipidemic effects were achieved in all the experimental group, however FP group proved to be the most potent one. The administration of the FP reduced the plasma total cholesterol, low-density lipoprotein cholesterol, triglyceride, and atherogenic index by 28.54, 33.25, 24.25, and 42.42 %, respectively, when compared to the saline administrated group. It also increased the high-density lipoprotein cholesterol level as much as 9.01 %. The sugar and amino acid compositions of FP were analyzed in detail.

This work was supported by the RRC program of MOIEC

P100010

Effect of pitavastatin on visceral fat obesity and glucose intolerance of spontaneously hypertensive hyperlipidemia rats with induced high serum glucose condition

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We have reported that spontaneously hypertensive hyperlipidemia rats (SHHR) fed by high fat diet (HFD) with 15 % sucrose (SUC) water loading develop visceral fat obesity (VSO) . We have investigated the effect of HMGCoA reductase inhibitor, pitavastatin (PIT) , on visceral fat and glucose intolerance on this model. Four months old male SHHR were fed by water with 100 mg/ L of nitric oxide synthase inhibitor (L-NAME) for a month, followed by HFD with 15 % SUC water loading and PIT 0.3 mg/ kg for 2 months. Intraperitoneal GTT (IPGTT) was performed with 1g/ kg of glucose under fasting condition, then the blood drawing was performed from tail vein. (SD rats for control.) SUC + HFD increased blood insulin and glucose with both SD and SHHR significantly. Visceral fat increased as well in SHHR, which was significantly suppressed by PIT. SUC + HFD worsened glucose intolerance in both SD and SHHR on IPGTT, PIT remarkably improved it especially in SHHR. SUC + HFD also increased serum cholesterol significantly in SHHR, which was suppressed by PIT. As conclusion, sucrose + HFD increased visceral fat and worsened glucose tolerance in SHHR, which was improved by PIT, maybe by related mechanism to serum cholesterol change.

P100011

Dealing with Dyslipidemia. A cross-sectional study on the usage of statins in hospital in Jakarta-Indonesia.

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A preliminary study on the pattern of the use of statins from May-July 2004 in three hospitals in Jakarta was conducted to see the responder rate of the patients. The inclusion criteria are 243 outpatients (127 male, 56 ±12 yrs and 116 female, 55 ±12 yrs) , diagnosed with dyslipidemia, treated with statins either as first choice or addon therapy and other lipid-lowering drugs. Atorvastatin is the most used statins as first choice (38.1 %) and as add-on (1.6 %) , followed by rosuvastatin (20.5 %) , fluvastatin (11.5 %) , pravastatin (10.7 %) ; whereas lovastatin (0.4 %) and simvastatin (3.7 %) were least prescribed. Patients' TC levels were reduced significantly (Δ±14 %, $p < 0.0001$) , while Tg levels were not significantly reduced (Δ±8 %) . Around 46 % cases (72 cases) met the NCEP ATP III goals on TC level. The combinations most used, with respect to atorvastatin, are fenofibrate (11.3 %) , pravastatin (9.3 %) , rosuvastatin (3 %) , and gemfibrozil (2 %) . Regarding rosuvastatin are gemfibrozil (9 %) , ciprofibrate (5 %) , and atorvastatin (3 %) . A large number, however, do not comply. Conclusion, the inappropriate usage of statins is due to various factors, will be discussed in the paper.

P100012

Mechanisms of Regulating cholesterol metabolism by Protocatechualdehyde, Ursolic acid and Quercetin

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In our study, CHO cells and BRL cells were cultured and exposed to different concentration of protocatechualdehyde, ursolic acid or quercetin for 24h, the inhibition of cholesterol biosynthesis was determined by MTT method in amphotercin B CHO cell model, the expression of cholesterol 7alphahydroxylase mRNA was examined by RT-PCR method in BRL cells. The results showed protocatechualdehyde 50 ～400 μg/ mL and quercetin 25 ～200 μg/ mL obviously increased OD value and cell viability in amphotercin B cell model, while protocatechualdehyde 50 ～400 μg/ mL and ursolic acid 1.25 ～10 μg/ mL up-regulated cholesterol 7alpha-hydroxylase mRNA expression in BRL cells. However, effects of quercetin on cholesterol 7alpha-hydroxylase mRNA expression and ursolic acid on CHO cell viability were not found. The results suggested that the decrease cellular cholesterol biosynthesis by protocatechualdehyde or quercetin and increase in conversion of cholesterol into bile acid by protocatechualdehyde or ursdic acid could lead to decrease cholesterol and low density lipoprotein-cholesterol levels in circulation, and they may have a synergism

P100013

Simvastatin inhibits plaque repture and subsequent thrombus formation in atherosclerotic rabbits with hyperlipidemia

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Atherosclerotic rabbits were performed by feeding high fat diet, then the rabbits were administered with simvastatin 1 mg·kg^{-1}·d^{-1} for 4 weeks, the rabbit atherosclerotic plaque rupture and thrombosis were triggered by injection of RVV and histamine. Results indicated that surface area covered by thrombus was 0.9 ± 1.1 mm^2 in control group, 78 ±53 mm^2 in model group, and 17 ± 12 mm^2 in simvastatin-treated group. Arterial plaque in model group showed obvious ulcers occurred and inflammatory cells infiltrated in shoulder area of plaque. Fibre cap on plaque in simvastatin-treated group was more thick and integrant than that in model group, and inflammatory cell infiltration was also decreased. Contents of cholesterol in abdominal aorta and TXB2 in thoracic aorta were decreased by 45.8 % and 24.2 %, respectively, while level of 6-keto-PGF1α and ratio of 6-keto-PGF1α/TXB2 in aorta were significantly increased. MMP-2 mRNA in abdominal aorta expressed less in simvastatin-treated group than in model group. These results suggested that simvastatin could increase plaque stability and inhibit thrombosis.

P100014

Study of some antioxidant enzymes and NO/NOS relationship on experimental hyperlipidemia rats after selerium and/or Vitamin E treatment

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OBJECTIVE: To investigate the effects of selenium (Se) and/or vitamin E (VE) on the relationship of some antioxidant enzymes and NO/NOS of heart, liver, kidney and serum in experimental hyperlipidemic rats. METHODS: High-fat-diet (HFD) was used to make experimental hyperlipidemic rats. In general, SD rats, divided into 5 groups: control; HFD; HFD + 0.4 mg/kg Se; HFD + 250 mg/kg VE; HFD + 0.4 mg/kg Se + 250 mg/kg VE, resepectively. The SOD, GSH-Px, CAT, NOS and NO activities or contents in hearts, livers, kidneys and serums were assayed by their assaying kits. RESULTS: SOD, GSH-Px and CAT activities were differently reduced in all the experimental tissues while NO contents and NOS activities reduced in heart and liver but increased in serum and kidney by HFD. Meanwhile, VE and/or Se can fight against or increase the SOD, GSHPx, CAT activities and NO contents in all the experimental tissues and increase and NOS activity in heart, liver and kidney, combined use of Se and VE were more effective. CONCLUSION: The effects of selenium and/or vitamin E on some antioxidant enzymes by HFD were related to the changes of NO and NOS.

KEY WORDS: selenium; vitamin E; antioxidant enzymes; NO/NOS

P100015

Taurine protects against low-density lipoprotein-induced endothelial dysfunction by DDAH/ADMA pathway

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Objective: To explore the involvement of dimethylarginine dimethylaminohydrolase (DDAH)/asymmetric dimethylarginine (ADMA) pathway in protective effect of taurine on endothelium. Methods: In vivo, endothelial dysfunction was induced by native LDL (4 mg/kg, i.v.). In vitro, damage of human umbilical vein endothelial cells (HUVECs) was caused by incubation with ox-LDL (100 μg/ml) for 24 h. Results: Pretreatment with taurine in vivo (60 or 180 mg/kg) significantly attenuated the reduction of endothelium-dependent vasorelaxation and NO level, and the elevated levels of ADMA, malondialdehyde (MDA) and tumor necrosis factor-alpha (TNF-alpha) induced by native LDL. In HUVECs, taurine (1 or 5 μg/ml) markedly attenuated the elevated levels of lactate dehydrogenase (LDH), ADMA, TNF-alpha and MDA, and inhibited the decreased level of NO and activity of DDAH induced by ox-LDL. Conclusion: taurine protects against endothelial dysfunction induced by native LDL in vivo or by ox-LDL in endothelial cells, and the protective effect of taurine on the endothelium is related to the decrease of ADMA level by increasing DDAH activity.

Key words: Asymmetric dimethylarginine; Endothelial cell; Low-density lipoprotein; Taurine

P100016

Balance between pro-and anti-inflammation cytokines of atherosclerosis induced by immunological and inflammatory stimulations in rats

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To observe the changes of rats aorta induced by immunolgic and inflammatory stimulation and probe cytokine of the aorta with Cytokine Antibody Array. To induce foam cell formation in the aorta of rat by repeat intraperitoneal injections zymosan every 4 days for 8 weeks and ovalbuimn, 2.5 mg/kg BW every one week for 5 times after initial subcutaneous sensitization simultaneously. All rats were feeded with a cholesterol-rich diet including control. Transmission Electron Microscope, Cholesterol Test Kit, Turbidimetry of Polyethylene Glycol and RayBio Rat Cytokine Antibody Array I were used to detect ultrastructural changes of aorta, serum total cholesterol (TC) levels, the changes of cytokines respectively. The ultrastructural changes were characterized by monocytes and smooth muscle cell migration with phagocytize lipid granule after 8 weeks. The TC levels were significantly higher than control ($p < 0.05$). Proinflammation cytokines for example IL-6, etc. increased during the process of AS. In conclusion The balance between pro- and anti-inflammation cytokines play an important role in immunological and inflammatory mechanism of AS.

Key Words: atherosclerosis, immunology, inflammation, Antibody Array

P100017

Effects of PNS on the formation of atherosclerosis in rabbits

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Panax notoginseng saponins (PNS) are the principal ingredient extracted from the traditional Chinese herb medicinal Panax notoginseng which has extensive effects on cardiovascular system, including atherosclerosis (AS), while the mechanisms are not clear. We therefore researched possible mechanisms of PNS in hypercholesteraemia rabbits. Methods: We measured the areas of AS in aorta, plasma TNF-α, total cholesterol (TC), triglyceride (TG), activity of postheparin Lipoprotein lipase (LPL) in hypercholesteraemia rabbits and hypercholesteraemia + PNS rabbits. Results: With the level of TNF-α decreased remarkable, the level of TC, TG, areas of AS decreased significantly in hypercholesteraemia + PNS group compared with hypercholesteraemia group, while the activity of LPL increased. These results demonstrated a possible association of increased postheparin LPL activity with AS inhibition role of PNS, through down-regulated the expression of serum TNF-α

Key words: atherosclerosis, Panax notoginseng saponins
This study was supported by NCF of China .№30470465, 30371768

P100018

Chronic Systemic Inflammation Accelerate the Formation of Atherosclerosis in Hyperchdesterolemic Rabbit

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Aim: It was well known that inflammation plays an very important role in the formation of atherosclerosis. This research was designed to testify whether chronic systemic inflammation can accelerate the formation of atherosclerosis plaque in hypercholesterolemic rabbit models. Methods: Created the inflammation adding hypercholesterolemic rabbit models by giving animals celiac injection of 10 mg/kg zymosan A (SIGMA) everyday. Tested the level of plasma cholesterol, LPL (lipoprotein lipase) and HL (hepatic lipase). Serum TNF-α was measured with ELISA. Hepatic mRNA for HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase was determined with RTPCR. Results: Treatment group have significantly increased area of atherosclerosis plaque in thoracic aortic vessels ($p < 0.01$). Treatment group have the elevation of serum TNF-α level ($p < 0.05$) and plasma concentrations of cholesterol ($p < 0.05$) and triacylglycerol ($p < 0.05$) in treatment group compared with control group. Conclusion: The increased area of atherosclerosis plaque in treatment group supports the importance of inflammation in atherosclerosis. The change of activation of LPL and HL and hepatic levels of mRNA for HMG-CoA reductase suggested the various effects of inflammation. A conclusion can be induced by the results that chronic inflammation can accelerate the formation of atherosclerosis by interfereing the metabolism of cholesterol through cytokines signal transport pathway.

Project supported by the grants from National Natural Science Foundation of China, No: 30470465 and 30371768

P11. Cardiovascular Pharmacology - Others

P110001

Cardiovascular Effects of Vitexin

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Vitexin isolated from prosopis farcta in a dose of 10^{-6} - 10^{-5} M/1dose dependeut positive inotropic effect in rabbit isolated atria which around 70 % of dopamine to on inotropic effect A comparative studies on diuretie activity of Furosemide vitexin has shown 90 % effect of this diuretic in both animals and humans in addition vitexin 0.5 mg/kg produced prolonged antihypertensive effect in mild chronic antihypertensive patients inboth sex compared with candesartan these effect not blocked by either B-antagonist or Antimuscarinic agents.

P110002

Drug Induced Long QT Syndrome and Triggered Cardiac Arrhythmias : Importance of biomarkers for Abnormal ventricular Repolarization

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We evaluated the relationship between drug-induced TdP and repolarization biomarkers in drug-induced long QT. Method and Results : In Langendorff-perfused rabbit hearts, dofetilide (0.001 to 0.1 M), a selective inhibitor of Ikr mimicking long QT2, increased the duration, reverse-use dependence, triangulation and instability of the action potential in a concentration-dependent manner and elicited early afterdepolarizations (EAD) in 4 out of the 6 hearts and TdP in 2 out of the 6 hearts. In anaesthetized male dogs with reduced ventricular repolarization reserve by dofetilide (0.05 mg/kg iv), HMR 1556, a selective inhibitor of Iks (0.25 + 0.5 mg/kg iv), markedly prolonged QTcV by 81 % and APD90 by 42 %, significantly increased Tpeak-Tend by 294 %, interventricular dispersion by 518 % and instability of QT by 169 % from baseline. These amplified biomarkers of ventricular repolarization in dogs were associated with 100 % incidence of EADs and 20 % incidence of TdP.

Conclusion : Drug-induced LQT2 is associated with marked biomarker increases in the abnormal ventricular repolarization. Based on these data, reduction in these repolarization biomarkers may be an important target for the prevention of TdP in LQT2.

P110003

The effects of various forms of estrogen on platelet aggregation induced by adrenaline and adenosine diphosphate.

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The impact of estrogens on the cardiovascular system and their ability to regulate platelet functions are remains controversial. Here, we investigated the effects of various forms of estrogen on platelet aggregation. Platelet rich plasma (PRP) was prepared from healthy volunteers. The study on platelet aggregation was assessed by using microplate reader. PRP was pre-incubated with 1, 10 and 100 nM of E_1, E_2 and E3 at 37 °C for 20 minutes and, then, co-incubated with normal saline, adrenaline (ADR) or adenosine diphosphate (ADP). Platelet aggregation was, then, measured every minute until 8 minutes. All forms of estrogen did not affected on platelet aggregation in untreated PRP. Only E1 and E3 can synergize the increased platelet aggregation by either ADR or ADP while the effects of E_2 on the increased platelet aggregation by either ADR or ADP depended on endogenous estradiol and platelet aggregated state. Thus, the rational use of these internal factors for estrogen used in clinical application, such as hormone replacement therapy, to evaluate thrombotic risk may have roles.

Acknowledgement : This study was support by Prasert Pasartthongosot Research Scholarships from Thai Medical Association.

P110004

Thrombolytic Effects of recombinant nattokinase on coronary thrombosis in miniature swine

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OBJECTIVE To study the thrombolytic effects of r-NK on coronary thrombosis induced by electrical stimulation in Chinese experimental miniature swine. METHODS Endoarterium was injuried and coronary thrombi were formed gradually through direct electrical stimulation on the coronary artery. Artery rechannelization was analyzed by coronary artery angiograph and multiple media graphic analysis. The experiments adopted epicardiogram mapping to measure the scope and degree of myocardial ischemia. And the size of myocardial infarction, serum creatine phospholkinase-MB (CK-MB) activity were detected. RESULTS RNK 0.25 - 0.5 mg kg^{-1} could improve coronary thrombolysis, lessen the thrombi area, and affiliate artery rechannelization. Furthermore, r-NK could alleviate the degree of myocardial ischemia (ST), narrow the ischemic area and inhibit the CK-MB activity. CONCLUSION R-NK could inhibit coronary thrombosis induced by electrical stimulation, improve thrombolysis, and alleviate myocardial damage subjected to ischemia reperfusion after artery rechannelizaiton.

Key words : recombinant nattokinase; miniature swine; coronary thrombosis; thrombolytic therapy

P110005

Identification of Amino Acid Residues Important for Sarpogrelate Binding to the Human 5-Hydroxytryptamine2A Serotonin Receptor.

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The purpose of this study was to examine the 5-HT2A receptor-Sarpogrelate interactions by site-directed mutagenesis. Based on molecular modeling studies, Aspartic acid (Asp) 3.32 and Tryptophan (Trp) 3.28 in the helix III and Trp6.48 in the helix VI of the 5-HT2A receptor were found to interact with Sarpogrelate. All of these residues were mutated to alanine (Ala). Asp3.32Ala and Trp3.28Ala mutants showed a markedly decrease in the binding affinity for [^{3}H] Ketanserin. So, it was not possible to find any Sarpogrelate affinity to the mutants using [^{3}H] Ketanserin. They also abolished 5-HT-stimulated formation of inositol phosphates (IP). On the other hand, Trp6.48Ala showed reduced binding affinity for both [^{3}H] Ketanserin (Kd 2 nM vs. 0.8 nM for native) and Sarpogrelate (pKi 5.71). It also showed the greatest decrease in sensitivity to Sarpogrelate (pKb 1.87) in inhibiting 5-HT-stimulated IP formation. These results provide direct evidence that Asp3.32, Trp3.28 and less importantly, Trp6.48 are responsible for the interaction between 5-HT2A receptor and Sarpogrelate. This research was supported by a grant from the promotion and Mutual Aid Corporation for Private Schools of Japan.

P110006

Effect of L-NAME on blood pressure regulation in mice overexpressing the adrenomedullin receptor component RAMP2

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Adrenomedullin (AM) is a vasodilator peptide, which acts via the AM_1 receptor, that comprises a seven transmembrane domain calcitonin receptor like receptor (CL) which interacts with one of three single transmembrane proteins termed receptor activity modifying protein1 (RAMP) 2. Overexpression of RAMP2 in C57BL/6 mice has no effect on basal blood pressure (BP) but enhanced the hypotensive and vascular relaxant responses to acute AM compared to wild type controls (WT). The aim of this study was to investigate the possible involvement of NO in the control of BP using RAMP2 overexpressing (TG) mice. BP was measured for 15 min prior to and 30 min after administration of L-NAME (10 mg/kg, i.p.) by tail cuff plethysmography. L-NAME induced a significant increase ($p < 0.01$) in BP in TG mice compared to vehicle-treated TG mice whereas no significant difference was observed in L-NAME vs. vehicle treated WT mice. These results suggest that the AM_1 receptor (CL/RAMP2) can influence BP in an NO-dependent manner.

Key words : mouse, BP, adrenomedullin

CT and NC are funded by the British Heart Foundation.

P110007

A new myocardial ischemia model in mini-pigs

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Objective: To investigate the preparation of myocardial ischemia model by using cardiac catheter to intervene thrombus in Chinese experimental mini-pigs. Method: Myocardial ischemia model of Chinese experimental mini-pigs were prepared by injection self-thrombus in the left anterior descending coronary artery(LAD), using guiding catheter through Carotid Artery. Coronary embolism, 30 dots body surface electrocardiogram, quantitative histology and hemodynamics of model animals were observed. Result: After 6 days, Model animals were embolized in the LAD, the extent and dot of ST segment raising in body surface electrocardiogram were obviously increase and they had large area myocardial infarction, the cardiac output(CO), stroke volume(SV), left cardiac work(LCW) were apparently degraded, the systemic vascular resistance(SVR) was remarkably raised. Conclusion: It is the first time to prepare myocardial ischemia model of Chinese experimental mini-pigs by using cardiac catheter to intervene thrombus.

Key words: self-thrombus; intervention; myocardial ischemia model; Chinese experimental mini-pig

P110008

LY2821 Inhibits Proliferation of Rat Aortic VSMCs by Modulating Cell Cycle Regulators

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The present study was designed to investigate the effects of LY2821on proliferation of rat aortic VSMCs in vitro. LY2821 potently inhibited the growth of rat aortic VSMCs and DNA synthesis induced by 5% FBS and 50 ng/ml PDGF-BB in a dose-dependent manner. To elucidate the inhibitory mechanism of VSMCs growth, cell cycle progression, apoptosis and signaling pathway were also investigated. LY2821 shows no effect on FBS- and PDGF-BB-induced intracellular early signal transductions such as ERK1/2, Akt and PLG-γ1. LY2821 blocked the FBS- and PDGF-BB-induced progression through G0/G1 to S phase of the cell cycle in synchronized cells without apoptosis. The expression of p27Kip1 in PDGF-BB-stimulated VSMCs inactivated cdk2 leading to G1 growth arrest. Taken together, these data suggest that LY2821 may inhibit the proliferation of rat aortic VSMCs proliferation by perturbing cell cycle progression, which may be due to the activation of p27Kip1 pathway. These results show that LY2821 may be developed as a potential antiproliferative agent for treatment of angioplasty restenosis and atherosclerosis.

P110009

Inhibitory Effect of Hesperetin, a Bioflavonoid, on Rabbit Platelet Aggregation

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In the present study, the antiplatelet activity of hesperetin was investigated in vitro and ex vivo. Hesperetin concentration-dependently inhibited washed rabbit platelet aggregation induced by collagen and arachidonic acid (AA), with IC_{50} of 20.5 ±3.5 and 69.2 ±5.1 μM, respectively, while has little effect U46619- or thrombin-mediated platelet aggregation, suggesting that hesperetin may selectively inhibited collagen-mediated signal transduction. Accordingly, hesperetin revealed blocking of the collagen-mediated PLG-γ2 activation, and caused a concentration-dependent decrease of arachidonic acid liberation, cytosolic calcium mobilization and serotonin release. It was also supported by the ex vivo platelet aggregation study that administration of hesperetin (100 mg/kg) potently inhibited collagen-induced platelet aggregation in rats. Furthermore, hesperetin inhibited AA-mediated platelet aggregation by interfering with COX activity as established by measuring the productions of TXA_2 and PGD_2 when arachidonic acid was added. Taken together, the present results provide a molecular basis for the antiplatelet activity of hesperetin, through inhibition of PLG-γ2 phosphorylation and COX activity.

Key words: Hesperetin; platelet aggregation; phosphdlipase C gamma2; cyclooxygenase

P110010

Effects of ethanolic extracts from Radix Morinda officinalis on the hemorheology and platelet aggregation in blood stasis rats

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Objective: Investigate the influence of ethanolic extracts from Radix Morinda officinalis(RMO) on the hemorheology and platelet aggregation in acute stress blood stasis rats. Methods: Acute stress blood stasis rats model was prepared by ice water stimulation and the main hemorheological indexes and the rate of platelet aggregation induced by ADP, COL and thrombin were detected. Three different concentrations (12,6,3 $g \cdot kg^{-1}$) of RMO were before given daily for five days via oral administration. Result: 12,6,3 $g \cdot kg^{-1}$ treatment of RMO not only significantly reduced the increase of whole blood viscosity at either high, middle or low shear rates, whole blood casson viscosity ($P < 0.01$) and Red-cell electrophoresis time ($P < 0.01$) but also restrained the rate of platelet aggregation ($P < 0.01$). In addition, 12,6 $g \cdot kg^{-1}$ treatment of RMO also decreased the whole blood reduction viscosity in acute stress blood stasis rats ($P < 0.01$, $P < 0.05$). Conclusion: RMO could significantly decrease the dense, viscosity, aggregation and coagulation in blood stasis rats, suggesting that it has the ability of blood-activating and tasis-eliminating.

Key words: RMO; blood stasis; hemorheology; platelet aggregation

P110011

Effect of aminoguanidine on inflammatory factor and neuronal apoptosis after focal cerebral ischemic injury in rats

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Objective To evaluate the effect of aminoguanidine(AG) on inflammatory factor and neuronal apoptosis after focal cerebral ischemic injury in rats. Methods Thirty male SD rats weighing 250-280g were randomly divided into three groups: 1. sham,ischemic and AG group. Focal cerebral ischemia was produced by middle cerebral artery occlusion (MCAO). The expression of TNF-αand the content of IL-1βwere measured. The Bcl-2 and Bax protein expression were also detected. Results The expression of TNF-αand the content of IL-1βand The percentage of apoptosis were markedly increased after MCAO. The expression of TNF-αand the content of IL-1βwere significantly lower in AG group than in IS group. The percentage of apoptosis cells and expression of Bax protein were markedly lower in AG group than in IS group. The expression of Bcl-2 protein was markedly higher in AG group than in IS group. Conclusion AG could inhibit the expression of TNF-αand the content of IL-1β, and protect neurons from apoptosis induced by focal cerebral ischemia through increasing the Bcl-2 protein expression and inhibiting the Bax protein expression.

Key words: Aminoguanidine; Brain ischemia; Apoptosis

P110013

INTERMEDIN AND RAMP1 EXPRESSION IS ATTENUATED BY ANTI-OXIDANTS IN A MODEL OF NITRIC OXIDE(NO) DEFICIENCY WITH CARDIOMYOCYTE HYPERTROPHY AND OXIDATIVE STRESS

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In myocardial ischemia and remodeling produced by NO synthase inhibition, up-regulation of adrenomedullin (AM) and receptor components, RAMP2 and 3, in hypertrophied cardiomyocytes is prevented by blood pressure (BP) lowering; that of RAMP1 and intermedin (IMD) is not. The hypothesis put forward is that IMD/RAMP1 is regulated by hypoxia and so we examined the effects of anti-oxidant intervention in this model. L-NAME(35 mg/kg/day) was given to rats for 8 weeks +/- Vitamin C + Tempol (each 25 mg/kg/day). Vitamin C/Tempol did not reduce systolic BP but in cardiomyocytes: (i) attenuated (by 42%) increased cell width and normalized expression of hypertrophic markers, sk-a-actin and ET-1, but not b-MHC, ANP or BNP; (ii) abolished a 3.6-fold increase in membrane protein oxidation and normalized expression of pro-oxidant NOX1, NOX2 (p22, p47) and anti-oxidant GPx, but not NOX2 (gp91) and SOD3; (iii) normalized expression of prepro-IMD and RAMP-1, but not prepro-AM, RAMP2 and 3. It is concluded that IMD/RAMP1 upregulation is induced by oxidative stress and so IMD may act in a negative feedback manner to reduce ischemic in-

jury and hypertrophic remodeling.
Intermedin/ RAMP1/ oxidative stress/ hypertrophy

P110014

effect of xanthotoxol on isolated guinea pig atria

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AIM: To study the physiological effect and its mechanism of xanthotoxol in the isolated guinea pig atria. METHODES: The contractile force of the isolated atria was determined by tension recording method. RESULTS: Xanthotoxol (XT) was isolated from the ethanol extracts of dried fruits of Cnidium monnieri. In the experiments on contractility of the left atria, XT (20, 40, 80 μmol L^{-1}) concentration-dependently decreased the contractile force. XT 80 μmol L^{-1} and Verapamil (Ver) 0.3 μmol L^{-1} significantly depressed the positive staircase phenomena, which was reversed by Ver but no by XT. However, the post-rest potentiation of myocardial contraction in the left atria was only markedly decreased by XT80 μmol L^{-1} but not by Ver 0.3 μmol L^{-1}. Furthermore, XT significantly reduced the sinus rates. XT 80 μmol L^{-1} not only attenuated the positive inotropic action but also delayed the following toxicity response induced by ouabain 0.2 μmol L^{-1} in the isolated left atria. CONCLUSION: XT decreased the contractile force and the spontaneous beats. XT not only blocked the voltage dependent calcium channel but also the receptor operated calcium channel in the isolated guinea pig atria.

P110015

Mechanisms of hypoxic vasoconstriction in the rat isolated basilar artery: role of Na-K-ATPase

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Objective: To explore the role of Na-K-ATPase in the basilar artery vasoconstriction under hypoxia. Methods: We measured the contraction of isolated rat basilar artery rings induced by KCl and U46619, a thromboxane A2 analog, using Multi Myograph System 610 M under hyperoxic (95% O2, 5% CO2) and hypoxic (95% N2, 5% CO2) conditions. Na-K-ATPase activity was assessed by test kit. Results: Vasoconstriction induced by KCl and U46619 was increased under hypoxia in dose- and time-dependent manner and reached the greatest response at 10 min after hypoxia. Pretreatment of ouabain (10-6 M), a Na-K-ATPase inhibitor, for 30 min attenuated the contraction induced by KCl under hypoxia. And both ouabain and K-free solution could reduce the hypoxic contraction caused by U46619. The Na-K-ATPase activity was decreased with prolonged anoxia, and also reached the lowest at 10 min after hypoxia. And after pretreatment of ouabain, the enzyme activity was further decreased. Conclusion: These results indicated that Na-K-ATPase was implicated in the hypoxic basilar artery vasoconstriction

Key words: Na-K-ATPase, hypoxia, basilar artery

P110016

Plasma 8-isoprostane is related to the extent of coronary stenosis in patients with coronary artery disease

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The purpose of the present study was to explore the relationship between 8-iso-prostaglandin F2alpha (8-iso-PGF2alpha) levels and the presence of coronary artery disease (CAD) and to also clarify whether 8-iso-PGF2alpha might add independently to measures of CAD extent. The study group consisted of 241 consecutive patients who were undergoing coronary angiography for suspected CAD. 8-iso-PGF2alpha levels were higher in the CAD(+) respect to the CAD(-) groups. A stepwise elevation in the 8-iso-PGF2alpha levels was found depending on the number of affected vessels ($P < 0.001$). The 8-iso-PGF2alpha levels showed a significant positive correlation with the numbers of >50% and >25% stenotic segments ($P < 0.001$) and the extent score of coronary stenosis ($P < 0.001$). The multivariate logistic regression analysis indicated 8-iso-PGF2alpha as an independent factor associated with CAD (odds ratio, 2.47; $P = 0.001$). The results suggested that 8-iso-PGF2alpha is associated with the presence of CAD in patients undergoing coronary angiography and is also related to the extent of coronary stenosis in Chinese population.

P110017

EFFECTS OF AMINOGUANIDINE ON THE EXPERIMENTAL CEREBRAL ISCHEMIC INJURY IN RATS

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Objecctives: To investigate the beneficial effect of aminoguanidine (AG) on cerebral ischemic injury in rats and the possible mechanism. Methods: The middle cerebral artery occlusion (MCAO) model was prepared. Gene expression of NOS after MCAO were examined by RT-PCR. The swelling, activity, NO, MDA, ATPase, SOD and GSH-Px in mitochondria were measured. LDH release, NO content, the cell viability by MTT stain and cellular morphology were used to evaluate the effect of AG. Results: Gene expression of iNOS was detectable only in the ischemia groups. The infarcted volume was significantly decreased in AG group. Administration of AG could ameliorate these injury induced by cerebral ischemia in rats. After ischemia, the swelling of mitochondria was markedly increased and the activity of mitochondria was decreased. The activities of ATPase, SOD and GSH-Px in mitochondria were markedly decreased, the contents of MDA and NO in mitochondria were markedly increased in MCAO rats. Administration of AG could inhibit the above changes. Administration of AG increased the cell viability and reduced the contents of LDH and NO. Conclusions: It may be concluded that AG have beneficial effect on ischemic cerebral injury.

P110018

Evidence for histamine as a neurotransmitter in the cardiac sympathetic nervous system

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The colocalization of histamine (HA) and norepinephrine (NE) immunoreactivities was identified within superior cervical ganglia neurons of the guinea pig. Coexistence of NE and HA was also visualized in the cardiac sympathetic axon and varicosities. Depolarization of cardiac synaptosomes with 50 mM K^+ stimulated endogenous HA release, which was not affected by Compound 48/80. Furthermore, K^+ evoked HA release was abolished by ω-conotoxin, but was not affected by lacidipine. Cardiac synaptosome HA exocytosis was augmented by the enhanced synthesis of HA or the inhibition of HA metabolism. HA H_3-receptor activation inhibited high K^+-evoked histamine release. The K^+-evoked endogenous NE release was attenuated by preloading the cardiac synaptosomes with L-histidine or quinacrine. These inhibitory effects were reversed by thioperamide or antagonized by α-fluoromethylhistidine. Our findings indicate that high K^+-evoked co-release of NE and HA may be inhibited by endogenous HA via activation of presynaptic HA H_3-receptors. The H_3-receptor may function as an autoreceptor, rather than a heteroreceptor, in the regulation of sympathetic neurotransmission, and HA may be a novel sympathetic neurotransmitter.

P110019

Morphological and pharmacological characterization of histamine in cardiac sympathetic nerve system of macaca mulatto monkey

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Objective To study the morphological and pharmacological characterization of histamine (HA) in cardiac sympathetic nerve system of macaca mulatto monkey. Methods Observed the co-localization of HA and norepinephrine (NE) in superior cervical ganglion (SCG) with double-labeled immunofluorescence, and detect the release of HA from the cardiac synaptosomes using ELISA. Results Co-localization of HA and NE was identified in the same neuron in SCG. Release of HA from synaptosomes with 50 mmol/L K^+ depolarization was detected. The release was Ca^{2+}-dependent and inhibited by ω-conotoxin, augmented by L-histidine and quinacrine. The K^+-evoked HA release was attenuated by HA H_3-receptor agonist (R)-α-methylhistamine, and the antagonist thioperamide inversed the effect of (R)-α-methylhistamine. Conclusions It reveals the further evidence for HA probably as a newly discovered neurotransmitter in cardiac sympathetic nerve system

Key Words: Histamine; Histamine H_3 receptor; sympathetic nerve system
Acknowledgement This work is supported by The Natural Science Foundation of

P. R. China (30300104). We would like to thank Prof. Jingshan Zhang and Mrs. Chen Dan for excellent technical assistance.

P110020

Anti-inflammatory Effect of fungus garden from odontotermes formosanus shirak

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Objective Study termite fungus garden with to resist inflammation result extract, offer science reliable experiment basis for confirming resisting inflammation function of termite's fungus garden. Methods Adopt two first cumbersome to cause little mouse auricle swelling, it is little for glacial acetic acid to bring out mouse abdominal cavity inflammation ooze out, cotton ball plant into, cause big mouse granulation organize hyperplasia and horn block up dish glue cause little mouse foot swelling model, observe resisting inflammation function of different extracts Results ABC three component have obvious result, B component level dosage and capillary penetrating function prominent to auricle swelling separately among them, suppressing rate is 64 %, 46 % and 53 %, 49 % respectively, the level dosage of group C is prominent to the swelling of the auricle and swollen function of granulation, suppressing rate is 67 %, 29 % and 24 %, 7 %. Conclusion Termite's fungus garden has obvious resisting inflammation function.

P110021

Mechanisms of growth inhibitory effects of lercanidipine on rat vascular smooth muscle cells

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Lercanidipine is a new third-generation lipophilic dihydropyridine L-type calcium channel antagonist with long duration and high vascular selectivity. The objective of this study was to investigate lercanidipine might be efficient for inhibiting the proliferation of rat vascular smooth muscle cells (VSMCs) in vitro and restenosis after balloon angioplasty in vivo. Lercanidipine inhibited VSMCs proliferation as demonstrated using trypan blue and XTT assays. Furthermore, lercanidipine appeared blocking of the FBS-inducible progression through G0/G1 to S phase of the cell cycle in synchronized cells. Lercanidipine dose-dependently reduced intracellular calcium in PDGF-stimulated VSMCs. In addition, lercanidipine inhibited the levels of phosphorylated extracellular signal-regulated protein kinase 1/2 (ERK 1/2) stimulated by FBS and PDGF. The levels of phosphorylated MAP kinase 1/2, the upstream of ERK 1/2, were also inhibited by lercanidipine. Besides, lercanidipine could significantly inhibit neointima formation following carotid artery injury by oral administration in the rat. Therefore, lercanidipine could be a viable strategy of the prevention of clinical restenosis. (Sponsor by NSC 94-2320-B-037-042)

P110022

Alprostadil protects against endothelin, cytokine with vascular restenosis

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Key words: alprostadil; balloon injury; endothelin; Cytokine

AIM: To evaluate the effects of alprostadil preventing RS after balloon injury and its mechanisms. METHODS Used a balloon injury to make the rat model of abdominal aorta endothelium injury. Three alprostadil dost (8、24、72 $\mu g \cdot kg^{-1}$) was injected via tail vein 5 days before operation, equal volume of normal saline was injected in model and sham groups. The blood samples were Collected after operation in each group at the 6th, 24th hour and 10th, 21st day. ET concentration of the plasma and IL-1β, IL-6, TNF-α concentration of the serum was measured by balanced method. RESULTS: Alprostadil could significantly decrease the plasma levels of ET ($P < 0.01$) in 24th hour groups. Compared with normal group, Alprostadil could dose-dependently decrease the maximum serum levels of IL-1β ($P < 0.01$). CONCLUSIONS The reduction of the plasma levels of ET and the serum levels of IL-1β, IL-6 and TNF-α plays an important role in the protective mechanisms against vascular RS of alprostadil.

P110023

Effect of SN-6, a novel benzyloxyphenyl derivative NCX inhibitor in cardiac ventricular myocytes

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We examined the effect of SN-6 on the NCX current and other membrane currents in isolated guinea pig ventricular myocytes with the whole-cell voltage-clamp technique. SN-6 suppressed the bi-directional NCX current in a concentration-dependent manner. However, SN-6 suppressed the unidirectional outward NCX current more potently than the inward NCX current. SN-6 and KB-R7943 suppressed the bi-directional NCX current more potentially at higher intracellular Na^+ concentrations. Intracellular application of trypsin via the pipette solution did not change the blocking effect of SN-6, implicating that SN-6 may not affect from the cytoplasmic side. Then, we checked the effects of 10 μM SN-6 on other membrane currents such as I_{Na}, I_{Ca}, I_{Kr}, I_{Ks}, I_{K1} and also on the action potential (AP). SN-6 inhibited I_{Na}, I_{Ca}, I_{Kr}, I_{Ks} and I_{K1} by about 10 %, 40 %, 30 %, 20 % and 10 %, respectively.

These results indicate that SN-6 inhibits NCX currents in a similar manner to that of KB-R7943. However, SN-6 affected other membrane currents less potently than KB-R7943 in guinea pig cardiac ventricular myocytes.

P110024

Herb-Drug Synergism: a Study of the Vasorelaxing Effects of Butylidenephthalide, a Constituent of Ligusticum chuanxiong, and Sodium Nitroprusside

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Prescription of an herb-drug combination is an ordinary practice in China. The aim of the present study is to examine the interaction between the vasorelaxing effects of butylidenephthalide (BDPH), a constituent of a common Chinese herb Ligusticum chuanxiong for cardiovascular diseases, and the NO donor sodium nitroprusside (SNP).

Vasorelaxation was examined using isometric force measurement in isolated rat aorta. BDPH and the L-type voltage-operated Ca^{2+} channel inhibitor nifedipine potentiated the SNP vasorelaxing response by 8 and 15-fold respectively (pEC_{50} comparison). BDPH and nifedipine applied together caused further augmentation to the SNP response by 3 to 4-fold. In the absence of extracellular Ca^{2+}, both BDPH alone and in combination with nifedipine potentiated the SNP response by 3-fold, while nifedipine alone produced no effect. A synergism between BDPH and SNP in causing vasorelaxation was observed. A general awareness of potential herb-drug interaction is much needed.

P110025

Losartan protects against myocardial ischemia-reperfusion injury via decreasing asymmetric dimethylarginine level in spontaneously hypertensive rats

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Objective: Elevated level of endogenous nitric oxide (NO) synthase inhibitor, asymmetric dimethylarginine (ADMA) is related to endothelial dysfunction in hypertension. We expored whether the improvement of endothelial function is involved in the protective effect of losartan on myocardial ischemia-reperfusion (I/R) injury in spontaneously hypertensive rats (SHR). Methods: Myocardial I/R injury was induced by 20 min of global ischemia and 30 min of reperfusion in isolated SHR hearts. Cardiac function was evaluated by left ventricular pressure and activity of creatine kinase in coronary effeluent. Endothelial function was reflected by acetylcholine-induced vasorelaxation. Results: In SHR, treatment with losartan (30 mg/kg) for 14 days signicficantly lowered blood pressure, elevated the plasma level of NO and decreased the concentration of plasma ADMA, concomitantly with improvement of endothelial function of thoracic aorta and restorement of I/R-induced cardiac dysfunction. Conclusion: losartan can improve endothelial function via decaresing level of ADMA in SHR, which may be involved in its protection against myocardial I/R injury.

Key wards: Hypertension; Losartan; Asymmetric dimethylarginine.

P110026

Comparision of Captopril and Enalapril in improvement of Endothelial Dysfunction induced by High Dieted Methionine

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AIM: To explore angiotensin-converting enzyme (ACE) inhibitors on endothelial dysfunction induced by methionine in rats. METHOD: 56 Male Sprague-Dawley rats were divided randomly into seven groups: Methionine, Captopril(15,30, 45 mg/kg), enalapril, N-acetylcysteine and control group. Drugs were administrated one time every day. After 30 days, endothelium-dependent (EDR) and non-dependent relaxation of thoracic aortic rings induced by acetylcholine and sodium nitroprusside and the biochemical index in plasma were examined. RESULTS: Methionine group inhibited Ach-induced EDR, decreased serum O level and activity of paraoxonase1(PON1) and SOD, increased serum MDA level, but had no effects on endothelium-independent relaxation compared with the control group. Treatment with captopril and enalapril attenuated inhibition of EDR, decreased MDA level, increased NO level and activity of PON1 and SOD compared with L-methionine group. CONCLUSION: Captopril exerted better effect than enalapril on endothelial dysfunction induced by methionine which may be related to scavenging oxygen free radicals and protection of PON1's sulfhydryl group.

Key words: ACE inhibitors, endothelial dysfunction, sulfhydryl group

P110027

Captopril Restores Endothelium-Dependent Relaxation Induced by Homocysteine-thiolactone in isolated Rat Aorta

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AIM:To explore effects of angiotensin-converting enzyme (ACE) inhibitors on endothelial dysfunction induced by homocysteine-thiolactone(HTL). METHODS: Endothelium-dependent (EDR) and non-dependent relaxation of thoracic aortic rings in rats induced by acetylcholine and sodium nitroprusside were examined. RESULTS: Exposure of aortic rings to HTL induced a significant inhibition of EDR, but not affected endothelium-independent relaxation. After incubation of aortic rings with captopril (3,10,30 umol/L), SOD and N-acetylcysteine prevented from the injuried of EDR caused by high HTL. Enalaprilat(3,10 μmol/L) had no difference with HTL about EDR, but enalaprilat(30 μmol/L) can restore the EDR response to HTL. Pretreatment with nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester and -SH group blocking agent phydroxymercurybenzoate blocked the protective of captopril and N-acetylcysteine. CONCLUSION: Captopril exerted better effect than enalaprilat against endothelial dysfunction by HTL which scavenged free radcals and have sulfhydryl group itself.

Key words: ACE inhibitors, endothelium-dependent relaxation, HTL

P110028

KMUP-1 displays relaxation effects on prostate via α1A-receptor blockade and enhanced expression of cGMP/PKG

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KMUP-1 has been demonstrated to raise cyclic nucleotides and to inhibit phosphodiesterases (PDEs). In receptor binding assay, KMUP-1 displayed a selective α1A-adrenoceptor blocking activity. In isolated rat prostate smooth muscles preconstricted with phenylephrine (10 μM), KMUP-1 (0.001-100 μM) also caused a concentration-dependent relaxation. This relaxation was attenuated by pretreatments with a soluble guanylyl cyclase inhibitor ODQ (10 μM), a PKG inhibitor Rp-8-pCPT-cGMPS (10 μM), a KATP channel blocker glibenclamide (1 μM), a voltage-dependent K^+-channel blocker 4-AP (100 μM), Ca^{2+}-dependent K^+-channel blockers apamin (1 μM) and charybdotoxin (100 nM). In rat prostate smooth muscles, KMUP-1 induced the expression of sGC and PKG proteins in a dose-dependent manner. KMUP-1 also augmented intracellular cyclic GMP levels, which was abolished in the presence of ODQ (10 μM). These results indicate that KMUP-1 activates sGC/cGMP/PKG pathway and selectively inhibits α1A-adrenoceptor, leading to the more relaxation efficacy on rat prostate, in comparison with other α1adrenergic blockers. KMUP-1 was suggested with potential clinical implications for the treatment of benign prostatic hyperplasia (BPH).

P110029

Novel anti-atherogenic lead compound-J18455

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Advanced glycosylation endproducts (AGE), formed by nonenzymatic reactions between carbohydrate and protein. Recent studies have demonstrated that AGE is involved in the pathogenesis of atherosclerosis (AS), diabetes, nurodegenerative diseases, renal failure, etc. Therefore, AGE has been proposed as therapeutic target for these diseases. We have established screening models for searching AGE inhibitors, breakers, receptor antagonists and have found an AGE breaker, J18455 (IC_{50} = 2ng/ml). Furthermore, we have also investigated anti-atherogenic effects of J18455 in rats fed with high cholesterol diet. The content of plasma cholesterol, triglyceride, LDL, HDL and SOD activity were recovered to control by treatment of J18455. Distinct AS plaques were formed in the thoracic aorta in model group, but the J18455-treated groups were not found AS plaques. Our results suggest that J18455 treatment can prevent the formation of AS and it could be presumed that AGE breakers may be benefit for the treatment of AS.

Key Words: Advanced glycosylation endproducts; Atherosclerosis; Lead compound

Acknowledgements: The study was supported by the National Natural Science Foundation of China (No. 30472015 and 30572182).

P110030

A NO/sGC/cGMP enhancer KMUP-1 reduces rat pulmonary artery hypertension, involving the inhibition activity on PKC and Rho kinase expression

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Pulmonary artery hypertension and increased pulmonary vascular resistance after cardiac surgery may increase morbidity and mortality. Reduced eNOS production and increased expression of Rho kinase and protein kinase C in pulmonary vessels have been implicated in the pathophysiology of pulmonary hypertension. In the present study, intraperitoneal and intravenous perfusion of KMUP-1 inhibited U46619-induced pulmonary artery hypertension and plasma oxygen consumption in rats. In isolated and U46619-preconstricted rat pulmonary arteries, KMUP-1 produced concentration-dependent relaxations. The ability of relaxations were reduced by pretreatement with PKC activator PMA, sGC inhibitor ODQ, nitric oxide synthase inhibitor, L-NAME, adenylate cyclase inhibitor SQ22536. Furthermore, KMUP-1 reduced Rho kinase and reversed the inhibited expression of eNOS. The relaxation effects of KMUP-1 on pulmonary artery might be mediated by the activation of NO/cGMP and inhibition of PKC/Rho kinase expression. KMUP-1 is suggested to be an effective therapeutic intervention for pulmonary anti-hypertension in the future.

P110031

Non-Steroidal Anti-Inflammatory Drugs antagonise the irreversible antiplatelet effect of aspirin

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Objective: To evaluate whether commonly used NSAIDs antagonise the antiplatelet effects of aspirin.

Methods: We assessed the effect of six NSAIDs (naproxen, ibuprofen, celecoxib, indomethacin, tiaprofenic acid and sulindac) on platelet function (PFA-100 epinephrine closure time [CEPI]), urine 11-dehydro-thromboxane B2 (TxB2), and urine 6-keto-prostaglandin F1alpha, in twelve healthy subjects in a multiple crossover study. The effect of each NSAID was assessed at the end of a twelve-hour dosing interval.

Results: At 12 hours post-dose, naproxen, tiaprofenic acid and sulindac significantly prolonged the PFA-100 CEPI closure time. Ibuprofen antagonised the antiplatelet effect of 300 mg of aspirin, mean CEPI 150s (95% confidence interval [CI] 123 to 178s), compared with 257s following aspirin + placebo (95% CI 207 to 307, $P = 0.03$). An interaction with aspirin also occurred with indomethacin ($P < 0.01$), tiaprofenic acid ($P < 0.05$) and naproxen ($P < 0.05$).

Conclusion: Naproxen and tiaprofenic acid have clinically significant antiplatelet activity at the end of a 12 hour dosing interval. Ibuprofen, indomethacin, tiaprofenic acid and naproxen antagonise the antiplatelet response to 300 mg aspirin.

Key words: aspirin, non-steroidal anti-inflammatory, interaction, platelets

Acknowledgements: Greenlane Research and Education Fund Board.

P110032

The regulation of norepinephrine on sodium pump activity and its α isoforms in guinea-pig ventricular myocytes

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The previous study has demonstrated that superfusion of ventricular myocytes (VM) with norepinephrine (NA) increases sodium pump current (IP). However, incubating VM with NA for 24 hours reduces the IP.

OBJECTIVE: To examine the molecular basis of the IP changes by NA in short term and long term regulation.

METHODS: The sodium pump activity was measured by using a coupled enzyme assay method. The expressions of α_1 and α_2 isoforms of sodium pump were evaluated by RT-PCR and Western blot. RESULTS: The activity of sodium pump was increased by incubation with NA for 10 minutes and was decreased for 24 hours. The α_1 isoform wasn't affected by NA in 10 minutes and 24 hours. The mRNA of α_2 isoform decreased when incubated with NA for 24 hours, which was abolished in the presents of prazosin and was not affected by the yohimbine. CONCLUSIONS: These results suggest that the change of the sodium pump activity is correspondent with the change of pump current and NA regulates the sodium pump activity by α_2 isoform through the α_1 receptor.

Key words: Na^+/K^+-ATPase; isoform; RT-PCR; Western blot

P110033

Asymmetric dimethylarginine modulates tissue factor coagulation pathway: role in acute coronary syndrome

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Objective: Endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) is an independent risk factor for cardiovascular diseases. We explored whether ADMA promotes acute coronary syndrome (ACS) via activating tissue factor (TF) coagulation pathway in monocytes. Methods: 113 patients with coronary artery diseases, including ACS (n = 77), and stable angina pectoris (SAP) group (n = 36), and 27 normal subjects were recruited. Human monocyte cell line THP-1 were treated with different concentrations of ADMA for various periods. Results: Plasma concentrations of ADMA and TF in patients with ACS were significantly higher than those in patients with SAP and in the control group. There were significant positive correlations between ADMA and TF as well as TF-mediated procoagulating activity, respectively. Treatment with ADMA significantly upregulated TF expression and increased TF-mediated procoagulating activity in THP-1 cells. Conclusion: ADMA increases procoagulating activity via upregulating TF expression in monocytes, which contribute to the development of ACS. (supported by postdoctoral funding from CSU)

Key words: Asymmetric dimethylarginine; Tissue factor; Acute coronary syndrome

P110034

Central α_7 nAChRs cardiovascular effects are mediated by vasopressinergic pathways in anaesthetized rats

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Rats (250-350g) were anaesthetised with α-chloralose (100 mg/kg; i.v.) neuromuscular blocked and artificially ventilated. The α_7 receptor agonist, PSAB-OFP (3 μmol/kg) given i.c.v. (n = 5) and i.c. (n = 5) evoked a significantly increased mean arterial blood pressure (MAP; 26 ±7 &38 + 8 mmHg) and renal nerve activity (RNA; 126 ±23 &130 ±30 %). In the presence of a V1 receptor antagonist (0.03μg/kg; n = 5) i.c.v. or i.c. these effects on RNA were blocked, although with i.c. administration PSAB-OFP caused an initial significant burst of RNA at 1 min (48 ±18 %). Furthermore for both routes of administration PSAB-OFP now evoked a significant decrease in MAP (10 ±2 and 11 ±2 mmHg). There was no evidence that V1 receptor antagonist had leaked out of the brain as the pressor response to i.v. vasopressin was unaffected. This data indicates that activation of central α_7 receptors causes a rise in MAP and renal sympathoexcitation due to central vasopressin release.

Supported by a BBSRC collaborative studentship with Eli Lilly Co. & Ltd, Surrey, UK.

P110035

Mechanisms of the relaxant effect of Danshen on rat isolated coronary artery

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This study investigates the actions of Danshen crude extract (*Salvia miltiorrhiza*) on rat isolated coronary artery rings precontracted with 1 μM 5-hydroxytryptamine (5-HT). Danshen produced similar concentration-dependent relaxation of the 5-HT-precontracted tone in intact and endothelium-denuded artery rings, and in those pretreated with a histamine H_2 receptor antagonist cimetidine (10 μM), a β adrenoceptor antagonist propranolol (100 nM), an adenylyl cyclase inhibitor SQ22536 (100 μM), a guanylyl cyclase inhibitor ODQ (10 μM), and a potassium channel inhibitor tetraethylammonium (TEA, 1 mM), but 10 mM TEA produced a significant right-ward shift of 2.2 fold on its concentration-response curve. Involvement of Ca^{2+} channels was investigated in artery rings incubated with Ca^{2+}-free buffer and primed with 60 mM KCl or 1 μM 5-HT for 5 min before adding $CaCl_2$ to elicit contraction. Pretreatment with 1 mg/ml Danshen or 100 nM nifedipine produced 80 to 100 % inhibition on the $CaCl_2$-induced contractions. These findings suggest the vasorelaxant effect of Danshen was produced by inhibiting Ca^{2+} channels and a minor component was mediated by the opening of K^+ channels.

Key words: Danshen; calcium channel, vaorelaxation; coronary artery

Acknowledgement: This research is supported by the Chinese University of Hong Kong.

P110036

Protective effect of Liuweidihuang Formula on the vascular endothelial cells from oxidative injury induced by oxidized low density lipoprotein

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Objective: To investigate the protective effects of Liuweidihuang Formula (LWDHF) on human umbilical vascular endothelial cells (HUVEC) injured by oxidized LDL (ox-LDL). Methods: The assay of the protective effects and mechanism of serum with LWDHF on injured HUVEC is based on the measurement of HUVEC proliferation ability by the use of MTT method and the HUVEC apoptosis rate with flow cytometry; meanwhile determining the level of malondialdehyde (MDA), the level of lactate dehyolrogenase (LDH), Nitric oxide (NO), as well as the activity of superoxide dismutase (SOD). Results: The rat serum with LWDHF increases the proliferation of HUVEC injured by ox-LDL and inhibits the apoptosis rate, it was also observed that the serum decreases the level of MDA, LDH and enhances the activity of SOD and the level of NO.

Conclusion: LWDHF could prevent vascular endothelial cells from oxidative injury induced by ox-LDL due to the antioxidant and inhibiting apoptosis properties.

Key word: LWDHF; HUVEC; ox-LDL

P110037

Studies of Preventive Action on Experimental Hyperlipidemia by 2,3-dioxoindoline

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Objective To study of preventive action of MW147 on the model of atherosclerosis in quail was established by hyper lipoidal forage. Methods The hyperlipidemia model in quails was induced exogenously by hyperlipoidal feed and oral administration of MW147 at the same time. The lipoid in serum was determined for 2, 5, 8 weeks after administration. Results MW147 20, 60, 120 mg.kg^{-1} signigicantly reduced serum TC、TG ($P < 0.05$, $P < 0.01$), and inhibited promotion of serum LDLC and apoB in varying degress ($P < 0.05$, $P < 0.01$). MW147 evidently rised the HDLC and apoA concentration ($P < 0.05$, $P < 0.01$). Conclusion MW147 have the preventive action of regulating lipicdemia on experimental hyperlipoidemia.

Key words: 2,3-dioxoindoline, Hyperlipidemia, Quail, Preventive action

P110038

Effects of myocardial ischemic injury on P2X3 receptor immunoreactivity and mRNA expression in rat stellate ganglia

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ATP is implicated in peripheral pain signaling by actions on P2X receptors. Little

is known about P2X3 involvement in cardiac nociception conditions. In the present study we have examined the changes of P2X3 expression in the stellate ganglion (SG) from naive rats and myocardial ischemic rat models. In the SG neurons of rats at 14 days after myocardial ischemic injury, the staining of P2X3 receptor appeared to be 219.87 ±7.59 (experimental group, n = 8) in the SG, more intense than those of naive rats, being 198.09 ±24.43 (control group, n = 8; p < 0.01) in the SG. The numerical density of neurons of the experimental group was higher than that of control group, being 2.51 ±0.15 and 5.79 ±0.26 (P < 0.01). The signals of P2X3 mRNA were 177.21 ±21.99 (experimental group, n = 7) and 148.52 ±32.12 (control group, n = 7; p < 0.01) respectively. The findings suggest that increased expression of P2X3 receptors in the SG may be implicated in the initiation or augmentation of cardiac nociceptive information.

Key words: P2X3 receptors, stellate ganglion, myocardial ischemia.

Acknowledgements: Grant supported by the National Nature Science Foundation of China (№30460040) to S. D. Liang.

P110039

Reduction in Superoxide Dismutases and Catalase Contributes to Oxidative Stress and Neurogenic Hypertension in Spontaneously Hypertensive Rats

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The present study assessed the hypothesis that augmented superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2) levels because of the reduction in superoxide dismutase (SOD), catalase (CAT) or glutathione peroxidase (GPx) in the rostral ventrolateral medulla (RVLM), where sympathetic premotor neurons are located, contribute to the pathogenesis of hypertension. We found that cupper/zinc SOD (SOD1), manganese SOD (SOD2) or CAT, but not GPx, mRNA or protein expression and enzyme activity in the RVLM of spontaneously hypertensive rats (SHR) was significantly lower than that in normotensive Wistar-Kyoto (WKY) rats, along with a significantly higher level of O^{2-} or H_2O_2. Microinjection of adenovirus encoding SOD1, SOD2 or CAT into the bilateral RVLM promoted a long-lasting reduction in arterial pressure in SHR, but not WKY rats; accompanied by an enhanced SOD1, SOD2 or CAT protein expression or enzyme activity and reduced O^{2-} or H_2O_2 level in the RVLM. These results suggest that downregulation of gene expression and enzyme activity of the antioxidant SOD1, SOD2 or CAT may underlie the augmented levels of O^{2-} and H_2O_2 in the RVLM, leading to oxidative stress and hypertension in SHR.

P110040

Phosphoinositide 3-kinase/Akt activates nitric oxide synthase II/peroxynitrite at rostral ventrolateral medulla during mevinphos intoxication

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The organophosphate poison mevinphos (Mev) induces cardiovascular toxicity via nitric oxide (NO) produced by NO synthase II (NOS II) in the rostral ventrolateral medulla (RVLM), the origin of sympathetic neurogenic vasomotor tone. We investigated the regulatory role of phosphoinositide 3-kinase (PI3K)/Akt signaling in this process. In Sprague-Dawley rats anesthetized with propofol, microinjection bilaterally of Mev into the RVLM induced an increase (Phase I) followed by a decrease (Phase II) in sympathetic vasomotor tone, alongside a progressive increase in Akt phosphorylation at Thr308 and Ser473, nuclear translocation of phospho-Akt, and NOS II or nitrotyrosine (an experimental marker for peroxynitrite) level in the ventrolateral medulla. Co microinjection bilaterally of PI3K inhibitors (Wortmannin or LY294002) into the RVLM significantly potentiated and prolonged the increased vasomotor activities during Phase I Mev intoxication, and blurted the augmented expression of phospho-Akt, NOS II or nitrotyrosine in the ventrolateral medulla. We conclude that PI3K/Akt signaling is upstream to NOS II/peroxynitrite expression in the RVLM during Mev intoxication.

Key words: mevinphos, NOS II, PI3K/Akt

P110041

Upregulation of nitric oxide synthase II by NF-kB via muscarinic receptor activation in rostral ventrolateral medulla during mevinphos intoxication

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The organophosphate poison mevinphos (Mev) elicits cardiovascular toxicity via nitric oxide (NO) produced by NO synthase II (NOS II) on activation of muscarinic receptors (MR) in the rostral ventrolateral medulla (RVLM), the medullary origin of sympathetic neurogenic vasomotor tone. The present study tested the hypothesis that the upregulated NOS II gene is induced transcriptionally by nuclear factor-kB (NF-kB), on activation of MR by the accumulated acetylcholine elicited by Mev in the RVLM. In adult Sprague-Dawley rats, co-microinjection of Mev and M2R antagonist or M4R antagonist significantly and dose-dependently suppressed the increase in DNA binding activity or nuclear translocation of NF-kB and surge in NOS II protein expression in RVLM, and alleviated hypotension, bradycardia or reduction in neurogenic sympathetic vasomotor activity during Mev intoxication. On the other hand, M1R antagonist or M3R antagonist was ineffective. We conclude that NO produced by NOS II, which is upregulated by NF-kB on activation of M2R and M4R by the accumulated ACh in the RVLM, underlies Mev-induced cardiovascular toxicity.

Key words: Mevinphos, NOS II, NF-kB

P110042

Heat shock protein 60 ameliorates cardiovascular fatality during experimental endotoxemia by an antiapoptotic action in rostral ventrolateral medulla of the rat

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The rostral ventrolateral medulla (RVLM) is the origin of a "life-and-death" signal that reflects central cardiovascular regulatory failure during brain stem death. Using an experimental endotoxemia model, we evaluated the hypothesis that heat shock protein 60 (HSP60) ameliorates cardiovascular fatality during brain stem death via an antiapoptotic action in the RVLM. Proteomic or Western blot analysis in Sprague-Dawley rats revealed a progressive decline in mitochondrial or elevation in cytosolic HSP60 in the ventrolateral medulla on intravenous administration of Escherichia coli lipopolysaccharide. Loss-of-function manipulations in the RVLM using anti-HSP60 antiserum or antisense hsp60 oligonucleotide exacerbated mortality by potentiating the cardiovascular depression during experimental endotoxemia, alongside intensified DNA fragmentation or augmented cytochrome c/caspase-3 cascade of apoptotic signaling in the RVLM. We conclude that HSP60 in the RVLM ameliorates fatal cardiovascular depression during endotoxemia via reduced activation of the cytochrome c/caspase-3 cascade of apoptotic signaling.

Key words: rostral ventrolateral medulla, heat shock protein 60, cardiovascular protection.

P110043

Sympathoexcitatory action of hypoxia-inducible factor-1/heme oxygenase-1 signaling cascade at rostral ventrolateral medulla in mevinphos intoxication

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The organophosphate poison mevinphos (Mev) induces its sympathoexcitatory phase (Phase I) of cardiovascular responses via nitric oxide (NO) produced by NO synthase I (NOS I) in the rostral ventrolateral medulla (RVLM), the origin of sympathetic vasomotor tone. This study evaluated the regulatory role of heme oxygenase-1 (HO-1) and its key transcription factor, hypoxia-inducible factor-1 (HIF-1), in this process. In Sprague-Dawley rats, significant hypoxia, along with nuclear translocation of HIF-1 and upregulated HO-1, heat shock protein 70 (HSP70), NOS I or protein kinase G (PKG) expression took place in the ventrolateral medulla during Phase I Mev intoxication. Pretreatment by microinjection of an anti-HO-1 antiserum or an antisense ho-1 oligonucleotide into the bilateral RVLM significantly blunted the augmented expression of HSP70, NOS I or PKG exhibited during this phase of Mev-induced increase in sympathetic vasomotor activities. Pretreatment with HO-2 antiserum or antisense ho-2 oligonucleotide, however, was ineffective. We conclude that HIF-1/HO-1 cascade regulates NOS I/PKG signaling via activation of HSP70 in the RVLM during the sympathoexcitatory phase of Mev intoxication.

P110044

Effects of lumbrokinase on thrombus and blood of experimental animals

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Aim To investigate the effects of lumbrokinase on the formation of thrombus of rats and blood biochemical parameters of rabbits and provide experimental evidences for clinical uses. Methods The effects of lumbrokinase on the weight of thrombus were observed by arteriovenous shut model in rats, and coagulation time of whole blood and the function of platelet were measured in healthy rabbits. Re-

sults Lumbrokinase could obviously decrease the weight of thrombus of rats in all groups. Lumbrokinase at the dose of 4 mg/kg could markedly inhibit platelet adhesion rate and aggression rate and prolong coagulation time of whole blood, however, the number of platelet was not influenced in rabbits. Conclusions Lumbrokinase could inhibit the formation of thrombus and the possible mechanisms were attributed to prolonging coagulation time of whole blood and affecting the function of platelet.

P110045

The probable pathway of low concentration of ouabain on intracellular calcium elevation in guinea ventricular mycytes

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AIM: The effects of low concentration of ouabain (OUA) on intracellular calcium concentration ($[Ca^{2+}]_i$) were investigated in guinea pig ventricular myocytes. METHODS: $[Ca^{2+}]_i$ was detected by confocal microscopy and represented by fluorescent intensity. RESULTS: OUA elevated $[Ca^{2+}]_i$ in a concentration-dependent manner, In Ca^{2+}-free Tyrode,s solution, which was lower than that in normal Tyrode,s solution. The effect of OUA 10-8 mol/l on $[Ca^{2+}]_i$ elevation was partly blocked by ryanodine (10-5 mol/l) in normal Tyrode,s solution, and completely blocked the elevation effects of OUA on $[Ca^{2+}]_i$ in Ca^{2+}-free Tyrode,s solution. OUA at low concentrations elevated the $[Ca^{2+}]_i$ in Na^+, K^+-free Tyrode,s solution to a similar degrece as in normal Tyrode,s solution. Genistein (GST) abolished the OUA-induced increases in $[Ca^{2+}]_i$ in a concentration-dependent manner, and 100 μmol/l GST can also abolished the elevation effects of both ryanodine 10-7 mol/l and BayK8644 on $[Ca^{2+}]_i$ in normal Tyrode, s solution. CONCLUSION: Low concentration OUA elevated $[Ca^{2+}]_i$ through tyrosine kinase pathway, invdlved in both intracellular and extracellular Ca^{2+} stores.

P110046

Effects of five stilbene compounds on the NO mediated vasodilation and their structure-activity relationship

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Objective To study the effects of five stilbene compounds, that is resveratrol (RES), diethylstilbestrol (DES), tetrahydroxystilbene-glucoside (THSG), trans-stilbene (TS) and stilbene water addition (SWA), on nitric oxide (NO)-mediate vasodilation and explore the structure-activity relationship. Methods In the rat thoracic aorta with and without endothelium, the vascular tension was observed. Results RES, DES and THSG (1～100 μmol·L^{-1}) could dose-dependently antagonize vessel contraction induced by phenylephrine (10 μmol·L^{-1}) with the potency of THSG > DES > RES. But TS and SWA (1～100 μmol·L^{-1}) could not markedly dilate vessel. The vasodilational effect of RES, DES and THSG could be strengthened by L-arginine (1 μmol·L^{-1}), while attenuated by methylene blue (1 μmol·L^{-1}). In addition, the vascular total NO content and NOS activity were increased by RES, DES Conclusion These indicate that diphenyl ethylene structure and existence of hydroxyl group in diphenyl are essential for vasodilational effect and the quantity and situation of hydroxyl group is important for their potencies.

Key words: stilbene, structure-activity relationship, NO

P110047

Asymmetric dimethylarginine inhibits intercellular communication in endothelial cells

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Objective: To explore the effects of endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine (ADMA), on intercellular communication in endothelial cells. Methods: Human umbilical vein endothelial cells were cultured and treated with different concentrations of ADMA (3-100 μM). Cell-cell communications were reflected by intercellular transmission of Lucifer yellow. Messenger RNA and protein expressions of connexin 43, one of the most important connexins expressed in endothelium, were determined by semi-quantitative RT-PCR, western blot and immunofluorescence, respectively. Results: Incubation of HUVECs with ADMA for 48 h concentration-dependently inhibited the cell-cell communication. Both mRNA and protein expressions of connexin 43 were decreased markedly in ADMA-treated endothelial cells. Conclusion: ADMA can inhibit the intercellular communication in endothelial cells, and this effect may be related to reduction of the expression of connexin 43 in endothelial cells.

Key words: Asymmetric dimethylarginine; Connexin 43; Endothelial cells

P110048

Involvement of DDAH/ADMA pathway in nicotine-induced endothelial dysfunction

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Objective: To determine the involvement of dimethylarginine dimethylaminohydrolase (DDAH)/asymmetric dimethylarginine (ADMA) pathway in nicotine-induced endothelial dysfunction. Methods: 18 smokers and 21 nonsmokers were recruited. Male SD rats were orally treated with nicotine (5 mg/kg/day) for 4 weeks. Human umbilical vein endothelial cells (HUVECs) were incubated with nicotine (10 μM) for 48 h. Results: The smokers had higher plasma levels of ADMA and von Willebrand factor than the nonsmokers. The level of ADMA was markedly increased in the nicotine-treated rats associated with a decrease in endothelium-dependent vasodilation. Nicotine caused a marked increase in the level of ADMA in HUVECs. Nicotine markedly downregulated both mRNA and protein levels of DDAH-II as well as DDAH activity in endothelial cells. The antagonists of alpha7 nicotinic acetylcholine receptor (alpha7 nAChR) blocked these effects of nicotine mentioned above. Conclusion: Nicotine modulates DDAH/ADMA pathway of endothelial cell via activation of alpha7 nAChR, which may be involved in endothelial dysfunction associated to smoking.

Key words: Asymmetric dimethylarginine; Nicotine; Endothelial function

P110049

Angiogenic potential of neural stem cells

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Neural stem cells (NSCs) are undifferentiated cells capable of both self-renewal and producing neurons and glial cells. They exist in various regions of the developing and adult central nervous system. We have shown that NSCs give rise to both endothelial cells and smooth muscle cells in vitro. NSCs were isolated from mouse embryonic day 12.5 (E12.5) cortex and cultured by neurosphere formation in serum-free medium in the presence of 20 ng/ml basic fibroblast growth factor (bFGF). To examine whether NSCs form vascular tube-like structures, NSCs were inoculated in collagen gels with 10% fetal bovine serum plus bFGF and incubated for 10 days. Vascular tube-like structures consisting of PECAM1- or VE-cadherin-immunoreactive cells were formed in the gels. Mbreover, the formation of vascular tube-like structures with a massive investment of alpha-smooth muscle actin-immunoreactive or GFAP-immunoreactive cells was occasionally observed. These results suggested that NSCs have a potential to form vascular tubes in vitro and perhaps in cerebral angiogenesis as well.

P110050

The effect of immmunsuppresor drugs of apo A and Apo B in renal transplant patients

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Transplantation is an optional treatment for end stage renal disease. Immunosuppresive drugs i.e Cyclosporine and prednisolone werw used after renal transplantation. Relationship of this drugs with lipid and lipoproteins level have been studied. Sixty four patients before and after transplantation for three months treatment with cyclosporine and prednisolon were elected and the level of apo A and apo B were measured. The result of study showed increase of apo B and decrease apo A levels in patients. Regarding this changes, we conclude that cyclosporine consumption could increase hyperlipidemia and cardiovascular risk in patients

P110051

Decreased release of endogenous CGRP release in nitroglycerin tolerance: role of ALDH-2

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Objective: To study the role of mitochondrial aldehyde dehydrogenase (ALDH-2) in reduction of endogenous calcitonin gene-related peptide (CGRP) release in nitroglycerin (GTN) tolerance. Methods: Tolerance was induced in vivo by pretreatment with GTN for 8 days in rats or in vitro by exposure of the isolated rat thoracic aorta and human umbical vein endothelial cells (HUVECs) to GTN. Results: GTN produced a depressor effect concomitantly with an increase in plasma CGRP, which was attenuated by pretreatment with GTN to induce tolerance or ALDH-2 inhibitor. Pretreatment with GTN or ALDH-2 inhibitor attenuated GTN-induced vasodilatation concomitantly with a decrease in the release of CGRP from the isolated thoracic aorta. Exposure of HUVECs to GTN increased the production of reactive oxygen species (ROS) and attenuated the activity of ALDH-2 as well as production of cGMP. Tolerance to GTN in HUVECs was restored in the presence of N-acetylcysteine or captopril. Conclusion: The reduction of endogenous CGRP release in GTN tolerance may be related to decreasing ALDH-2 activity by stimulation of ROS formation.

Key words: Nitroglycerin; Aldehyde dehydrogenase; Calcitonin gene-related peptide

P110052

The effects of crocetin on the primary culture of a cardiac myocytes injury induced by doxorubicin.

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AIM: To elucidate the protective mechanism of crocetin to rat myocardial cells injured by doxorubicin.

METHOD: Rat myocardial cells were used to investigate the cardiotoxicity of doxorubicin (DOX). The effects of crocetin on the activity of CPK, the MDA level, the depolarization of mitochondrial membrane potential (MMP) and the percentage of cardiac myocytes apoptosis were assayed. RT-PCR was used to examine mRNA expression of cytochrome c oxidase I (COI), COII, COIII and observed the effect of crocetin on the change. The crocetin effect on the oncolytic activity of DOX against SMMC-7721 and A549 cells in vitro were determined.

RESULT: Compared with the model group, crocetin could inhibit the MDA concentration dependently, relieve the decrease of MMP, inhibit the CK release, and decrease the cell apoptosis. Crocetin could descent the concentration and distribution of doxorubicin in the cardiomyocyte cells. The mRNA of COII was decreased, however no notable changes of COI and COIII in the model group. The crocetin had no effect on the oncolytic activity of DOX. CONCLUSION: Crocetin could reduce the cardiotoxicity of DOX.

Key words: Crocetin; Doxorubicin; Cell Culture; Cardiotoxicity

P110053

Effect of endothelium and cGMP on vasorelaxant effect of 17 βestradiol in human saphenous vein

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In this study the acute relaxant effects of 17 β-estradiol (E_2) and role of endothelium and cGMP on this effect has been investigated on human saphenous vein (HSV). Rings of HSV were prepared and equilibrated in Krebs solution under 3 g tension for 60 min. In the various experiments, the HSV rings were contracted by PGF2αor KCl. Afeter stabilized contraction, E_2 applied for 40 minutes in the presence or absence of endothelium and different inhibitors. Relaxation was expressed as percent reversal of contraction. E_2 elicited concentration-dependent relaxation of KCl and PGF2αinduced active tone in HSV rings. Incubation of veins with methylen blue or L-NAME reduced the relaxant effect of E_2 significantly. This reduction was disappeared by denuding endothelium. However, when intact tissues were incubated with indomethacin, KT5823, cycloheximide or puromycin the vasorelaxant effect of E_2 on PGF2αinduced contraction was not modified significantly. These results suggest that E_2 induces dose dependent relaxant effect in HSV, at least partially, by nitric oxide production and this relaxant effect is independent of cGMP, cycloxygenase or genomic pathways.

Key words: Human saphenous vein, 17βestradiol,

P110054

The characterization of the vascular effects of ghrelin on rat aorta.

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Objective: Ghrelin is a novel GH-releasing peptide, isolated from the rat stomach, The aim of this study is to characterize the direct effect of ghrelin on isolated rat aorta. Methods: Rats were injected with ghrelin to measure the mean arterial pressure (MAP). Ghrelin was tested for vasodilator effects on rat isolated aortae. Intracellular calcium ($[Ca^{2+}]_i$) level was determined by using microfluorometer. Results: Ghrelin injection decreased the MAP however it did not affect the phenylephrine, endotelin, or KCl-induced contractions in rat aorta. We have shown earlier that ghrelin stimulated cAMP production and inositol phosphate (IP) accumulation in rat aorta. Ghrelin has not changed the $[Ca^{2+}]_i$ levels despite the fact that it has increased the accumulation of IP. Activation of the two counteracting mechanisms could be the reason why no effects have been observed. Conclusion: GH-dependent mechanisms or supression of sympathetic activity or the direct effect of the ghrelin on cardiac functions can be the cause of ghrelin's vasodilator effect

Key words: Ghrelin, cAMP, $[Ca^{2+}]_i$
Acknowledgement: This study was supported by Gazi University Scientific Projects Foundation (Project code: 02/ 2005-17)

P110055

Synchronized oscillations of $[Ca^{2+}]i$ in endothelial and smooth muscle cells in rat mesenteric small arteries exposed to cyclopiazonic acid (CPA)

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The mechanisms leading to vasomotion in the presence of inhibitors of the SERCA pump were investigated in isolated rat mesenteric small arteries. Isometric force, membrane potential and confocal images of Ca^{2+} were obtained in smooth muscle (SM) and endothelial (ED) cells. During stimulation with noradrenaline, CPA induced oscillations of tone with a low frequency and high amplitude. The oscillations were unaffected by ryanodine but the amplitude was reduced by indomethacin and increased with L-NAME. The oscillations were inhibited by nifedipine, and the frequency increased about 3 times by removal of the ED, by charybdotoxin plus apamin. The oscillation of tone was associated with oscillations of membrane potential in ED and SM cells which were in phase and oscillations of Ca^{2+} which were in antiphase. The data suggest that inhibition of SERCA causes synchronization between ED and SM which leads to antiphase oscillations of Ca^{2+} in two cell types and thus oscillation in tone.

Key words: CPA, oscillation, membrane potential, artery.
Acknowledgments: K. Skaarup and J. Andresen for technical assistance, the Danish Heart Foundation for financial support.

P110056

Effects of evodiamine and rhynchophylline on Angiotensin II-induced Proliferation in rat Vascular Smooth Musde Cells

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To study the effects of evodiamine (Evo) and rhynchophylline (Rhy) on the proliferation in cultured rat's vascular smooth muscle cells (VSMCs), the growth-arrested VSMCs were stimulated with Angiotensin II (Ang II) 1.0 μmol L^{-1} and the proliferation of VMSC was evaluated by cell counting, MTT assay, the content of nitric oxide (NO) and the activity of nitric oxide synthase (NOS) were determined, and the expressions of c-myc mRNA, HRG-1 mRNA and cNOS mRNA were detected by RT-PCR. The results showed that additions of Rhy (3×10^{-7} to 1×10^{-5} M) or Evo (1×10^{-7} to 1×10^{-5} M) could significantly reduce the increasing cell number induced by Ang II by 16% to 38% or by 12% to 31%, respectively. At the same time, Rhy or Evo could decrease the elevating expression of c-myc mRNA, and increase the content of NO, average activity of NOS, and the expressions of cNOS mRNA and HRG-1 mRNA compared with the

control. It is concluded that Rhy and Evo can inhibit the proliferation of VSMCs stimulated by Ang II, which may be related to their activating effect on the activity of cNOS, resulting in the increasing formation of NO.

Key words : rhynchophylline ; evodiamine ; vascular smooth muscle cell ; proliferation

P110057

Inhibitory effects of isorhynchophylline on platelet aggregation and thrombosis

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For investigating the inhibitory effect of isorhynchophylline (Isorhy) on platelet aggregation and thrombosis, the model of mice administered (iv) with a mixture of collagen with epinephrine (C+E) and the rat thrombogenesis model of artery-vein bypass were used; In vitro, the rat's platelet aggregation induced by ADP and Thrombin (Thr) was examined, $[Ca^{2+}]i$, the thromboxane B2 (TXB2), cAMP and 6-keto-PGF1α in rabbit platelet were monitored. The results showed that Isorhy 5 and 10 mg kg^{-1} inhibited the thrombosis of the model, reduced the 5 min-mortality of the "C+E" treated animals at the doses of 50 and 100 mg kg^{-1}; Isorhy administered in vivo or in vitro inhibited the platelet aggregation induced by ADP and Thr; additions of Isorhy (0.65 and 1.3 mM) depressed the Ca^{2+} influx and the $[Ca^{2+}]i$ elevation induced by ADP and Thr, reduced the TXB2 generation induced arachidonic acids, increased the cAMP level and the 6-keto-PGF1α generation. It is concluded that Isorhy can inhibit the platelet aggregation and thrombosis, which may be related to its increasing effect on cAMP generation and inhibiting effect on $[Ca^{2+}]i$ and TXB2 generation.

Key words : isorhynchophylline ; platelet aggregation ; thrombosis

P110058

Cardiac Overexpression of Insulin-Like Growth Factor-1 Attenuates Senescence-Associated Cardiac Diastolic Contractile Dysfunction and Protein Damage

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Aging is accompanied with cardiac dysfunction and IGF-1 deficiency. We examined the effect of cardiac overexpression of IGF-1 on cardiac contraction in young (2 mo) and old (24 mo) mice. Contractile function was evaluated including peak shortening (PS), time-to-PS, time-to-relengthening (TR90) and maximal velocities of shortening/relengthening. Intracellular Ca^{2+} was measured by fura-2. Protein levels of advanced glycation endproduct (AGE), protein carbonyl, Ca^{2+} regulatory proteins phospholamban (PLB) and Na^{+}-Ca^{2+} exchanger (NCX) were assessed by Western blot. Aging prolonged TR90 and elevated resting intracellular Ca^{2+} without any other indices. Aged cells exhibited a steeper PS in response to enhanced stimulus frequency compared with young myocytes. IGF-1 attenuated aging-induced alterations with little effect in young mice. AGE and protein carbonyl were higher in aged mice which was not affected by IGF-1. NCX and PLB were decreased and enhanced, respectively by aging, which was ablated by IGF-1. Our data strongly suggest beneficial role of IGF-1 in aging-associated alterations of cardiac diastolic function and Ca^{2+} regulation protein.

Key words : IGF-1, myocytes, aging, cardiac contraction

P110059

MODULATION OF NITRIC OXIDE DONORS ON THE NA+/CA2+ EXCHANGER IN RAT ISOLATED AORTA

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The Na^{+}/Ca^{2+} exchanger (NCX) is a bi-directional transmembrane ion transporter that is involved in regulating the intracellular $[Ca^{2+}]$ in most tissues. Lowering the concentration of extracellular sodium ($[Na^{+}]o$) results in contraction of rat aortic rings by inducing Ca^{2+} inflow through NCX. Previous studies have suggested that in the presence of low $[Na^{+}]o$, nitric oxide is released from endothelial cells and inhibit NCX. The aim of the present study was to examine the effects of sodium nitroprusside (SNP) on low-$[Na^{+}]o$-induced contraction of endothelium-denuded aortic rings isolated from male Sprague-Dawley rats. 30 nM SNP produced a greater relaxation response in rings precontracted with low $[Na^{+}]o$ (1.18 mM) than thromboxane A2-mimetic U46619 ($n=5$, $P<0.05$) or 80 mM KCl ($n=5$, $P<0.001$). These results indicate that constriction by Ca^{2+} entry through NCX is highly sensitive to inhibition by nitric oxide which may explain why the endothelium dampens NCX mediated constriction.

P110060

TROWAGLERIX, A SNAKE VENOM PROTEIN FROM TROPIDOLAEMUS WAGLERI IS A POTENT PLATELET AGGREGATION INDUCER ACTING ON COLLAGEN RECEPTOR

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Trowaglerix (Tx), a snake venom protein with potent platelet-activating activity, was purified from Tropidolaemus wagleri venom. Under non-reducing condition, it migrates as a protein with mass more than 175 kDa protein on SDS-PAGE. Upon reduction, it exhibits two subunits with masses of 14 and 15 kDa, respectively. Tx induced platelet aggregation of human washed platelets and platelet-rich plasma in a dose-dependent manner (EC50 = 10.3 and 10.6 pM, respectively). PP2, piceatannol, and Wortmannin inhibited Tx-induced aggregation, indicating that activation of Src, Syk, and PI3K are involved in its activation process. By flow cytometric analysis, we found that Tx inhibited the binding of anti-GPVI mAb, but not GPIb mAb, toward platelets. Tx induced tyrosine phosphorylation of platelet lysates with a profile similar to that produced by collagen and convulxin, involving a time-dependent tyrosine phosphorylation of proteins including FcR γ-chain, Syk, Src, LAT, phospholipase Cγ2. Taken together, Trowaglerix is a heterodimeric multimer, which activates platelets mainly through acting on GPVI, leading to platelet aggregation.

Key words : snake venom protein, platelet aggregatopm, Glycoprotein VI agonist

P110061

Modulation of the nonadrenergic noncholinergic vasodepressor responses by alpha2-adrenoceptors in pithed rats.

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It is known that resistance blood vessels are mainly innervated by both sympathetic and sensory nerves, which modulate the resistance vascular tone through the release of norepinephrine and calcitonin gene-related peptide, respectively. Activation of sensory nerves results in a vasodepressor response that is termed nonadrenergic noncholinergic (NANC).

On this basis, the present study set out to investigate the possible role of alpha2adrenoceptors modulating the NANC vasodepressor responses produced by electrical stimulation. For this purpose, male Wistar pithed rats were given i.v. continuous infusion of hexamethonium (2 mg/kg.min) and methoxamine (15 μg/kg.min). Under these conditions, electrical stimulation (0.56-5.6 Hz) of the spinal cord (T_9-T_{12}) resulted in frequency-dependent decreases in diastolic blood pressure; these vasodepressor responses, which remained unaffected by an i.v. continuous infusion of saline, were significantly inhibited by clonidine (10 μg/kg.min). Since this inhibition was antagonized by rauwolscine (300 μg/kg, i.v.), our results suggest that activation of alpha2adrenoceptors located on sensory nerve terminals can inhibit the NANC vasodepressor responses.

P110062

Evidence for the presence of GPRC6A in the rat mesenteric artery

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GPRC6A is an "orphan", G protein-coupled receptor (related to the calcium sensing receptor) which is activated by basic amino acids, Al(3+) and the calcimimetic NPS-568 (see Pi et al., 2005, J Biol Chem 280:40201-9). The aim of this study was to investigate the possible presence of GPRC6A in rat mesenteric artery (RMA). In sharp micro-electrode recordings, L-ornithine (0.3 mM) produced an endothelium-dependent hyperpolarisation and potentiated the hyperpolarisation to the calcimimetic calindol (100, 300 nM). The effects of both L-ornithine and calindol were abolished by the IKCa channel inhibitor TRAM-34 (0.01 mM). Similar effects (TRAM-34 sensitive hyperpolarisation and potentiation of calindol effect) were produced by Al(3+) (0.1 mM). RT-PCR using mRNA extracted from RMA produced an amplicon of the predicted size, which was sequenced and confirmed as GPRC6A. Furthermore, the protein was also i-

demified by Western blot using a selective polyclonal antibody. We conclude that GPRC6A is present in RMA endothelial cells and may play a role in the regulation of vascular tone.

Funded by the British Heart Foundation

Key words: GPRC6A, IKCa channel, Calindol, Mesenteric Artery

P110063

Isoliquiritigenin, a flavonoid from licorice, relaxes guinea-pig tracheal smooth muscle in vitro **and** in vivo: **role of cyclic GMP and L-type Ca^{2+} channels**

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Aim: To evaluate the effects of isoliquiritigenin (ISL) on the responses to contractile agonists in guinea-pig tracheas and the mechanisms underlying these effects. Methods: The effects of ISL on muscle tone in vitro were studied by measuring isometric tension, while the effects on cytosolic Ca^{2+} concentrations were studied by measuring the spectra of fura-2 loaded in guinea-pig tracheal smooth muscle cells. In vivo the protective effects of ISL on bronchospasm induced by bronchoconstrictors was measured. Results: ISL induced concentration-dependent relaxation responses in guinea-pig trachea precontracted with Ach, which was attenuated by pretreatment with charybdotoxin. Relaxation response was also attenuated by ODQ, but not reduced by SQ 22536. ISL significantly prevented KCl-induced $[Ca^{2+}]i$ rise. In vivo experiment ISL significantly prolonged the latency time of intratracheal administration of histamine and Ach-induced collapse and inhibited the increase of lung overflow induced by intravenously histamine. Conclusion: These data indicate that ISL activates sGC and increases intracellular cyclic GMP, leading to the opening of K^+ channels and blockade of L-type Ca^{2+} channels and resulting tracheal relaxation.

Key words: Isoliquiritigenin; tracheal smooth muscle cells; cyclic GMP; L-type Ca^{2+} channels

P110064

The Effects of Bumetanide on Human Umbilical Artery Contractions

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Umbilical circulation is very important for normal fetal growth and viability. We have investigated in vitro effects of bumetanide, a loop diuretic and a Na-K-2Cl cotransport (NKCC1) inhibitor, on serotonin, histamine and KCl-induced contractions in human umbilical artery (HUA). Rings of HUA segments from vaginal deliveries with normal term pregnancies were suspended for isometric tension recording in organ baths. Cumulative concentration-response curves to serotonin (10^{-8} - 10^{-4} M), histamine (10^{-8} - 10^{-4} M) and KCl (5-80 mM) were performed in the absence (control) or in the presence of bumetanide (10^{-5} - 10^{-3} M). The contracting agents caused concentration-dependent contractions of HUA. Bumetanide pretreatment, concentration-dependently, decreased the sensitivities and maximal contractions of HUA to serotonin and histamine. The highest concentration of bumetanide, 10^{-3} M, inhibited the maximum contractions to serotonin and histamine, extent to approximately 60 %. This findings raises the possibility that NKCC1 may play a role in the regulation of the feto placental vascular tone.

Key words: human umbilical artery, bumetanide

P110065

CHARACTERIZATION OF SMOOTH MUSCLE RELAXATIONS TO NITROXYL ANION IN NITRERGICALLY INNERVATED TISSUES

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This aim of this study was to characterize relaxations to nitroxyl anion in nitrergically innervated tissues, in comparison with free radical nitric oxide (NO) and nitrergic nerve stimulation. Relaxant responses of isolated tissues from SD rats to nitroxyl anion donor Angeli's salt (AS) were recorded in vitro. AS produced a concentration dependent relaxation in anococcygeus, gastric fundus, urethra and corpus cavernosum, which was inhibited by the soluble guanylate cyclase (sGC) inhibitor ODQ, but unaffected by the NO-scavenger carboxy-PTIO. L-cystein significantly inhibited AS-induced relaxations in the rat anococcygeus and gastric fundus but not in the urethra. In the rat anococcygeus, AS-induced relaxation was inhibited by the myosin phosphatase inhibitor calyculin A, and enhanced by the Cu^{2+} chelator cuprizone, but was not affected by inhibitors of cytochrome P450, tyrosinase and mitochondiral complex II and III. The results indicate that sGC is important in mediating nitroxyl anion induced relaxations in nitrergically innervated tissues, although the bioactivation mechanism of AS remains to be elucidated.

P110066

Short and Long-Term Effect of Isoprenaline on Na^+, K^+-ATPase Expression in Guinea-pig Ventricular myocytes

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Our previous studies have demonstrated that guinea-pig ventricular myocytes acute or prolonged exposure to isoprenaline(Iso) can decrease and increase Na/K pump current (Ip), respectively, which are targeted to the α1 isoform of the Na^+-K^+ ATPase. The purpose of the current study was to characterize the molecular basis of the effect of Iso on Na,K-ATPase in guinea-pig ventricular myocytes. Methods: The expression of α1 isoform of Na^+,K^+-ATPase was evaluated by Western blot. Result: short term exposure(10 min) of Iso to isolated guinea pig ventricular myocytes decreased α1 isoform cell surface expression, without change in total α1 isoform levels. Long term exposure(24h) of Iso increased Na^+,K^+-ATPase α1 iso form expression. Propranolol abolished the above effects. Conclusions: These results suggested that altering the distribution and expression of α1 isoform may be the molecular basis of Iso affecting Na^+,K^+-ATPase activity.

Key words: Na^+/K^+-ATPase; Isoprenaline; Western blot.

P110067

Role of PKC-induced actin polymerization in the regulation of uterine artery contractility: effect of pregnancy

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Previous studies demonstrated that PKC-induced contractions of the uterine artery (UA) independent of changes in $[Ca^{2+}]i$. The present study tested the hypothesis that actin polymerization was a mechanism of PKC-induced UA contractions, which was downregulated by pregnancy. UAs were isolated from nonpregnant (NP) and near-term pregnant (P) sheep. PKC activator PDBu-induced contractions and actin polymerization were measured simultaneously in the same UAs with/without actin polymerization inhibitor cytochalasin B. PDBu-induced contractions were significantly higher in NPUA than PUA. Cytochalasin B inhibited PDBu-induced contractions in NPUA, but not PUA. The ratio of globular and filamentous-actin (G/F) levels in NPUA was significantly lower than that in PUA. Activation of PKC failed to affect the G/F-actin ratio in PUA, but decreased it in NPUA, which was blocked by cytochalasin B. In addition, immunohistochemical study showed that PDBu increased F-actin fluorescence density. In summary, this study has demonstrated that actin polymerization regulates PKC-induced contractions of the UA, and pregnancy attenuates the PKC-actin polymerization signal pathway. (Support by NIH HL57787 and TRDRP 14FT-0075)

P110068

Direct effects of estrogen and progesterone on PKC-mediated contractions of the uterine artery

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Previous studies demonstrated that PKC-induced contractions of the uterine artery (UA) decreased during pregnancy. The present study examined the direct effects of estrogen and progesterone on the adaptation of PKC-mediated contractions to pregnancy in UAs isolated from nonpregnant (NP) and pregnant (P) sheep. Tissues were treated with 17β-estradiol (E_2), progesterone (P_4), the E_2 inhibitor ICI 182780, and the P_4 inhibitor RU486 for 20 min (acute) or 48 hrs (chronic), and the PKC activator PDBu-induced contractions were determined. In acute studies, the hormones and the inhibitors had no effects on PDBu-induced contractions in NPUA or PUA. In chronic studies, E_2, P_4, or combination of E_2 and P_4, significantly inhibited PDBu-mediated contractions in NPUA. In accordance, ICI 182780 and RU486 significantly increased PDBu-induced contractions in PUA, and PDBu-induced contractions of PUA after treatment were not significantly different from that of NPUA. The results demonstrate a key role of the hormones in the downregulation of PKC-induced contractions of the UA during pregnancy, which is likely mediated by a direct genomic mechanism of the hormones. (Support by NIH HL57787 and TRDRP 14FT-0075)

P110069

Cl^--dependent DNA Synthesis Induced by Thrombin In Vascular Smooth Muscle Cells Role of Extracellular-Signal Regulated Kinase 1/2

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Thrombin-induced increase in DNA synthesis was dependent on Cl^-. We asked whether phosphorylation of ERK1/2 induced by thrombin was Cl^--dependent in vascular smooth muscle cells (VSMC). With 120 mEq/L of [Cl^-], thrombin (0.03 U/ml)-induced peak increase in ERK1/2 phosphorylation was greatly attenuated in 20 mEq/L of [Cl^-]. Thrombin-induced phosphorylation of MEK1/2 and Ras was also Cl^--dependent. No obvious change in morphology or mitochondria dehydrogenase activity was observed in VSMC with high vs. low [Cl^-]. In contrast, thrombin and A23187-induced Ca^{2+} transients were not dependent on Cl^-, suggesting Cl^- may act downstream of Ca^{2+} signaling. In addition, kinase activity of MEK1/2 was attenuated by 30% after replacing Cl^- with bicarbonate or gluconate; whereas protein expression of MKP-1, a phosphatase that dephosphorylates ERK1/2 and MEK1/2, was enhanced after replacing Cl^- replacement. Our results suggested that Cl^- may enhance ERK1/2 phosphorylation to enhance DNA synthesis in VSMC.

P110070

Protective Effect of Dauricine on Restenosis after Thoracic Aorta Balloon Injury in Rats

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Dauricine(Dau), a bisbenzylterrahydroisoquinoline alkaloid, has many pharmacologic effects, including antiarrhythmic and anti-ischemic effects. In order to investigate the effect of Dau on restenosis after artery balloon injury, we used a rat model of thoracic aorta balloon injury in vivo and an insulin-induced vascular smooth muscle cells (VSMC) proliferation model in vitro. Using these models, we observed the effects of different dosages of Dau. The thoracic aorta wall and intimal area morphology were examined by HE staining. Apoptosis of VSMC was measured by TUNEL assay. Protein and mRNA expressions of p27, bcl-2, and bax in cultured VSMCs were measured by immunohistochemistry and RT-PCR respectively. Dau significantly increases the apoptosis of VSMCs and markedly increases the expression of p27 and bax while bcl-2 expression is decreased in a dose dependent manner. Dau has a protective effect on restenosis after arterial endothelial injury by inhibition of the proliferation and enhancement of apoptosis in VSMC.

Key words: Dauricine; restenosis; apoptosis; vascular smooth muscle cell
Acknowledgement: SFC of Hubei province

P110071

Anthocyanins from soybean seed coat inhibit the expression of TNF-a-induced genes associated with ischemia/reperfusion in endothelial cell by NF-kB-dependent pathway

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Myocardial damage due to reperfusion of ischemic tissue is caused primarily by proinflammatory cytokine, tumor necrosis factor-alpha (TNF-a). We examined the inhibition of the expression of some inflammatory genes associated with ischemia-reperfusion (I/R) injury by anthocyanins isolated from black soy bean seed coat in TNF-a-treated bovine aortic endothelial cells. In addition, its potential use on I/R-injury was investigated using rats subjected to 30 min occlusion of left descending coronary artery followed by 24 h reperfusion. Western blot analysis and luciferase activity assay showed that anthocyanins inhibited TNF-a-induced VCAM-1, ICAM-1, and COX-2 levels, which is through NF-kB dependent pathway. Further, anthocyanins protected myocardiac injury from I/R in rats. It is suggested that anthocyanins from black soybean seed coat[(cyanidin-3-glucoside(72%), delphinin-3-glucoside (20%) and petundin-3-glucoside(6%)] can be used as useful drug to modulate cardiovascular disorder.

P110072

Nicotinic receptors in the dorsal facial area regulate carotid arterial blood flow in cats

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We determined nicotinic acetylcholine receptor (nAChR) subtypes in dorsal facial area (DFA) in the medulla that regulate common carotid arterial blood flow (CCABF) in cats. Microinjections of nicotine (a non-selective nAChR agonist) or choline (a selective α_7-nAChR agonist) into DFA elicited a dose-dependent increase in CCABF. Nicotine-induced CCABF increase was dose-dependently attenuated by prior microinjections of α-bungarotoxin and methyllycaconitine (α_7-nAChR antagonists), mecamylamine (a relatively selective $\alpha_3\beta_4$-nAChR antagonist) and dihydro-β-erythroidine (a preferential $\alpha_4\beta_2$-nAChR antagonist) in DFA. Choline-induced CCABF increase was dose-dependently attenuated by α-bungarotoxin and mecamylamine, but not by dihydro-β-erythroidine. Microinjections of muscarinic agonists did not affect the basal nor change the nicotine-induced increase in CCABF. In conclusion, α_7, $\alpha_4\beta_2$, and $\alpha_3\beta_4$ subunits of nAChR are present on DFA neurons. Activations of them increase CCABF. Muscarinic receptor on DFA are not involved in regulation of CCABF. These nAChR subtypes in DFA may be important in regulating CCABF.

Key words: carotid, cholinergic, parasympathetic, brainstem.

P110073

Peroxisome proliferators-activated receptor γ mediates proliferation of rat vascular smooth muscle cells induced by advanced glycation end products

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We investigated the effect of peroxisome proliferator-activated receptor gamma (PPARγ) on proliferation in rat vascular smooth muscle cells (VSMCs) induced by advanced glycation end products (AGEs). Primary cultures of VSMCs from aorta were exposed to AGEs of different concentrations (0, 50, 100, 200, 400 mg/L) and different times prior to co-treatment with pioglitazone, a PPARγ activator and AGEs. MTT assay was adopted for the quantification of the cell proliferation ratio and PPARγ expression was determined by RT-PCR and western blot. AGEs increased the proliferation of VSMCs (0.47 ± 0.01 vs 0.64 ± 0.10, 0.74 ± 0.09, 0.85 ± 0.06 and 0.82 ± 0.09 respectively, $P < 0.05$). AGEs treatment to VSMCs decreased mRNA and protein levels of PPARγ also in a time- and dose-related manner ($P < 0.05$). Pioglitazone increased PPARγ mRNA and protein levels and decreased the AGEs induced proliferation of VSMCs. Activating PPARγ in VSMCs, pioglitazone may play a role in antiatherosclerosis. The reduction in PPARγ expression may be implicated VSMCs proliferation and pathogenesis of atherosclerosis in patients with diabetes mellitus.

Key Words: peroxisome proliferator-activated receptor γ, advanced glycation end products, pioglitazone

P110074

Inhibition of inducible nitric oxide synthase (iNOS) augments cardiac contraction to dobutamine in rats with type 2 diabetes

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Cardiac contractile dysfunction is a common occurrence in type 2 diabetes. We examined if iNOS contributes to cardiac dysfunction in 20 week old Zucker diabetic (type 2) rats. Conscious Zucker diabetic and Zucker control rats (n = 7 per group) were studied after 24h recovery from halothane anesthesia and surgical preparation that involved insertion of catheters into the iliac arteries and veins, and the left ventricle (LV). Both groups had similar LV pressure (LVP) and +dp/dt. Dobutamine dose-dependently (1-30 μg/kg, min) increased LVP and +dP/dt in both groups; but the responses were less ($P < 0.05$) in the diabetic than control rats. Immunostainings (proteins) of iNOS and eNOS were higher in the hearts of the diabetic than control rats. Administration of 1400 W (iNOS inhibitor; 3 mg/kg and 3 mg/kg/h, i.v) did not alter responses to dobutamine in the control rats, but augmented ($P < 0.05$) the effects of dobutamine on LVP (but not +dP/dt) in the diabetic rats. Therefore, iNOS contributes to cardiac contractile dysfunction in Zucker diabetic rats.

Key words: diabetes, iNOS, cardiac contraction
Supported by the Heart & Stroke Fdtn of BC & Yukon and the Canadian Diabetes Assn.

P110075

Ferulic acid inhibits P-selectin expression and von Willebrand Factor secretion in stimulated endothelial cells

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Abstract: Objective To study the effects of ferulic acid (FA) on P-selectin expression and von Willebrand Factor (vWF) secretion in human umbilical vein endothelial cells (HUVEC). Methods HUVEC were pretreated by FA(0.62, 0.41,

0.21 mM) and activated by 300μM H_2O_2. The effects of FA on P-selectin expression and vWF secretion were detected by flow cytometry and sandwich enzyme linked immunosorbent assay respectively. Results The mean fluorescence intensity of P-selectin expression in FA(0.62, 0.41, 0.21 mM) treated HUVEC was lower than that of model HUVEC (without exception $P < 0.05$). The level of vWF in the culture supernatant in FA (0.62, 0.41, 0.21 mM) treated HUVEC was lower than that of model HUVEC ($P < 0.01$, $P < 0.01$, $P < 0.05$). Conclusions FA can inhibit expression of P-selectin and secretion of vWF in HUVEC activated by H_2O_2. This can contribute to its effects on prevention and treatment of thrombosis and ischemia-reperfusion injury.

Key words: ferulic acid, endothelial cell, P-selectin, von Willebrand Factor.

P110076

Coenzyme Q10 confers cardiovascular protection against mevinphos intoxication by ameliorating bioenergetic failure and hypoxia in rostral ventrolateral medulla of rats

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Coenzyme Q10 (CoQ10) is a highly mobile electron carrier in the mitochondrial respiratory chain. We evaluated the cardiovascular protective efficacy of CoQ10 at the rostral ventrolateral medulla (RVLM), where sympathetic vasomotor tone originates and where the organophosphate poison, mevinphos (Mev) acts to elicit cardiovascular intoxication. In Sprague-Dawley rats, microinjection bilaterally of Mev into the RVLM induced progressive hypotension and minor bradycardia, alongside selective depression of the activity of NADH cytochrome c reductase (enzyme marker for Complexes I+III) or cytochrome c oxidase (enzyme marker for Complex IV) in the mitochondrial respiratory chain, reduction in ATP concentration or tissue hypoxia in the RVLM. The Mev-induced hypotension, bioenergetic failure or hypoxia was significantly reversed when CoQ10 was co-administered bilaterally into the RVLM with the organophosphate poison. We conclude that CoQ10 confers cardiovascular protection against acute Mev intoxication by ameliorating the selective dysfunction of respiratory enzyme Complexes I and IV in the mitochondrial respiratory chain, the reduced ATP level and the induced tissue hypoxia in the RVLM.

P110077

α_{1A}-Adrenoceptors contrd blood pressure in the mouse mesenteric vascular bed

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The pressor action of the α_{1A}-adrenoceptor agonist, A61603 (N-[5-(4,5-dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methanesulfonamide) or phenylephrine, and their blockade by selective α_1-antagonists in the mouse isolated mesenteric vascular bed were evaluated. A61603 is a full agonist with 40 fold higher potency in elevating perfusion pressure in mesenteric bed compared to phenylephrine (partial agonist that showed ≈ 65% effect). The α_{1A}-antagonist RS 100329 (5-methyl-3-[3-[4-[2-(2,2,2,-trifluoroethoxy) phenyl]-1-piperazinyl] propyl]-2,4-(1H)-pyrimidinedione), displaced with high affinity the agonist curves to the right in a concentration-dependent manner; while the alpha1D-adrenoceptor antagonist BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-8-azaspiro[4.5] decane-7,9-dione), did not displace A61603 neither phenylephrine-induced pressor effect.

Data indcate that the mouse mesenteric vascular bed expresses α_{1A}-adrenoceptors and suggest it as a model to study α_1-adrenoceptors in gene knockout or overexpression.

Key words: α_{1A} adrenoceptor, mouse mesenteric vascular bed
Conacyt grant 47481, Fundacion Miguel Alemán, PAPIIT IN322005

P110078

The effect of dilating coronary artery on canine by injection of garlicin

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Method: 1. Using six canines, directly administrating garlicin in left coronary artery(LCA) with 0.15 mg and 1.5 mg two dosages respectively, adopting contrast examination before and after administration, and making film photography, measuring the diameter of left anterior decending branch (LAD) on screen, calculating the ratio of dilatation. 2. Using 5 canines, with hypophysin continuously dropping in vein to make coronarospasm model, according design of super latin square, administrating five drugs in LCA in turns: normal saline(NS), solvent of garlicin, garlicin, puerarin、nitroglycerin, measuring and calculating methods are same. Results: Compared with NS, two dosages of garlicin have no notable dilatating action after administration shortly, but dilatating LAD at the end of diadtolic phase(EDP) after 10 minutes at 0.15 mg dosage, ($P < 0.05$)。In experimental coronarospasm, Garlicin and nitroglycerin can dilatate LAD at the end of systole(ESP), ($P < 0.05$); They also can dilatate LAD at EDP. Compared garlicin with nitroglycerin ($P > 0.05$). Conclusion: Garlicin have delay dilatating action on normal coronary artery at EDP and can dilatate LAD in both EDP and ESP in experimental coronarospasm.

P110079

Evaluation of Hypoglycemic and Cardiovascular Effects of KS-C370G on Normal and Streptozotocin-induced Type 1 Diabetic Rats

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It is well known that the complication of cardiovascular disease is a major cause of death in diabetic patients. Here we examined the hypoglycemic and cardiovascular effects of a synthetic caffeic acid derivative "KSC370G" on normal and streptozotocin-induced type 1 diabetic rats. In Wistar and diabetic rats, KS-C370G was found to decrease plasma glucose. The effect in Wistar rats was associated with the increase of plasma insulin and glucose utilization as revealed by the intravenous glucose tolerance test. In addition, KS-C370G was found to increase the coronary flow on Langendorff perfused rat hearts of Wistar and diabetic rats. Since the increase of coronary flow was partly suppressed by L-NAME, it may be related to the increase of NO release. In rat thoracic aorta, KS-C370G shifted the dose/response curve of phenylephrine to the right probably via antagonism of α_1 receptors. In diabetic rats, chronic therapy with KS-C370G (3 mg/kg, i.p., b.i.d.) for 4 weeks resulted in an increase of basal coronary flow. In conclusion, KS-C370G was found to have hypoglycemic activity and beneficial effects on coronary flow of diabetic rats.

Key words: Caffeic acid, Diabetes, Coronary artery.

P110080

Anthocyanins inhibit the expression of TNF-α-induced genes associated with ischemia/reperfusion in endothelial cell by NF-κB-dependent pathway

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We examined the inhibition of the expression of some inflammatory genes associated with ischemia-reperfusion (I/R) injury by anthocyanins isolated from black soy bean seed coat in TNF-α-treated bovine aortic endothelial cells. In addition, its potential use on I/R-injury was investigated using rats subjected to 30 min occlusion of left descending coronary artery followed by 24 h reperfusion. Western blot analysis and luciferase activity assay showed that anthocyanins inhibited TNF-α-induced VCAM-1, ICAM-1, and COX-2 levels, which is through NF-kB dependent pathway. Further, anthocyanins protected myocardiac injury from I/R in rats. It is suggested that anthocyanins from black soybean seed coat can be used as useful drug to modulate cardiovascular disorder.

P110081

Involvement of endothelial COX metabolites in AVP-induced contraction in the rat basilar artery

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The present experiments were undertaken to clarify the pharmacological nature of [Arg8]-vasopressin(AVP)-induced contraction in the rat basilar artery in vitro. The basilar artery of Sprague-Dawley rats was used as a spiral preparation. AVP (0.003 nM to 0.1 μM) produced a concentration-dependent contraction which was decreased by the vasopressin V1 receptor antagonist ([Pmp1, Tyr(Me)2]-Arg8-vasopressin at 0.1 to 0.3 nM) in a concentration-dependent manner. The contraction by AVP (0.03 nM) was abolished by pretreatment with saponin (0.4 mg/ml). The contraction by AVP (0.3 nM) was significantly attenuated by a PLA2 inhibitor (manoalide) and a COX-2 inhibitor (NS-398, L-745337 and Celecoxib), but not by a COX-1 inhibitor (flurbiprofen), thromboxane A2 (TXA2) synthetase inhibitor (OKY-046) or TXA2 receptor antagonist (ONO-3708). These results indicated that the contraction induced by the lower concentrations of AVP in the rat basilar artery is endothelium-dependent and that the contraction is mediated by the vasopressin V1 receptors and is due to endogenous

contractile arachidonic acid metabolites generated mainly via COX-2 pathway.
Key Words : vasopressin ; rat basilar artery ; EDC.

P110082

ENDOTHELIAL DYSFUNCTION INDUCED BY GENETIC DELETION OR INHIBITION OF THE Mas RECEPTOR

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Mas is an endogenous receptor for the endothelium-dependent vasorelaxant angiotensin (Ang)-(1-7). We investigated the impact of altered Ang-(1-7)/Mas axis on endothelial function. In isolated mesenteric arteries of Mas-deficient mice, Ang-(1-7)-mediated relaxation was impaired compared to matched wildtype controls and was similar to that of isolated wildtype vessels exposed to the Ang-(1-7) receptor blocker A779. Furthermore, the vasorelaxant response to bradykinin (BK) and acetylcholine were reduced or completely inhibited, respectively, while endothelium-independent relaxation by sodium nitroprusside was unaltered. In cultured human endothelial cells, pre-treatment with A779 for 24 or 72 h blunted BK-mediated NO release, but unaffected endothelial NO synthase levels. Finally, in mesenteric arteries isolated from wildtype mice subjected to one-week minipump infusion of A779, BK-induced relaxation was significantly impaired. In conclusion, lack of Mas functionality is linked to generalized endothelial dysfunction, highlighting a pivotal role for Mas in preserving normal reactivity and pointing at Mas agonists as promising tools to treat cardiovascular diseases characterized by endothelial dysfunction.

P110083

N-Allylsecoboldine as a novel agent prevents acute renal failure during endotoxemia

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Blockades of cytokines and oxygen radicals release are considered to be beneficial in reducing multiple organ injury and increasing the survival rate in sepsis/septic shock. Thus, we examined the protective efficacy of N-allylsecoboldine, an antioxidant and α1-antagonist, in rats treated with endotoxin. Pretreatment of LPS-treated rats with N-allylsecoboldine significantly attenuated the hypotension, hypoglycemia, TNF-α and inhibited the iNOS protein expression in the renal cortex. N-allylsecoboldine improved the endotoxemia-induced organ injury as demonstrated from the conspicuous recovery of marker enzymes in the LPS-treated rats. Endotoxemia was associated with renal dysfunctions, as indicated by decreases in renal blood flow, urinary potassium excretion, and renal nitrate clearance, which were alleviated by N-allylsecoboldine. In addition, a lower dose of N-allylsecoboldine decreased the mortality of LPS-treated mice. This study demonstrates N-allylsecoboldine's ability to avail against acute renal failure and increase survival rate during endotoxemia. These beneficial effects may be attributed to the inhibition of iNOS expression, TNF-α production, and free radical scavenging activities.

P110084

Effects of Intermittent Hypobaric Conditions on Chronically Exercised Rat Hearts

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Objective : A new approach for enhancing athletic performance is to expose intermittent hypoxia while exercise in normobaric conditions ; we aimed to measure in vitro cardiac functions of rats in a setting which simulate this approach. Methods : The Wistar rats in group 1 stayed in a hypobaric cabinet 2 hours a day and 5 days a week for 9 weeks. Hypoxia simulated the PO_2 pressure in 3000 meters of altitude. They swam 30 minutes/day for 4 days for 9 weeks. Group 2 stayed and exercised in normobaric conditions. Groups 3 and 4 simulated groups 1 and 2 respectively but these groups did not exercise. Then hearths were perfused in Langendorff apparatus where their basal and 7.5, 12.5 and 75 mg/L dobutamine treated cardiac performance were measured. Results : Diastolic function deteriorated in group 1 (-dp/dt max 982 ±443 vs. 1511 ±224 that of group 2, p = 0.040). Basal heart rate of group 2 was lower than group 4 (p = 0.018) and that group had higher peak systolic pressure after dobutamine induction (at 7.5 mg/L concentration was 119 % of baseline, p = 0.019). Conclusions : Swimming in normobaric conditions enhanced cardiac functions. However, intermittent hypobaric conditions deteriorated cardiac performance.

P110085

PROTEOMIC ANALYSIS OF THE RAT ROSTRAL VENTROLATERAL MEDULLA, A NEURAL DETERMINANT FOR BRAIN DEATH

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Our laboratory revealed previously that the rostral ventrolateral medulla (RVLM) is intimately related to the "life and-death" process. This study sets the stage for screening for the multiple pro-life and pro-death programs that may be engaged by the RVLM during the progression towards death. Tissues collected from the ventrolateral medulla of Sprague-Dawley rats under minimal experimental perturbations were subject to two-dimensional electrophoresis and MALDI-TOF mass spectrometry-peptide map fingerprint analysis. The two-dimensional electrophoretic gel (pI: 3-10; Mr: < 94 kDa), on silver staining or colloidal Coomassie Brilliant Blue staining, showed approximately 530 or 230 protein spots. Of 200 spots selected for in-gel digestion followed by database search using measured peptide masses resulted in the identification of 148 proteins in 188 spots. These include structural proteins, or proteins related to transcription and translation, intermediary metabolism, chaperones, signal transduction, apoptosis, protein turnover, and oxidative stress. This information shall form the foundation in our search for the pro-life and pro-death proteins at the RVLM that may participate in brain death.

P110086

Hydrogen sulfide facilitates carotid sinus baroreceptor activity in anesthetized male rats

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Aim: To study the effect of hydrogen sulfide (H_2S) on carotid baroreceptor activity (CBA). Methods : The functional curve of carotid baroreceptor (FCCB) was constructed and the functional parameters of carotid baroreceptor were measured by recording sinus nerve afferent discharge in anesthetized male rats with perfused isolated carotid sinus. Results : H_2S (25, 50, 100 μmol/L) facilitated CBA, which shifted FCCB to the left and upward. There was a marked increase in peak slope (PS) and peak integral value of carotid sinus nerve charge (PIV) in a concentration-dependent manner. Pretreatment with glibenclamide (20 μmol/L), the above effects of H_2S on CBA were abolished. Pretreatment with Bay K8644 (500 nmol/L) eliminated the role of H_2S on CBA. An inhibitor of cystathionine γ-lyase (CSE), DL-propargylglycine (PPG; 200 μmol/L) inhibited CBA in male rats and shifted FCCB to the right and downward. Conclusion: Exogenous H_2S exerts a facilitatory role on isolated CBA through opening K_{ATP} channels and further closing the calcium channels in vascular smooth muscle. Endogenous H_2S may activate the activity of the CBA in vivo.
Key words : hydrogen sulfide; K_{ATP} channel opener; glibenclamide; baroreceptor

P110087

Effects of genistein on neuronal discharges in paraventricular nucleus of rat hypothalamic slices[1]

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Aim: To study the effects of genistein (GST) on paraventricular nucleus (PVN) neurons. Methods : Using extracellular recording technique. Results : In response to the application of GST (10, 50, 100 μmol/L) into the perfusate, the spontaneous discharge rates (SDR) of neurons were decreased in a dose-dependent manner. The G protein-coupled inwardly rectifying K^+ (GIRK) channels antagonist, tetraethylammonium (TEA 1 mmol/L) blocked the inlibitory effect of GST (50 μmol/L). Pretreatment with L-glutamate (L-Glu, 0.2 mmol/L) led to a marked increase in the SDR of neurons in an epileptiform pattern. The increased dis-

charges were also suppressed after GST (50 μmol/L) was applied. Application of N^G-nitro-L-arginine methyl ester (L-NAME, 50 μmol/L) augmented the SDR of neurons, then GST (50 μmol/L) applied reduced the increased SDR of neurons. Conclusion: GST can inhibit the electrical activity of paraventricular nucleus neurons by activating GIRK which induce K^+ outward current and then engender the cell membrane hyperpolarization, and increasing production of NO, which indicated that GST play a protective role on the central neurons.

Key words: paraventricular nucleus; GST; TEA; L-NAME

Project supported by the National Natural Science Foundation of China (No 304005) and the Natural Science Foundation of Hebei Province (No 303467).

P110088

Effects of rosiglitazone on rats with metabolic syndrome

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To investigate the effects of rosiglitazone (RSG) on metabolic syndrome (MS) rats. MS model was produced by two-kindey, one-cliped male SD rats fed with high fructose. sham operation group were fed with a common diet. At the end of 8 weeks, to verify MS model success by detecting the related indexes. Subsequently, MS rats were randomly divided into 2 groups: MS group and MS + RSG group, rats were fed with a common diet for 3 weeks. The results indicate: (1). At 8 weeks, compared with sham operation group, hypertension, hyperglycemia, hyperinsulinmia, insulin resistance and hyperlipidemia appeared in the MS group. (2). At 11 weeks, in MS + RSG group, Systolic blood pressure (SBP), triglyceride (TG), fasting blood sugar (FBS) and fasting serum insulin (FSI) remarkably reduced, total cholesterol (TC), high density lipoprotein cholesterol (HDLC) and insulin sensitive index (ISI) significantly elevated; While other two groups, the above variables did not change significantly compared with those of at 8 weeks. These findings suggest that rosiglitazone can reduce SBP, improve insulin resistance and correct the abnormality of sugar and lipid metabolism.

Key words: rosiglitazone; metabolic syndrome

P110089

Propofol Attenuate Hyperglycemia induced Cardiomyocyte Hypertrophy In Cultured Neonatal Cardiomyocytes in Rats

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We investigated whether propofol, an intravenous anesthetic with antioxidant properties could protect cardiomyocytes from hyperglycemia (HG)-induced cardiomyocyte hypertrophy (MH). Cultured neonatal rat cardiomyocytes were exposed to normal (5.5 mmol/L, LG) or high concentration of glucose (25.5 mmol/L, HG), HG in the presence of 12.5 mM propofol or 50 mM propofol, respectively, for 48 hours. Myocyte cross-sectional area was measured by immunocytochemistrical analysis. Myocyte protein content was determined by measuring incorporation of [^{3}H]-Leucine. Reactive oxygen species (ROS) were detected by fluorescence of dihydroethidium (DHE) staining. HG enhanced protein production and significantly increased myocyte crosssectional area compared to LG (1.7 fold of LG, $P < 0.01$ LG) that was significantly attenuated by propofol at 50 mM (1.3 fold of LG, $P < 0.01$ vs HG) but not at 12.5 mM (1.5 fold of LG). Propofol attenuation of HG induced MH was associated with a decrease in ROS production. In conclusion, propofol effect in attenuating HG induced cardiomyocyte hypertrophy may be attributed to its antioxidant property.

P110090

14-3-3 protein is involved in lipopolysaccharide-induced cardiomyocyte injury

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Objective: To study the role of 14-3-3 protein and isoforms in lipopolysaccharide (LPS)-induced cardiomyocyte injury. Methods: Primary neonatal SD rat cardiomyocytes were treated with LPS or TNF-α, and the expression of 14-3-3 protein and mRNA were investigated by Western blot and RT-PCR, respectively. TNF-α in the medium was measured by ELISA. Results: LPS and TNF-α up-regulated the expression of 14-3-3 protein and 14-3-3 ζ mRNA. Additionally, the level of TNF-α in medium was increased in LPS-treated cells ($p < 0.05$). However, there were no changes in the expression of 14-3-3 ζ mRNA. Conclusions: 14-3-3 protein is involved in LPS-induced cardiomyocyte injury.

Key words: cardiomyocyte; lipopolysaccharide; 14-3-3 protein

Acknowledgement: This work was supported by a grant from Natural Science Foundation of China (№:30460048)

P110091

The Alternation of NO and NOS mRNA Expression in Type 2 Diabetes Rats and the Protective Effect of Valsartan

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Objective To investigate the alteration of nitric oxide (NO), NO synthase (NOS) mRNA expression and the role of Valsartan at different stage of type 2 diabetes. Methods The models were streptozotocin and high-energy diet treated rats. 12 weeks later, four groups: normal controls (NC), diabetes controls (DC), Valsartan (8, 24 mg/kg/d, 8 weeks, ig) treated diabetes were studied. At 12th and 20th weekend, such indices as cardic function, endothelium-dependent vasodilation (EDVD), ultrastructure of myocard. and aorta, concentration of NO, NOS mRNA expression were measured. Results In DC, cardic function and EDVD declined, ultrastructure of myo. and aorta changed, NO increased at 12th but decreased at 20th weekend. Besides, iNOS mRNA expression up-regulated at 12th and 20th weekend, eNOS mRNA expression down-regulated only at 20th weekend. Valsartan regressed the aggravation and accommodate NO level, as well as NOS mRNA expression. Conclusion The abnormality of NO and NOS mRNA expression might be relative to the cardiovascular complication of diabetes. Valsartan played a protective role partially through adjusting the system of NO.

Key words: diabetes, nitric oxide; Valsartan The Research Found of the Department of Education in Jiangsu.

P110092

Effects of urotensin II on the electrical activity of paraventridar neurons in rat hypothalamic slices

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Aim: To study the effects of urotensin II (UII) on paraventricular nucleus (PVN) neurons. Methods: Using extracellular recording technique. Results: In response to the application of UII (0.3, 3.0, 30.0, 300.0 nmol/L) into the perfusate, the spontaneous discharge rates (SDR) of neurons were decreased in a dose-dependent manner. Pretreatment with bicuculline (BIC, 100 μmol/L), a specific $GABA_A$ receptor antagonist led to an increase in the SDR of neurons in an epileptiform pattern. The increased discharges were not significantly changed after UII (3.0 nmol/L) was applied. Pretreatment with picrotoxin (PIC, 50 μmol/L) led to an increase in the SDR of all neurons. The increased discharges were also not influenced by the applied UII (3.0 nmol/L) in neurons. Application of N^G-nitro-L-arginine methyl ester (L-NAME, 50 μmol/L) augmented the SDR of neurons, while UII (3.0 nmol/L) applied led the augmented SDR of all neurons to be decreased. Conclusion: UII decrease neuronal excitability of PVN neurons by potentiating $GABA_A$ receptor-mediated Cl^- current, may be involving the mediation by nitric oxide.

Key words: hypothalamic slices; UII; bicuculline; picrotoxin

P110093

Delayed protection and mechanism of Sodium Ferulate on cultured rat cardiomyocytes subjected to anoxia reoxygenation injury

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Aim: To investigate delayed protection and mechanism of Sodium Ferulate (SF) on cultured cardiomyocytes subjected to anoxia-reoxygenation (A/R) injury. Methods: The primary cultured neonatal rat cardiomyocytes were pretreated with SF (3.36, 1.68, 0.84 mmol/L) or SF (3.36 mmol/L) and PD98059 (50 umol/L), Glibenclamide (0.1 mmol/L) and L-NAME (0.1 mmol/L) respectively for 3 hours, and subjected to A/R injury after 24 hours. Viability and ultrastructure of myocytes, LDH activity in medium, expression of HSP70 of myocytes were measured. Results: Pretreatment with SF decreased LDH activity, increased cell viability, and upregulated HSP70 expression in a concentration-dependent manner. The delayed protective effects of SF were partly abolished by PD98059, Glibenclamide and L-NAME respectively, with the down-regulation in HSP70 expression. Conclusion: SF has a potent delayed cardioprotection against A/R injury, and its mechanism appears to be related to up-regulation of HSP70 expres-

sion mediated by activation of MAPK pathway, production of NO and opening of ATP sensitive potassium channels.

Key Words : Sodium Ferulate, delayed protection, cardiomyocyte, HSP70

P110094

Protective Effects of Sasanquasaponin Preconditioning Mediated by Bradykinin on Isolated Rat Hearts

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Aim: To study the preconditioning effects and mechanisms of Sasanquasaponin (SQS) on isolated rat heart subjected to anoxia-reoxygenation (A/R) injury. Method: Isolated rat hearts were perfused in Langendorff mode, and with SQS 0.1, 1, 10 umol/L or with HOE140 1 umol/L and SQS 1 umol/L for 15 min, then subjected to A/R injury. Heart rate, coronary flow (CF), left ventricular pressure and its first derivative were recorded. The activities of LDH, CPK, GSH-Px, SOD and the contents of MDA in CF solutions or myocardium, the area of myocardial infarction were measured. Results: SQS 0.1, 1, 10 umol/L preconditioning could make heart functions improved, moreover, the activities of LDH and CPK, contents of MDA and the area of myocardial infarction decreased, whereas, the activities of GSH-Px, SOD increased on the heart subjected to A/R injury, but after treating with HOE140, the protective effects of SQS were mainly cancelled. Conclusion: SQS can induce the cardioprotective effects of pharmacological ischemic preconditioning and the mechanisms may be relative with the enhancement of the activity of bradykinin system.

Key word: Sasanquasaponin, Ischemic preconditioning, Bradykinin, Isolated rat heart

P110095

Hypoxia preconditioning up-regulates 14-3-3 protein through activation of ERK1/2 in neonatal rat cardiomyocytes

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To determine if hypoxia preconditioning up-regulates 14-3-3 protein in rat myocardiocytes and the upregulation is involved in extracellular signal-regulated protein kinase 1/2 (ERK1/2). A delayed preconditioning model was established by using cultured neonatal rat cardiomyocytes. PD98059 was used to modulate ERK1/2 activation. Injury was evaluated by measuring cell viability and LDH release. Expression of 14-3-3 protein was measured by Western blot. Increased cell viability and decreased LDH release were observed in cardiomyocytes treated with hypoxia preconditioning and the delayed protection was abolished by pretreating with PD98059. The expression of 14-3-3 protein was significantly increased in 24 h after hypoxia preconditioning, which also suppressed by PD98059. The findings suggest that hypoxia preconditioning up-regulated 14-3-3 protein in cultured neonatal rat myocardiocytes and ERK1/2 activation was involved in the up-regulation of 14-3-3 protein.

Key word: 14-3-3 protein; hypoxia preconditioning; cardiomyocyte; ERK1/2

Acknowledgement: This work was supported by a grant from Natural Science Foundation of China (№: 30460048).

P110096

Beneficial effects of n-hexacosanol on STZ-induced diabetic rat aorta smooth muscle

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Objectives: Vascular dysfunction is a major complication of diabetic mellitus. In this study, we investigated the effects of n-hexacosanol (FA) on the contractile responses to norepinephrin (NE) and KCl and the relaxation induced by acetylcholine (ACh) on the diabetic rat aorta. Methods: Eight weeks old male SD rats were divided into 5 groups. One was as age-matched control group and the others were induced diabetes by streptozotocin (50 mg/kg i.p.) and were maintained without treatment. Four weeks after the induction of diabetes, one group of diabetic rats was immediately sacrificed to perform experiments, while the other three groups were treated with vehicle or FA (2 or 8 mg/kg, i.p. every day) for the following 4 weeks. Results: The contractions induced by NE or KCl were augmented and the relaxation produced by ACh was reduced in the diabetic rat aorta. The hyperreactivity to NE and the reduced relaxation were recovered to control level with the treatment with FA. The levels of insulin and glucose were unchanged with FA. Conclusion: Our data indicate that FA can improve the diabetes-induced hyperreactivity and impairment of relaxation of the diabetic rat aorta.

Key word: aorta, streptozotocin

P110097

Prevention of Vascular Smooth Musde Calcification by Thyroid Hormone

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Thyroid hormones have marked cardiovascular effects in vivo. However, their direct effects on vascular smooth muscle cells have been unclear. We examined the effects of 3',3,5-triiodo-L-thyronine (T3) on the expression of calcification-associated genes in rat aortic smooth muscle cells (RAOSMCs). Quantitative RT-PCRs revealed that a physiological concentration of T3 (15 pmol/L free T3) increased mRNA level of matrix Gla protein (MGP). In RAOSMCs transiently transfected with a luciferase reporter gene driven by the MGP promoter, T3 significantly stimulated luciferase activity. Aortic smooth muscle tissues from methimazole-induced hypothyroid rats (400 mg/L drinking water, 4 weeks) also showed a 68% decrease in the MGP mRNA level, as well as a 33% increase in calcium content, compared to that from the control euthyroid animals, whereas hyperthyroidism (T3 0.2 mg/kg, i.p., 10 days) upregulated MGP mRNA and reduced calcium content. Our findings suggest that a physiological concentration of thyroid hormone directly facilitates MGP gene expression in smooth muscle cells via thyroid hormone nuclear receptors, leading to prevention of vascular calcification in vivo.

P110098

GLYCOGEN SYNTHASE KINASE-3-BETA INHIBITORS ATTENUATE THE ORGAN INJURY/DYSFUNCTION CAUSED BY HEMORRHAGIC SHOCK

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Glycogen synthase kinase 3b (GSK-3b) is a serine/threonine protein kinase involved in the modulation of the inflammatory response. Dysregulation of GSK-3b has been implicated in the pathogenesis of several diseases including sepsis. Here, we investigate the effects of two chemically distinct GSK-3b inhibitors, TDZD-8 and SB216763, on the circulatory failure and organ injury/dysfunction associated with hemorrhagic shock. Male Wistar rats were subjected to hemorrhage (sufficient to lower mean arterial blood pressure to 35 mmHg for 90 min) and subsequently resuscitated with shed blood for 4 h. Hemorrhage and resuscitation resulted in renal dysfunction and hepatic injury; this was abolished by treatment with either TDZD-8 (1 mg/kg i.v.) or SB216763 (0.6 mg/kg i.v.). In addition, TDZD-8, but not SB216763, attenuated the increase in plasma levels of the proinflammatory cytokine IL-6 caused by hemorrhage and resuscitation. Neither of the inhibitors however affected the delayed fall in blood pressure caused by hemorrhagic shock. Thus, inhibition of GSK-3b may represent a novel therapeutic approach for the therapy of hemorrhagic shock.

Key Words : Glycogen synthase kinase 3b, hemorrhagic shock, rat

P110099

Endogenous hydrogen sulfide contributes to the cardioprotective effects of preconditioning with endotoxin, but not ischemia, in the rat

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Here we investigate whether the cardioprotective effects of pre-conditioning (PC) with endotoxin (LPS) or ischemia are due to endogenous hydrogen sulfide (H_2S). In male Wistar rats, two cycles (5 min) of PC with ischemia followed by regional myocardial ischemia-reperfusion resulted in a significant reduction (50%) in infarct size. When compared to vehicle-treated animals, 16 h pre-treatment with LPS (1 mg/kg i.p.) resulted in a significant reduction (41%) in infarct size. Administration of the irreversible cystathionine-gamma-lyase inhibitor, DL-propargylglycine (PAG, 50 mg/kg), which prevents the formation of H_2S, did not affect the cardioprotective effect afforded by ischemic PC, but abolished the cardioprotective effects afforded by LPS. Administration of 5-hydroxydecanoate (5 mg/kg) also abolished the cardioprotective effect of LPS. These findings demonstrate that the delayed cardioprotective effects afforded by LPS, but not ischemia, in the rat are largely due to the formation of endogenous H_2S, which in

turn may cause cardioprotection by causing the opening of mitochondrial K_{ATP}.

Key words: PAG, ischemia-reperfusion
Supported by the William Harvey Research Foundation.

P110100

Continuous luminal flow attenuates basal release of NO but augments that of EDHF in the rabbit carotid artery.

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The influence of continuous luminal flow on endothelial function in rabbit carotid artery was examined by comparing contractions to phenylephrine (PE) ($3x10^{-8}$ - $3x10^{-5}$ M) and relaxations to acetylcholine (ACh) (10^{-6} M) in the absence and presence of endothelium in segments with (5 and 50 ml/min) or without (static rings) flow. Flow shifted the concentration-response curve to PE to the left and reduced tissue cGMP content when compared to tissues without flow. Treatment with nitro-L-Arginine methyl ester (L-NAME, 10^{-4} M) and removal of the endothelium abolished differences in sensitivity to PE and tissue cGMP content between flow and non-flow conditions. Acetylcholine-evoked relaxations were increased in perfused segments. L-NAME nearly abolished the acetylcholine-evoked relaxation in static rings, whereas half of the relaxation remained in segments exposed to flow. This remnant relaxation was blocked by apamin (10^{-7} M) plus 1-[(2-chlorophenyl) diphenyl methyl]-1H-pyrazole (TRAM-34, 10^{-7} M). Thus, in the rabbit carotid artery sustained flow reduces basally released endothelial NO, and unmasks an ability of acetylcholine to release EDHF.

P110101

Experimental setups made of plastic influences the effect of reboxetine on vascular contractions evoked by field stimulation.

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The actions of reboxetine, a norepinephrine re-uptake inhibitor (Rasmussen and Nedergaard, JPET, 306:995-1002), were studied on contractions evoked by electrical field stimulation in the isolated rabbit carotid artery. The isolated tissue baths and holders were made of either plastic (polymethylmethaacrylate) or glass (Pyrex). In the setup made of plastic, but not glass, reboxetine (10^{-9} - 10^{-6} M) and cocaine (10^{-6} - 10^{-5} M) were unable to enhance contractions. Reboxetine (10^{-8} M) and cocaine (10^{-5} M) completely prevented the blocking action of bretylium (10^{-6} M) on contractions in both the plastic and glass setup. Bretylium (10^{-4} M) did not inhibit neurogenic contractions in the plastic setup, when the setup was first exposed (30 min) to reboxetine (10^{-6} M) followed by repeated washes with distilled water (12 h). In contrast, bretylium (10^{-6} M) completely blocked contractions in the glass setup, when the setup was exposed (30 min) to reboxetine (10^{-6} M) and repeatedly washed with ethanol (12 h). These findings suggest that reboxetine binds strongly to plastic from where it is released into the solution.

Supported by the Danish National Research Council.

P110102

The reactivity to contracting agents is impaired in rat carotid subjected to pressure overload

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The remodelling of vascular wall, as consequence of several physiological and/or pathological conditions, is responsible for vascular lumen narrowing and loss of arterial elasticity. In this study we report functional changes in vascular reactivity to contracting agents induced by pressure overload. The pressure overload was performed by transverse aorta stenosis (TAS group) applying a silver clip between the two carotids in male Wistar rats. Vascular stenosis produced pressure overload to the heart and right carotid (RC) but not on left carotid (LC) and systemic circulation. After 14, 28, 42 and 56 days rats were sacrificed and carotids excised for in vitro study. The phenylephrine (PE; 0.3 μM), angiotensin II (0.1 μM) or potassium chloride (40 mM) contractions were significantly ($P < 0.05$) reduced in both RC and LC of TAS group compared to sham or naive group. This effect could be related either to an increase in the expression of eNOS and/or by an impairment of calcium homeostasis throughout the voltage dependent channels. Our data indicate that the overload in blood pressure produces impairment of reactivity as well as morphological remodelling of the vascular wall.

P110103

Dan-Shen ameliorate oxidative stress in endothelial cells via an NO-dependent mechanism

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Salvianolic acid A, Salvianolic acid B, Tanshinone IIA, Tanshinone I, Dihydrotanshinone I and Cryptotanshinone were isolated from Dan-Shen (DS), the root of Salvia miltiorrhiza Bunge (Labiatae). We tested the hypothesis that DS protects the endothelium by a NO-mediated mechanism in endothelial cells.

Salvianolic acid A, Tanshinone IIA, Dihydrotanshinone I and Tanshinone I (1100 μmol/l) could increase in NO release and the eNOS protein expression, which release could be blocked by the NOS inhibitor L-NMMA.

It is observed that H_2O_2 (800 μmol/l) increased the level of NO and iNOS activity and expression (protein level) in ECV-304 cells. Pretreatment with 3100 μmol/l Salvianolic acid A, Dihydrotanshinone I and Tanshinone I resulted in a significant recovery from H_2O_2-induced cell damage, which decreased iNOS protein expressions and overall nitrite generation.

Our results show that L-homocysteine at micromolar levels increases lipid peroxidation in cultured EC, which is dependent on superoxide that involves eNOS. Treatment with 0.1-100 μmol/l Salvianolic acid A and B significantly resulted in a significant recovery from L-homocysteine-induced cell damage which decreased eNOS protein expressions and overall nitrite generation. Thus, DS actively protects EC from oxidative stress via an NO-dependent mechanism.

P110104

Effects of rosiglitazone on collagen I/III and secretion of TGFβ1 of vascular smooth musde cell induced by high glucose

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The aims of this study were to explore the effects of rosiglitazone (RSG), thiazolidione peroxisome proliferator activated receptor-γ (PPAR-γ) activator, on the expression of collagen I/III-mRNA and secretion of transforming growth factor-β1 (TGFβ1) of rat aortic vascular smooth muscle cells (VSMCs) cultures in vitro induced by high glucose. The expression of collagen I/III-mRNA of VSMCs from rat thoratic aorta cultured in vitro was determined by RT-PCR method, the levels of TGFβ1 in the supernatants were measured by enzyme-linked immunabsorbant assay (ELISA). A 48-h incubation of VSMCs with high glucose (22 mmol/L) exhibited increasing effect on the expression of collagen I/III-mRNA of VSMCs and stimulated the protein secretion of TGFβ1. After the 0.5-h incubation of VSMCs in the co-presence of RSG (10 μmol/l) with high glucose, RSG remarkedly reversed those effect. These results showed that RSG executes its protective effects on high glucose-induced VSMCs by reducing the expression of collagen I/III and the secretion of TGFβ1.

Key word: rosiglitazone; high glucose; VSMC; collagen I/III; TGFβ1

P110105

Effect of propfol on the activation of Nudear factor-κB and expression of inflammatory cytokines during myocardium mischemia/reperfusion injury in rats

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Objective: To investigate the protective effect of propofol in myocardium mischemia/reperfusion (M/R) injury. Methods: Rat M/R injury was induced by occluding the left main coronary artery for 30 min and reperfusing for 2h. Propofol was intravenously given 15 min before ischemia. NF-κB activation and its inhibitory protein, I-κB were determined by Westernblot. The concentrations of TNF-α, IL-1β in serum were evaluated by ELISA. The cardiac amount of mRNA codfying for ICAM-1 and iNOS were investigated by RTPCR. Results: Compared with the sham contrd group, NF-κB activity in myocardial nuclei was markedly increased and cytosolic I-κB was decreased in I/R group. The concentrations of TNF-α, IL-1β, and the expression of ICAM-1, iNOS were increased. Electron microscopic examination showed more serious injury of myocardium ultrastructure in I/R group. Administration of propofol attenuated NF-κB activation and reduced the inflammatory response and alleviated the ultrastructure injury. Conclusion: Propofol could inhibit NF-κB activation and down-regulate the inflammatory gene expression in M/R injury, which may be one of the molecular mechanisms of its cardioprotection.

Key words: propofol, ischemia/reperfusion, inflammation

P110106

EFFECTS OF BOTHROPS MARAJOENSIS VENOM IN BLOOD PRESSURE, ELETROCARDIOGRAPHIC PARAMETERS AND PERFUSED HEART.

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Bothrops marajoensis is a snake found in marajo island located in North Brazil. In the present study the effects of the whole venom (BmWV) was evaluated in arterial blood pressure (MAP), eletrocardiographic (ECG) and in the isolated perfused heart. The MAP was registered by means of a transducer and the ECG was registered using subcutaneous platinum electrodes. The isolated heart was perfused in an open Langendorff system. The injection of BmWV (1, 3, 30 and 100 µg/Kg) induced a dose-related decrease in MAP (from 104.3 ±11.5 mmHg to 85.1 ± 17, 78 ±17, 55.7 ±8.1 and 45.9 ±7.5 mmHg, respectively). The main ECG alteration was an atrioventricular blockade with an atrial tachycardia, QRS widening and ST elevation and extra-systoles (less than 25%). The injection of BmWV in the perfused heart at concentrations ranging from 0.01 to 10 µg induced, cardiac depression with 75.2 ±11% ($p < 0.05$) reduction in amplitude contraction with an increase of 88.1 ±4.5% in coronary perfusion pressure ($p < 0.05$). The cardiac frequency was not altered in vitro. This data suggests that the cardiovascular effects of BmWV are related to a direct cardiac depression with coronary spasm and also to indirect cardiac depression.

P110107

Impaired cardiac function after aortic constriction in transgenic mice with heart-directed overexpression of protein phosphatase inhibitor-2

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It has been suggested that a higher expression and activity of protein phosphatase type-1 (PP1) may contribute to the dephosphorylation of cardiac proteins, which then triggers the development of heart failure. Conversely, cardiac-specific overexpression of inhibitor-2 (I-2), which inhibits PP1 activity, can increase protein phosphorylation and contractility. To study a potential benefit of I-2 overexpression, we subjected mice overexpressing I-2 (TG) and wild-type (WT) mice to transverse aortic constriction (TAC). Banded mice were compared to sham-operated mice ($n = 5$-8). After four weeks of TAC, cardiac hypertrophy was comparable in TG and WT. In left-ventricular cardiac catheterization, the maximum rate of contraction (+dP/dt) was depressed by 62% in TG-TAC and only by 24% in WT-TAC compared to corresponding sham mice ($p < 0.05$). Biochemical analyses revealed that pressure overload upon TAC was accompanied by a higher PP1 activity in TG-TAC compared to WT-TAC, independently of PP1 protein expression. Thus, these findings suggest that the inhibition of PP1 by activation of I-2 is insufficient in reducing the progression of cardiac remodeling and heart failure.

Key words: PP1, inhibitor-2, contractility

P110108

Protective effects of crocin on cultured calf aortic endothelial cells injured by low-density lipoproteins(LDL)

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Objective To study protective effects of crocin on cultured calf aortic endothelial cells injured by low-density lipoproteins (LDL). Methods Bovine aortic endothelial cells was incubated with crocin for 12 hours, and then cultured with LDL for 24 hr, the activity of LDH, NO in culture media and activity of NOS in endothelial cells were measured. ;The atherosclerosis formation was induced by hyperlipidamic diet, after the 9th week, the level of serum LDL and NO were measured. Results Compared with control, LDL group could decrease activity of NO in culture media and activity of NOS in endothelial cells, endothelial cells was preincubated with crocin, the effecs of LDL were inhibited; Compared with the model group, crocin can reduce the level of LDL and elevation NO concentration. Conclusion It indicated that crocin had protective effects on cultured calf aortic endothelial cells injured by low-density lipoproteins.

Key words: Crocin; Endothelial cell (EC); LDL; NOS; NO

P110109

Pleiotropic phenotype of a genomic knock-in of an RGS-insensitive G184S GNAI2 allele

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Regulators of G protein Signaling (RGS proteins) speed the turn-off of G protein signals and inhibit signal transduction but the in vivo roles of RGS proteins remain poorly defined. To overcome the redundancy of RGS functions and reveal the total contribution of RGS regulation at the Galphai2 subunit, we prepared a genomic knock-in of the RGS-insensitive G184S GNAI2 allele. The Galphai2 G184S knock-in mice show a dramatic and complex phenotype affecting multiple organ systems (heart, myeloid, skeletal, and CNS). Both homozygotes and heterozygotes show a lower than Mendelian penetrance and decreased body weight. Other phenotypes include shortened long bones, a markedly enlarged spleen, elevated neutrophil and monocyte counts, an enlarged heart, and behavioral hyperactivity. Heterozygous Galphai2 +/ G184S mice show some but not all of these abnormalities. Thus, loss of RGS actions at Galphai2 produces a dramatic and pleiotropic phenotype which is more evident than the phenotype seen for individual RGS protein knockouts.

Key words: RGS proteins, Galphai2, knock-in mice model

Acknowledgements: This work was supported by NIH Grants R01-GM89561 (RRN), NIH-T32 HL078530 6(XH).

P110110

Geranylgeranylation is necessary in Na^+/Ca^{2+} exchanger mRNA increase by lisophosphatidylcholine in H9c2 cells.

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Cardiac Na^+/Ca^{2+} exchanger (NCX1) expression levels change under various pathophysiological conditions. However, its mechanism is unknown. We previously showed that fluvastatin (Flv), an HMG-CoA reductase inhibitor, decreased NCX1 mRNA and protein by inhibiting a small G-protein, RhoB in H9c2 cardiomyoblasts (2005). Conversely, lisophosphatidylcholine (LPC) increased NCX1 mRNA and protein by activating RhoB. RhoB requires isoprenylation for its activation with either farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP). Here, we investigated which isoprenoid is involved in the effect of LPC. Treatment of H9c2 cells with Flv for 24 hours decreased NCX1 mRNA by 40%. Addition of GGPP or FPP to Flv restored NCX1 mRNA to control level. No difference was observed between GGPP and FPP. When LPC was applied with Flv, NCX1 mRNA remained decreased. However, when LPC and GGPP, but not FPP, were applied simultaneously, NCX1 mRNA increased to a level significantly higher than the control. Furthermore, a GG-transferase inhibitor, but not F-transferase inhibitor, inhibited the effect of LPC. We conclude that geranylgeranylation but not farnesylation of RhoB is involved in the effect of LPC on NCX1.

P110111

Activation of Fas-mediated death in human aorta smooth muscle cell in the presence of 7-ketocholesterol

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We investigated whether 7-ketocholesterol, one of the major cholesterol oxides in the lesions, altered resistance of HVSMC to Fas-mediated death pathway. Crosslinking of Fas receptor with agonistic anti-Fas antibody (CH11) in the presence of 7-ketocholesterol induced death in human aorta smooth muscle cells (HAoSMC) as detected by morphology, viability, and DNA fragmentation. The agonistic anti-Fas antibody, however, did not induce death in the presence of 7α-hydroxychlesterol or cholesterol. The HAoSMC death was significantly inhibited by an antagonistic Fas receptor (FasR) antibody and by expression of dominant negative Fas-associated death domain containing protein (DN-FADD) using adenoviruses. Activation of caspase-3 was observed in HAoSMC destined to death. HAoSMC death was significantly inhibited by pharmacological caspase inhibitor, z-VAD and z-DEVD, and baculovirus caspase inhibitor p35. 7-Ketocholesterol impaired mitochondrial transmembrane potential and ATP production. Overexpression of bcl-xL also significantly inhibited HAoSMC death. In dying HAoSMC, bax was translocated from the cytosol to mitochondria and cytochrome c was re-

leased from mitochondria into the cytosol.

P110112

Role of H2S in the cardiovascular system and its possible interaction with NO.

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The present study aims to examine the role of hydrogen sulfide (H2S) in the cardiovascular system and its possible interaction with nitric oxide (NO). Male Sprague-Dawley rats (250-300g) were anaesthetized and cannulated for blood pressure measurement and drug delivery. In one study, exogenous H2S (as NaHS solution) and NO (as sodium nitroprusside, SNP, solution), separately and as a mixture, were given as a bolus intravenously. In another study, NaHS was infused at different rates and blood pressure monitored. Both NaHS and SNP caused a dose-dependent decrease in mean arterial pressure (MAP) when given separately. There was no change in MAP when NaHS and SNP were given together at doses causing a fall in blood pressure. Low rate of NaHS infusion (10 μmol/kg/min) caused an increase in MAP, while a higher rate of NaHS infusion (25 μmol/kg/min) caused a transient increase in MAP followed by a rapid drop. We conclude that the interaction between NO and H2S may be important for regulating the effect of each mediator on MAP. We also note a novel role of H2S as a possible vasoconstrictor at low concentrations.

Nitric oxide, hydrogen sulfide, blood pressure

We thank A*STAR for P.M's Graduate Scholarship.

P110113

The role of NO as a neurotransmitter in the cerebral vasculature

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Neural control of smooth muscle tone affects tissue functions. We have reported that dilating transmitter derived from nerves innervating blood vesssels, penile corpus, GI tract etc. is nitric oxide (NO). In anesthetized dogs and monkeys, electrical stimulation (ES) of a pterygopalatine ganglion (PPG) dilated cerebral arteries only in the stimulated side. NO synthase inhibitors abolished the dilation. Surgical denervation at the PPG instantly constricted the cerebral artery. In rats, ES of the nerve bundles from the PPG increased the cerebral blood flow, which was inhibited by NO synthase inhibitors. After FITC-dextran (10 kD) was systemically infused in anesthetized dogs, ES was applied to one side of the PPG. The fluorescent intensity in certain areas of the brain was higher in the stimulated side. Similar findings were histochemically obtained. T1-weighted MRI enhanced by gadolinium-DTPA during ES in the monkey showed higher signal intensities in certain brain regions in the stimulated side. These findings suggest that nitrergic nerve derived from PPG, tonically dilates the cerebral artery to maintain the cerebral circulation. Further, the nitrergic nerve seems to regulate the BBB permeability.

P110114

ROLE OF OXYTOCIN IN THE NATRIURESIS INDUCED BY CENTRAL ANGIOTENSIN II

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The mechanisms of central angiotesnin II induced natriuresis is unknown. We assessed the role of oxytocin (OX) in the natriuretic action induced by IVT-AngII or renin, using atosiban (AT). Sprague-Dawley male rats were treated: 1. AT (500 μg/kg, s.c.); 2. L-NAME (20 mg/kg, i.p.) or 3. Vehicle (s.c.). Half an hour after, animals received IVT-AngII, renin or saline. Rats were placed into metabolic cages and urine was collected at 1, 3, and 6- hr. Na^+ and K^+ was determinate by flame photometry and cGMP by radioimmunoassay. AngII-IVT reduced urinary volume, and increased urinary Na^+, K^+ and cGMP excretion. AT blunted these effects. The increase in urinary cGMP was independent of NOS activity, since L-NAME did not alter IVT-renin natriuresis. Our results support the concept that oxytocin mediates the natriuretic action of brain renin angiotensin system (CDCH06-30-5390-2004 and IIF-10/2005).

Key words: oxytocin, natriuresis, angiotensin II, renin

P110115

Phosphatidylinositol 3-kinase: a potential therapeutic target in oxidative stress and platelet aggregation

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Phosphatidylinositol 3-kinase (PI 3-kinase) is a central mediator of a number of important leukocyte functions such as chemotaxis, phagocytosis and activation of NADPH oxidase. In the present study the ability of PI 3-kinase to produce reactive oxygen species (ROS) in neutrophils and whole blood was investigated by the use of three different PI 3-kinase inhibitors. Inhibition of PI 3-kinases by wortmannin, resveratrol or LY-294002 decreased oxidative stress and platelet aggregation at comparable doses. A possible link that could explain the antioxidant and antiplatelet actions of PI 3-kinase inhibitors is that a decrease in oxidative stress would enhance the availability of nitric oxide which inhibits platelet aggregation. Our study shows that PI 3-kinases are involved in the formation of ROS and also mediate platelet aggregation; therefore, members of this key enzyme family might represent important therapeutic targets in inflammations which involve impaired platelet behaviour and production of ROS.

Key words: PI-3 kinase, platelet aggregation, oxidative stress, signaling.

Acknowledgment: We thank Higher Education Commission, ICCS, and The Aga Khan University, Karachi, for support.

P110116

Asymmetric dimethylarginine induces apoptotic death in vascular smooth muscle cells: a preliminary result

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Objective: Endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) is recently ascribed as a novel pro-atherogenic molecular. The aim of the present study was to investigate the effect of ADMA on apoptosis of vascular smooth muscle cells (VSMC). Methods: Rat primary VSMC were cultured and treated with different concentrations (1-30 μM) of ADMA for various periods (24-72 h). Cell viability and apoptosis were evaluated by MTT test and DNA fragmentation analysis, respectively. Results: As shown by MTT, ADMA decreased cellular viability of VSMC in a dose- and time-dependent manner. Apoptotic DNA fragments were observed when VSMC is treated with 10 μM ADMA for 48 h. Conclusion: ADMA has cell toxic effect and induces the apoptosis in VSMC, which may contribute to its pro-atherogenic activity. The precise mechanisms involved in such effects of ADMA need to be further investigated.

Key word: Asymmetric dimethylarginine; Apoptosis; Vascular smooth muscle cells

P110117

Influence of aspirin alone and in combination with ticlopidine on bleeding time, platelet count and hematocrit in rats

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Compared with aspirin, ticlopidine can cause life-threatening hematological adverse reactions. The aim of this study was to evaluate the effects of aspirin alone and in combination with ticlopidine on bleeding time, platelet count and hematocrit. Twenty four Wistar rats were divided in three groups and they recived i.p. one of the following treatments for 4 days: group I-control, group II-aspirin 50 mg/kg, group III-aspirin and ticlopidine combination 50 mg/kg + 125 mg/kg. Bleeding time was significantly prolonged in the aspirin-treated group compared to control ($p < 0.001$). Also, bleeding time was significantly prolonged in group treated with aspirin and ticlopidine combination compered to control ($p < 0.001$) and aspirin alone ($p < 0.05$). Group treated with aspirin and ticlopidine combination was the only one with slightly decreased platelet count. The hematocrit was significantly decreased only in group treated with aspirin and ticlopidine combination compared to control ($p < 0,05$). Based on obtained results it can be noticed that values of hematological parameters were lower in group treated with aspirin and ticlopidine combination compared with aspirin alone.

Key words: aspirin, ticlopidine, rats

P110118

CARDIOVASCULAR PROFILES OF THE YOUNG MALAYSIAN HYPERTENSIVE PATIENTS: PRELIMINARY FINDINGS

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Introduction: Peripheral vascular resistant is characteristically elevated in hypertension because of alterations in structure and function of small arteries. A cross-sectional study on the cardiovascular profiles was carried out by using the HDI Pulsewave Analysis device. Methodology: There were 24 young hypertensive and 15 normotensive subjects included in the study. There were significant differences on the following cardiovascular parameters ($P < 0.05$): 1) Cardiac Ejection Time (288.2 ±45.0 vs 324.0 ±33.8 msec, patient vs control), 2) Stroke Volume Index (42.5 ±6.6 vs 49.7 ±10.5 ml/beat/m2), 3) Estimated Cardiac Output (5.9 ±0.9 vs 5.2 ±0.9 L/min), and 4) Artery Elasticity Index of Small Artery (5.8 ±2.4 vs 9.4 ±3.9 ml/mmHgx100). Other cardiovascular parameters showed no significant differences including Stroke Volume, Cardiac Index, Artery Elasticity Index of Large Artery, Systemic Vascular Resistance and Total Vascular Impedance ($P > 0.05$). In conclusion, this study revealed that there were some pathological changes mainly in the compliance of the small arteries. These changes partly support the benefit of using vasodilator in the treatment of young hypertensive subjects.

P110119

Activation of Dopaminergic and Glutamatergic Neurotransmission Invdved in Cardiovascular Changes Induced by Intrategmental Microinjection of DiMe-C7

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Local microinjection of the substance P analogue DiMe-C7 (10 fmol) into the ventral tegmental area (VTA) caused increases in the mean arterial blood pressure (MAP) and heart rate (HR) in chloral hydrate anaesthetized rats. The pressure response was associated with the increases in the dopamine (DA) level in the central nucleus of amygdala (CeA; 140 ±6%), the plasma vasopressin (VP) concentration (8.6 ±1.0 vs. 22.5 ±2.4 pg/ml) and the inhibition of baroreflex response (BRR; 66 ±5%). Bilateral pretreatment with the DA antagonist, haloperidol, into the CeA blunted the increases of MAP, HR, and the inhibition of BRR after DiMe-C7 microinjection. However, the pretreatment had no effect on the release of VP. Bilaterally pretreated a nonselective ionotropic glutamate receptors antagonist, kynurenic acid (2.5 fmol), into the supraoptic nucleus (SON) blunted the increases of MAP, HR and VP release after DiMe-C7 microinjection, and had no effect on the baroreflex inhibition. Our results suggested that the dopaminergic pathway and glutamatergic pathway are independently contributed to the cardiovascular changes induced by intrategmental DiMe-C7 injection.

Key words: DiMe-C7, baroreflex response, vasopressin

P110120

Effect of endogenous male sex hormone deprivation on vascular superoxide dismutase function in aorta and mesentery rat arteries.

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This study examines if endogenous male sex hormones influence the vascular Cu/Zn-superoxide dismutase (SOD) function. For this, aorta and mesenteric arteries from control and castrated (CX) male Sprague-Dawley rats were used to analyse: (1) superoxide anion generation; (2) vasodilator response to the nitric oxide donor, sodium nitroprusside (SNP), and (3) expression and activity of endogenous Cu/Zn-SOD. Orchidectomy increased the generation of superoxide anions in both arteries. SNP-induced relaxation was enhanced by the SOD mimetic tempol only in arteries from CX rats. Endogenous Cu/Zn SOD expression was increased in aorta and unaltered in mesenteric segments from CX rats. However, Cu/Zn-SOD activity was increased in both arteries from CX rats. These results show that orchidectomy increased the superoxide anion production, which are involved in the vasodilator response to SNP only in arteries from CX rats. Orchidectomy increased the Cu/Zn-SOD expression only in aorta, and SOD activity in both arteries, probably to compensate for the increased superoxide anion formation.

Key Words: orchidectomy; Cu/Zn-SOD; superoxide anion.

Support: FIS (PI020335, PI051767 and C03/01) and DGCYT (SAF2005-05760).

P110121

Involvement of Thromboxane A2 in the Acetylcholine-induced response in rat aorta. Role of endogenous male sex hormones.

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This study investigates whether endogenous male sex hormones affect the participation of thromboxane (TXA2) in the acetylcholine (Ach)-induced response in rat aorta. For this purpose, aorta from control and orchidectomized male Sprague Dawley rats were used to analyze: (1) vascular response to Ach; (2) vascular response to the TXA2 mimetic U-46619 and, (3) the basal and Ach stimulated production of TXB2, the stable TXA2 metabolite. The Ach-induced relaxation was unaltered by the TXA2 synthesis inhibitor, furegrelate, in arteries from control rats while was increased in aortas from orchidectomized rats. In intact vessels, the concentration dependent contraction induced by U-46619 was similar in arteries from both rat groups. The basal and Ach-stimulated TXB2 release was increased in arteries from orchidectomized animals. These results show that TXA2 production is increased in aortas from orchidectomized rats compared to their controls, and that this prostanoid is functionally involved in the vasodilator response to Ach only in arteries from orchidectomized rats.

Key Words: Orchidectomy; TXA2, rat aorta.

Support: FIS (PI051767 and C03/01) and DGCYT (SAF2005-05760).

P110122

CHEMICAL PRECONDITIONER 3-NP HAS NO ANTIOXIDANT EFFECT

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Objective: In our previous studies, 3-nitropropionic acid(3-NP), a chemical preconditioner, was found to induce a long-term myocardial protection against ischemia-reperfusion injury in rats. In the present study, we have investigated the cardioprotective effect of 3-NP depend on whether scavenging free-radicals or increasing total antioxidant capacity (TAC). Methods: In cell-free experiments, inhibition of peak chemiluminescence (CL) of hypochlorous anion (-OCl) or hydrogen peroxide (H_2O_2) by 3-NP was measured by using flow injection analysis-luminol chemiluminescence (FIA-CL). For isolated rat heart experiments, hearts were isolated 1, 2, 3, 4 or 5 days after 3-NP (20 mg/kg, i.p.) injection. TAC was measured in plasma samples. Results: 3-NP did not displayed a significant inhibitory effect on the peak CL-induced by H_2O_2 or -OCl. In addition, 3-NP administration did not increase the total antioxidant capacity. Conclusion: 3-NP might not have a direct or indirect antioxidant effect.

Key words: rat heart, 3-NP, total antioxidant capacity, FIA-CL.

Acknowledgement: This study was supported by Gazi University Scientific Projects Foundation (Project code: 11/2003-01) and L'oreal.

P110123

DEXAMETHASONE INCREASE NORADRENALINE AND DECREASE THROMBOXANE RELEASE IN MESENTERIC ARTERIES FROM SPONTANEOUSLY HYPERTENSIVE RATS.

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This study examines the mechanisms involved in the decreased contractile response induced by electrical field stimulation (EFS) in dexamethasone-treated (DEX) mesenteric arteries from spontaneously hypertensive rats (SHR). The responses to: EFS adding either L-NAME (100 μM) or Capsaicin (0.5 μM), calcitonin gen-related peptide (CGRP) (0.1nM-0.1 μM), sodium nitroprusside (SNP) (0.1nM-10 μM) and noradrenaline (NA) (0.1nM-0.1 μM) were analysed. The [^{3}H]-NA and thromboxane (TXA2) release induced by EFS and COX-2 expression were studied, and the participation of TXA2 in the decreased response to EFS was analysed in the presence of furegrelate (10 μM). DEX did not affect vasomotor responses to NA, SNP or CGRP. The effect of DEX on EFS response was not affected by L-NAME or capsaicin and was reverted by furegrelate. DEX increased [^{3}H]-NA release and decreased COX-2 expression. The results indicate that the net effect of DEX is mediated by increased NA and decreased TXA2 release. Sensory and nitrergic innervation did not seem to participate in the DEX effect.

Key words: Dexamethasone, Hypertension. Supported by FIS (PI051767 and C03/01) and (SAF2005-05760).

P110124

Higenamine reduces apoptotic cell death by induction of heme oxygenase-1 in rat myocardial ischemiareperfusion injury

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Higenamine is known to reduce ischemic damages by unknown mechanism(s). The protective effect of higenamine on myocardial I/R-induced injury was investigated. Ligation of rat left anterior descending coronary artery for 30 min under anesthesia was done and followed by 24 h reperfusion before sacrifice. I/R-in-

duced myocardial damages were associated with mitochondria-dependent apoptosis as evidenced by the increase of cytochrome c release and caspase-3 activity. Administration of higenamine (bolus, i.p) 1 h prior to I/R-injury significantly decreased the release of cytochrome c, caspase-3 activity, and Bax expression but up-regulated the expression of Bcl-2, HO-1, and HO enzyme activity in the left ventricles, which were inhibited by ZnPP IX, an enzyme inhibitor of HO-1. In addition, DNA-strand break-, immunohistochemical-analysis, and TUNEL staining also supported the anti-apoptotic effect of higenamine in I/R-injury. Most importantly, administration of ZnPP IX inhibited the beneficial effect of higenamine. Taken together, it is concluded that HO-1 plays a core role for the protective action of higenamine in I/R-induced myocardial injury.

P110125

Protective effects of rosiglitazone on endothelial function in insulin-resistant rats

Hong-Yan LING[1], Bi HU[1], Shui-Dong FENG[2], Shou-Hong ZHOU[1], Duan-Fang LIAO[3*]. 1. Department of Physiology, Nanhua University, Hengyang 421001, China. 2. Department of Epidemiology, Nanhua University, Hengyang 421001, China. 3. Division of Pharmacoproteomics, Institute of pharmacy and pharmacology, Nanhua University, Hengyang 421001, China. To investigate the effect of rosiglitazone (RSG) on endothelial relaxation function in insulin resistance (IR) rats. Male SD rats were randomly assigned to four groups for 8 weeks: a normal diet (C), a normal diet + RSG (C+R), a high fructose diet (IR), a high fructose diet + RSG. At sacrifice, physiological and biochemical parameters and vascular function test were examined. The results find: (1) Ach-induced relaxation was depressed significantly in the IR group, and the effect was reversed by RSG; after L-NAME pretreatment, ACh-induced relaxation was further impaired, the effect was partly reversed by RSG. (2) SNP-induced relaxation did not differ significantly among the groups. (3) IR rats exhibited an increase in SBP, serum insulin, triglycerides levels and aorta MDA concentration and a decreased HDL, NO levels and SOD activity as compared with CON or C+R rats; these parameters were reversed by RSG. These findings suggest that RSG can protect vascular endothelial function in IR rats, the effect might be associated with an increased ability of anti- oxygen free radicals and release of NO.

Key words: rosiglitazone; insulin resistance; endothelial function; nitric oxide; oxygen free radicals

P110126

Urotensin II inhibits carotid sinus baroreflex in anesthetized male rats

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Aim: To study the effects of urotensin II (UII) on carotid sinus baroreflex (CSB). Methods: The functional curve of carotid sinus baroreflex was measured by recording the changes of arterial pressure in anesthetized male rats with perfused isolated carotid sinus. Results: UII (30.0, 300.0, 3000.0 nmol/L) shifted the functional curve of the baroreflex to the right and upward in a concentration-dependent manner, with a reduction in peak slope (PS) and a reflex decrease (RD) in mean arterial pressure (MAP), which indicates that UII exerts an inhibitory effect on the CSB. Pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME, 100 μmol/L) cannot eliminate the inhibitory effect of UII (300.0 nmol/L) on CSB. The role of UII (300.0 nmol/L) on CSB was totally abolished by pretreatment with BIM-23127 (3.0 μmol/L), an antagonist of human and rat urotensin II receptors. Conclusion: These data suggest that urotensin II (UII) play an inhibitory role on the isolated CSB. The effect mediated by UII receptors in the vascular smooth muscle, and locally nitric oxide might not be involved.

Key words: urotensin II; carotid sinus; baroreflex; L-NAME

P110127

Urocortin II (UcnII) Increases Contractility of Mouse Ventricular Myocytes via Corticotropin-Releasing Factor Type-2 Receptor (CRF2)

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Objective: UcnII is a CRF-like peptide and highly selective for CRF2. The purpose of this study was to determine if UcnII exerts a positive inotropic effect in mouse ventricular myocytes. Methods: Changes in cell length of isolated myocytes (fractional shortening, FS) were measured by an edge detection system. Western blots were carried out on myocyte extracts with antibodies against total and phosphorylated phospholamban (PLN). Results: UcnII enhanced cell shortening in a time- and concentration-dependent manner. The inotropic effect of UcnII was maximal after 15 min at 100 nM (UcnII group n = 5 vs. control group n = 4: FS 136 ±24 % of initial control vs. 71 ±7 % of initial control, P < 0.05). The inotropic actions of UcnII were eliminated by antisauvagine-30 (10 nM), a CRF2 antagonist (UcnII + antisauvagine-30 group n = 4 vs. UcnII group n = 5: FS 67 ±11 % vs. 136 ±24 % of initial control, P < 0.05). In addition, UcnII increased phosphorylation of PLN in a concentration-dependent manner (1-100 nM). Conclusion: UcnII increases contractility in mouse ventricular myocytes via CRF2-mediated phosphorylation of PLN in a concentration-dependent manner.

P110128

Effects of angiotensin II type 1 receptor blockade on transforming growth factor-β1 in the process of developing of heart failure in rats

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We tested the hypothesis whether inhibition of the angiotensin AT1 receptor losartan, acting through myocardial expression of TGF-β1 mRNA gene, type I and type III collagen mRNAs, is sufficient to attenuate myocardial remodeling and improve hemodynamic function in rats with heart failure (HF). Four groups of rats were studied: sham controls, HF vehicle rats, HF rats treated losartan, sham rats treated with losartan. Losartan (10 mg/kg/day) was administered orally to rats from the 1st to 6th. week after the operation. Treatment with losartan increased the survival rate of rats after HF (92% versus 65% for water-treated sham). TGF-β1 mRNA, collagen type I and collagen type III expression were increased by 1.4-fold, 1.82-fold and 1.73-fold in HF, respectively, and were blunted by losartan. The findings indicate that the mechanisms by which angiotensin AT1 antagonist attenuates myocardial remodeling and improves function may be attributable by direct inhibition of the TGF-β1 mRNA, collagen type I and collagen type III mRNA expression levels.

P110129

Acute and Chronic Effects of Nicotine on NO Release in Bovine Coronary Artery Endothelial Cells

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Endothelium-derived NO is a key modulator of vasodilation in the cardiovascular system. The investigation of the relationship between nicotine and NO release could reveal an important aspect of nicotine in cardiovascular system and provide cellular mechanisms to understand tobacco smoking-associated cardiovascular disease. In the present study, we examined the effect of nicotine on basal NO release and agonist-induced NO release in cultured bovine coronary artery endothelial cells. The results showed that incubation of bovine coronary artery endothelial cells in the presence of 10 uM nicotine for 24 hours and 48 hours caused a significant decrease in the basal release of NO as compared to control cells. In contrast, nicotine showed no effect on the ATP-induced NO release. Accordingly, nicotine did not affect ATP-induced intracellular Ca^{2+} release. Further studies demonstrated that nicotine decreased eNOS expression after 24 h treatment. The results suggest that nicotine induces a reduction of NO release in coronary artery endothelial cell, which is mediated by inhibition of eNOS expression.

P110130

Endogenous hydrogen sulfide is cardioprotective against myocardial ischemia in rat

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The role of hydrogen sulfide (H_2S) in myocardial infarction (MI) has not been previously studied. We therefore investigated the effect of H_2S in a rat model of MI in vivo. Animals were randomly divided into 3 groups (n = 80) and received either vehicle, 14 mol/kg of NaHS or 50 mg/kg propargylglycine (PAG) everyday for 1 week before surgery and the treatment continued for a further 2 days after MI. The mortality was 35% in vehicle-, 40% in PAG- and 27.5% in NaHS-treated (p < 0.05 vs vehicle) groups, respectively. Infarct size was 52.9 ±3.5 % in vehicle-, 62.9 ±7.6 % in PAG- and 43.4 ±2.8 % in NaHS-treated (p < 0.05 vs vehicle) groups. In the hypoxic vascular smooth muscle cells, we found that cell death was increased under the stimuli of hypoxia but the increased cell death was attenuated by the pre-treatment of NaHS (71 ±1.2 % cell viability in hypoxic vehicle vs 95 ±2.3 % in non-hypoxic control, p < 0.05). In conclusion, endogenous H_2S was cardioprotective in the rat model of MI and the results suggest

that H_2S might provide a novel approach to the treatment of myocardial infarction.

P110131

Physiological role of Gs-triggered, cAMP-independent activation of large-conductance Ca^{2+}-sensitive K^+ (MaxiK) channel in smooth muscle relaxation.

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Molecular mechanisms responsible for the cAMP-independent relaxations mediated through G_s coupled receptors (prostacyclin receptor, IP-R; beta2-adrenoceptor, beta2-AR) were investigated in guinea-pig aorta (AOR) and tracheal (TRA) smooth muscles. cAMP-independent relaxation was elicited by beraprost (AOR, IP-R) or isoprenaline (TRA, beta2-AR) in the presence of SQ22,536 (adenylyl cyclase inhibitor), which almost totally inhibited tissue cAMP elevation by both agonists. In both relaxant responses, the SQ22,536-insensitive relaxation was significantly inhibited by MaxiK channel-selective blocker, iberiotoxin (IbTx). In side out patches from AOR smooth muscle cells, beraprost in the presence of channel internal side of GTP significantly increased the open probability (P_o) of MaxiK channel with a slope conductance of about 200 pS. MaxiK channel P_o was also increased by the treatment with a G_s activator, cholera toxin, which produced IbTx-sensitive relaxant response in the presence of SQ22,536 in both AOR and TRA smooth muscles. These results suggest that Gs-triggered direct activation of MaxiK channel substantially accounts for cAMP-independent smooth muscle relaxations mediated through IP-R and beta2-AR.

P110132

Oxidized low density lipoprotein induces apoptosis in human umbilical vein endothelial cells: potential role of reactive oxygen species

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Studies have shown that oxidized low density (ox-LDL) elicits both necrotic and apoptotic cell death and several mechanisms have been investigated. Ox-LDL induces reactive oxygen species (ROS) formation in different types of cells. This study was designed to determine whether there is an association between apoptosis and the production of ROS. After exposure to ox-LDL (50, 100, and 150 μg/ml respectively) for 18 hours, HUVECs exhibit typical apoptotic characteristics both determined by transmission electron microscopy and flow cytometry analysis. Ox-LDL increases intracellular ROS formation including superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2) in a dose and time dependent manner. Pretreatment with Vitamin C, apocynin or catalase could significantly reduce ROS production and ox-LDL induced apoptosis while indomethacin or allopurinol had no effect. These results suggest that ROS production play an important role in ox-LDL induced apoptosis and removal of ROS may account for the anti-atherosclerosis effects of some free radical scavenger drugs.

Key words: Oxidized low density lipoprotein; Apoptosis; Reactive oxygen species; Human umbilical vein endothelial cells

P110133

Protective mechanism of notogisenoside-Rg1 in hippocampi of Focal cerebral ischemia reperfusion injury in rats

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Objective: To study the effect of notogisenoside-Rg1 on brain-derived neurotrophic factor(BDNF) in hippocampi after ischemia reperfusion injury and to investigate whether Rg1 can upregulate BDNF of the positive neurons and enhance the amount of the positive neurons. Methods: SD rats were divided into model group; Rg1 group and nimodipine group. The animal model was made with thread-occluded method and the brain tissue were sliced and were stained with the immunohistochemical techniques. The content of BDNF of the positive neurons and the amount of the positive neurons in hippocampi were observed and counted. We also observe the nervous deficit symptoms. Results: Rg1 treated groups could obviously attenuate nervous symptoms and increase BDNF as well as the positive neurons in the hippocampi after ischemia reperfusion injury. The effect of Rg1 was superior to that of the nimodipine or equate it. Conclusion: Rg1 could upregulate the expression of BDNF and increase the amount of positive neurons in hippocampi. It treated cerebral ischemia by the protection of BDNF on neurons injured in the ischemia-reperfusion, which can be one of the protective mechanism of Rg1 on focal cerebral ischemia reperfusion injury.

P110134

QTcvx, an individualized QT correction, minimizes QT over-correction by QTcv (Van de Water) at extreme tachycardia in telemetric dogs

Meng Heping*, David Dodd, Charles Kasiewski. sanofi-aventis

Objectives: To modify QTcv (Van de Water) formula to minimize the over-correction of QT interval at high heart rate (HR). Methods: The ECGs were measured after 6 telemetric dogs were dosed with formoterol po. QTcv formula was modified by re-defining the fixed value of 87 as a variable. Original QTcv = QT-87*[(60/HR)-1] Modified QTcv (QTcvx) = QT-x*[(60/HR)-1] where x ranges from 5 to 150. Results: An optimum x value for each individual dog should have a slope near 0 in a regression analysis of HR-QTcvx. It could be determined after a series of regression analysis of HR-QTcvx (x = 5-150). The optimum x values were 6, 36, 47, 48, 55 and 83 for the 6 dogs, respectively and the correlation coefficient was lower than 0.065 for each dog. Comparing QTcv and QTcvx with the same QT/HR data set revealed that QTcv was prolonged by 12% with 3 mg/kg formoterol po (HR: ~200 bpm) while the QTcvx was not changed significantly. Conclusions: Individualized QT correction with QTcvx could minimize the mathematical over-correction by QTcv when HR was at 150-200 bpm in telemetric dogs. It better reflected the true QT changes independent of HR changes and necessary for future data analyses.

Key words: QTcv, dog, heart rate

P110135

Formoterol-induced extreme tachycardiac affects computerized ECG/QT measurement in telemetric dogs

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Objectives: To examine if Ponemah software could measure ECG/QTc properly during tachycardia in dogs and if QT correction formulas (Fridericia: QTcf and Van de Water: QTcv) could properly calculate QTc at extremely high heart rate (HR). Methods: Telemetric dogs were given formoterol at 1, 3 or 10 mg/kg po. Lead II ECG and blood pressure were measured for 24 h. Results: Formoterol induced tachycardia (202, 303 and 268% of the pretreatment value for 1, 3 or 10 mg/kg groups, respectively). At 10 mg/kg, formoterol resulted in severe deformation of ECG waveforms, resulting in an improper marking of waveforms by the Ponemah software. When the HR was near 200 bpm, a prolongation of QTcf by 20% and QTcv by 9-12% was observed, respectively. Regression analyses indcated that the prolonged QTcf and QTcv were at least partially due to a mathematical over-correction, rather than a pharmacological effect. Summary: Ponemah software could properly mark and measure ECG/QT waveforms when HR was below 200 bpm. At a HR range between 150 and 200 bpm, a mathematical over-correction by QTcf and, to less extent, by QTcv, suggesting the limited value of these formulas.

Key words: dog, ECG, QT

P110136

Protective effects of astragaloside IV on primary cultured cardiomyocytes injured by adriamycin

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Objective: To investigate the protective effects of astragaloside IV (AST) on primary cultured newborn rats cardiomyocytes injured by adriamycin (ADR). Methods: 2 mg/L ADR was used to damage cultured newborn rats cardiomyocytes. The cells were randomly divided into four groups: untreated group; ADR group (2 mg/L); AST group (20 mg/L); AST + ADR group. The mitochondrial membrane potential (MMP) was detected. The activity of lactate dehydrogenase (LDH) and sarcoplasmic reticulum Ca^{2+}-ATPases (SERCA) were also measured. Results: The cardiomyocytes were injured severely, but not in AST + ADR group. Comparing with that of untreated group, the activity of SERCA and MMP significantly decreased in ADR group ($p < 0.05$) and LDH activity was significantly increased ($p < 0.05$). AST pretreatment markedly increased the MMP and the activity of SERCA and reduced LDH activity ($p < 0.05$). Conclusion: AST can protect cardiomyocytes from ADR injury.

Key words: astragaloside IV; adriamycin; cardiomyocytes;

P110137

Protective effects of Astragaloside IV on cortical ischemia-reperfusion injury and expression of APE/Ref-1 in rats

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Objective: To study the effects of Astragaloside IV (AST) on cortical ischemia-referfusion (I/R) injury and expression of apurinic/apyrimidinic endonuclease/redox-factor 1 (APE/Ref-1) in rats. Methods: Focal 1h cerebral ischemia followed by 6h reperfusion was induced by middle cerebral artery occlusion in rats. AST, 1 and 4 mg/kg, was injected intraperitoneally into rats 0.5h before ischemia. Results: At the end of 6h, brain tissues were removed. Score of behavior and infarct volume of brain tissue were reduced significantly in both AST treated groups comparing with untreated group. The levels of MDA, an indicator of lipid peroxidation, were decreased markedly while the levels of SOD, an antioxidant enzyme, were increased markedly in cortical tissues with AST treatment. The protein levels of APE/Ref-1, a multifunctional protein involved in DNA repair, were increased markedly in AST treated groups comparing with untreated group. Conclusions: We suggest that AST have neuroprotective effects due to its antioxidant properties and its regulation of APE/Ref-1. APE/Ref-1 may be a drug target in treating (I/R) injury.

Keyword: Astragaloside IV; ischemia-reperfusion injury; APE/Ref-1; neuroprotective effects

P110138

The differential expression of vanilloid receptor and its function in myocardial ischemia rats

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Objective: To examine the vanilloid receptor 1 in thoracic dorsal root ganglions innervating cardiac response to ischemia. Methods: Myocardial ischemia was produced by occlusion of the left coronary artery in rats. The differential expression was evaluated by qPCR method. Results: The VR1 expression level was peaked at 6 hours post-infarct significantly and persisted for at least 4 days post-infarct. The systolic function of LVSP and LV+dp/dtmax in capsaicin-pretreated group was reduced by 46% ($P < 0.01$) and 26.4% ($P < 0.05$) respectively vs. control group. And the diastolic function of LVEDP and LVdp/dtmin in capsaicin pretreatment group was dropped by 58.8% ($P < 0.01$) and 21.5% ($P < 0.05$) respectively vs. control group. In control group, administration of capsaicin by epicardium significantly lowered cardiac function of LVSP, LV+dp/dtmax, LVEDP and LV-dp/dtmax by 39.3% ($P < 0.05$), 44.3% ($P < 0.05$), 35.3% ($P < 0.05$), and 44.6% ($P < 0.05$) respectively. Conclusion: Pretreatment of capsaicin impaired the function of VR1 to mediate sympathetic cardiac response.

Key Words: vanilloid receptor, myocardial ischemia

Acknowledgement: Project of Mega 6 Human Resource Summit, Bureau of Education, Jiangsu Province

P110139

Increased atherosclerotic lesion area in apoprotein E deficient mice superimposed by experimental renal hypertension

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It is known that hypertension is associated with an increased risk for atherosclerosis, however, little is known about the mechanisms underlying the interaction of hypertension and atherosclerosis. Thus, we developed a mouse model to assess the hypothesis that hypertension accelerates atherosclerotic lesion formation. When apoprotein E (ApoE)-deficient mice (8 wks) were submitted to 2 kidney 1-clip (2K1C) operation, arterial pressure was elevated 1-2 wks after renal arterial clipping, and remained high until 16 wks. In the histopathdogical and immunohistochemical analyses, animals with hypertension for 8 weeks showed a high incidence of foam cell accumulation in the intima of aortic sinus. The foam cells exhibited positive staining for anti monocyte/macrophage antibody and lipids, suggesting that the origin of these cells can be attributed to lipid-laden macrophages. This study shows that renal hypertension augments the development of atherosclerosis in apoE-deficient mice. The mechanisms could be direct effects of renal ischemia-derived humoral factors on cellular processes in the vessel wall or the result of hypertensive state.

Key words: Atherosclerosis, Hypertension, Hyperlipidemia, Foam Cell

P110140

Arginase Inhibition Prevents Nitrate Tolerance by Conserving L-arginine Levels

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Objective: We explored whether arginase inhibition could prevent nitrate tolerance by conserving intracellular L-arginine levels. Methods: Rat isolated aortic rings and mesenteric arteries were set-up for isometric recording. Responses to repeated applications of sodium nitroprusside and acetylcholine were obtained in the absence and presence of L-arginine or the arginase inhibitors L-NOHA (NG-Hydroxy-L-arginine) and BEC ((S)-(2-boronethyl-L-cysteine). Repeated application of both acetylcholine (ACh) and sodium nitroprusside (SNP) resulted in either a significant rightward shift (aorta, ACh; $\pm$log EC50 first vs second application: 7.74 $\pm$0.09 vs 7.26 $\pm$0.08; $P < 0.05$) or a dampening of the maximal dilatation (SNP; first vs second application: 98.56% $\pm$0.77 vs 89.61 $\pm$2.37; $P < 0.05$). These decreased responses were no longer observed in the presence of L-arginine or the arginase inhibitors (ACh; -log EC50 absence vs presence of BEC: 7.20 $\pm$0.08 vs 7.29 $\pm$0.14; P = ns; SNP: 97.25% $\pm$1.27 vs 96.6 $\pm$ 2.08; P = ns) used. Conclusion: Arginase inhibition prevents nitrate tolerance by conserving L-arginine stores, possibly reducing uncoupled eNOS and superoxide production.

P110141

Micro-domanial Interaction of Na+-Ca2+ Exchange with L-Type Ca^{2+} Channel in the Rat Ventricular Myocytes

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It is still unclear whether Na^+-Ca^{2+} Exchange (NCX), a major Ca^{2+} extrusion mechanism transporting 3 Na^+ against 1 Ca^{2+}, locates in the dyadic cleft or not in the heart. We pursue the proportion of NCX in the same Ca^{2+} micro-domain with L-Type Ca^{2+} Channel by pharmacologically isolating the inward NCX current contaminated in the ICaL in the isolated single rat ventricular myocytes patch-clamped in a whole cell-mode with or without internal dialysis with high BAPTA. 1. ICaL was activated with a 200 ms-step pulse from 60 mV to 0 mV repeated every 10 sec. SR Ca^{2+} release was blocked by 10 uM ryanodine and/or 10 uM thapsigargin. Ca^{2+}-dependent inactivation of ICaL was removed by 3 uM Bay K 8644. 2. In the absence of BAPTA, 0 Na suppressed ICaL by 34% in charge influx. 3. In the presence of 10 mM BAPTA, 0 Na still suppressed the ICaL by 16% in charge influx. From these results, it is concluded that at least 45% of NCX activity is concentrated in the same Ca^{2+} microdomain with L-Type Ca^{2+} Channel and increases the ICaL by simply producing inward current and also by slowing Ca^{2+}-dependent inactivation in the rat ventricular myocytes.

P110142

4-Hydroxynonenal induces vascular smooth muscle cell apoptosis via mitochondrial ROS formation

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Lipid peroxidation and its product such as 4-hydroxynonenal (HNE) is known to affect redox imbalance during various vascular dysfunction, however, little is known about the mechanisms by which HNE induce VSMC apoptosis. To investigate the mechanisms of apoptotic response to HNE, we tested the possibility that mitochondria are a potential source of HNE-dependent reactive oxygen species (ROS) formation in VSMC. Exposure of VSMC to HNE at various concentrations (0.1-10 uM) caused an augmented apoptosis in a dosedependent manner, and this was associated with an increased production of ROS. Both the enhanced ROS formation and apoptosis by HNE exposure were blunted by mitochondrial function inhibitors, rotenone (0.5 uM), stigmatellin (0.1 uM) and KCN (1 mM), but not by other oxidase inhibitors involving NADPH oxidase, xanthine oxidase and cyclooxygenase. In support of this concept, mitochondrial function-deficient (rho0) VSMC showed a substantial decrease in ROS formation stimulated by HNE. Taken together, these results suggest that mitochondrial dysfunction plays a key role in mediating HNE-induced VSMC apoptosis through an increased mitochondrial production of ROS.

P110143

Enhancement of endothelium-dependent relaxation in the aorta of apolipoprotein-E-deficient mice

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Apolipoprotein-E-deficient (apoE KO) mice are considered to be one of models for atherosclerosis. In this study, endothelium-derived effects on the aortas of apoE KO and its control, C57BL/6 wild type (WT) mice were compared by measuring tension and cyclic GMP (cGMP) at 2-4 months of age. In both mice, phenylephrine (PE, 10 micro M) induced more potent contraction in endothelium-denuded aortic rings than endothelium-intact strips. The PE-induced contractions in endothelium-intact aortas of apoE KO mice were less potent than those of WT mice. A pretreatment with NO synthase inhibitor (LNA) showed a tendency to intensify the effects of PE on endothelium-intact strips of the both mice. In the presence of LNA, dicrofenac, cyclooxygenase inhibitor, did not additionally increase the PE-induced contraction of the both mice. Basal level of cGMP was higher in aortas of apoE KO mice than WT mice. A pretreatment with LNA decreased the aortic cGMP level in apoE KO mice. These results suggest that endogenous relaxing mechanism probably due to endothelium-derived NO is rather enhanced in early stages of atherosclerosis.

P110144

Antagonism of PAR1 mediated antithrombotic activity in extracorporel arteriovenous shunt in the rat.

Létienne Robert*, Calmettes Yannick, Perez Michel, Le Grand Bruno. Centre de Recherche Pierre Fabre The aim of this study was to determine a putative role of PAR1 in thrombosis using a conventional arteriovenous shunt model in rat. The mean occlusion time was 616 ±21 s in the presence of vehicle. An histological analysis of thrombin confirmed the presence of platelets. The mean occlusion time of the shunt was measured in the presence of two selective PAR1 antagonists, (SCH-203099, 0.63-1.25 mg/kg, ER-112787, 0.63-5 mg/kg), a PAR4 antagonist (YD-3, 1.25 mg/kg) and a fibrinogen receptor GPIIb/IIIa antagonist, (abciximab, 0.16-1.25 mg/kg) administered i.v. 10 min before the opening of the shunt. SCH-203099, ER-112787 and abciximab statistically significantly and dose-dependently by prolonged the occlusion time (maximal variation 31 ±6, 30 ±9 and 69 ±14 %, respectively). On the other hand, YD-3 was devoid of antithrombotic activity in this model. Even at high doses, PAR1 antagonists were devoid of hemodynamic effects. To conclude, PAR1 antagonists exerted an antithrombotic activity, in the same range of potency as a GPIIb/IIIa antagonist, demonstrating that the PAR1 plays a pivotal role in the platelet aggregation-induced acute arterial thrombosis.

P110145

Anti-ischemic activities of F 15845, a new blocker of the persistent cardiac sodium current.

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F 15845 is a persistent sodium current blocker which was tested in two in-vitro models of cardiac ischemia. F 15845 (0.01 to 10 µM) was tested in veratrine-induced diastolic contracture of rat left isolated atrium. F 15845 reduced diastolic contracture with IC_{50} of 0.14 µM (n = 16) without affecting basal developed tension. At 10 µM, F 15845 fully prevented diastolic contracture (95.5 ±1.8 % inhibition, n = 4, P < 0.001) with an effect on basal developed tension (-22.2 ± 3.5 % variation, n = 4, P < 0.001). In addition, F 15845 was tested in global ischemia-reperfusion model of isolated perfused guinea-pig heart. At low concentration, no effect of F 15845 was observed on left ventricular basal function and heart rate. Higher concentrations (1 and 10 µM) reduced left ventricular pressure. During global ischemia, F 15845 reduced diastolic contracture with an IC_{50} of 0.64 µM (n = 30). These results demonstrated that F 15845 is a potent and effective compound in preventing veratrine- and ischemic diastolic contractures mediated by activation of persistent sodium current.

Key words: F 15845, sodium current

P110146

F 15845: a novel antianginal persistent sodium channel blocker

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The potential antianginal activity of F 15845 was evaluated in two models of myocardial ischaemia-induced ST segment changes, a supply ischaemia model in anesthetized rabbits subjected to a transient coronary occlusion and a demand ischaemia model in anesthetized dogs with coronary stenosis subjected to left atrial pacing. In the rabbit model, F 15845 produced highly effective, dose-dependent inhibition of ischaemia-induced ST segment elevation following i.v. (ED50 0.05 mg/kg) or oral (ED50 0.13 mg/kg) administration, without hemodynamic effects. The oral anti-ischemic activity remained significant 4 hours after a single administration of F 15845. Furthermore, F 15845 showed additive effects when co-administered with atenolol, ivabradine and nitrate derivatives. In the canine model of demand ischaemia-evoked ST segment changes, F 15845 (0.16-0.63 mg/kg) inhibited ischaemia-induced ST segment elevation from 0.16 mg/kg i.v. in the absence of cardiac hemodynamic and electrocardiographic effects. In conclusion, F 15845, a novel persistent sodium channel blocker, exerts potent antianginal activities without any hemodynamic cardiac effects.

P110147

The role of classic β-adrenoceptors in dobutamine-induced vasorelaxation in rat isolated aorta

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In present study, the role of β1- and β2-adrenoceptors in dobutamine (DBU)-induced vasorelaxation was investigated in rat isolated aorta. Dilatory response of endothelium intact rings to DBU was significantly higher than the endothelium denuded rings, especially in submaximal concentrations. Pretreatment with propranolol (PRO) (2×10^{-7} M) caused a partial inhibition of relaxant response to DBU in endothelium intact rings whereas relaxant response did not differ significantly in endothelium denuded rings. This concentration of PRO has shown to block just classic β1- and β2-adrenoceptors. It is concluded that DBU could relax rat aorta with both endothelium dependent and independent mechanisms. Although classic β adrenoceptors contribute the endothelium dependent relaxation but they are not involved in endothelium independent vasodilation induced by DBU.

Key words: Dobutamine, β adrenoceptors, rat aorta

P110148

EFFICIENCY OF CALCIUM CHANNEL BLOCKERS AT CHEMICAL AND OCCLUSION MYOCARDIAL ISCHEMIA

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The dihydropyridine calcium antagonists, which are used for treatment of cardiac insufficiency, angina pectoris, arterial hypertension, name preparations of the first line. However, always they do not appear effective. Influence of nifedipine, fenigidin, foridon is studied on the myocardium contractile function and coronal blood circulation at an occlusion (coronal artery ligation) and chemical (poisoning by the antichdinesterase preparations-POS) myocardial ischemia on anaesthetized dogs at artificial ventilation of lungs, wide thoracotomy and pericardiotomy, left heart ventricle catheterization, coronal artery selection. Their high efficiency is shown at an occlusion myocardial ischemia. The calcium antagonists potentiate negative inotropic effect and inhibition of coronal blood circulation, caused by POS, which conditioned, obviously, by ability of POS to lock not only potential-dependent but also ligand-sensitive calcium channels.

Key words: ANTAGONISTS OF CALCIUM, POS, ISCHEMIA

P110149

ALTERATION OF ENDOTHELIAL FUNCTION OF CORONARY ARTERIES UNDER HYPOXIA-REOXYGENATION: COMBINED EFFECT WITH ST THOMAS CARDIOPLEGIA AND TEMPERATURE

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We examined the effect of hypoxia-reoxygenation (H-R) with/without St Thomas cardioplegic solution (ST) on endothelium-derived hyperpolarizing factor (EDHF)-mediated function in porcine coronary microarteries (PCMA). PCMA were incubated in Krebs (I) or ST (II), either at 37°C (A) or 4°C (B), with exposure to hypoxia (PO_2 < 5 mmHg, 30/60 min in IA, 60 min in IB, IIA and IIB) followed by 30-min reoxygenation. In the presence of inhibitors of nitric oxide and prostacyclin, bradykinin-induced, EDHF-mediated relaxation and hyperpolarization were studied. H-R reduced EDHF-mediated relaxation in IA (30 min: 59.9 ±1.6 % vs. 81.2 ±3.5 %, $p < 0.05$; 60 min: 44.4 ±6.0 % vs. 82.7 ± 7.4 %, $p < 0.001$), IIA (28.9 ±1.8 % vs. 78.1 ±3.0 %, $p < 0.001$), IB (49.3 ±3.0 %, $p < 0.001$) and IIB (43.1 ±2.6 %, $p < 0.001$) with more reduction in IIA than in IIB ($p < 0.001$). EDHF-mediated hyperpolarization decreased after 60 min H-R (5.5 ±0.03 vs. 9.2 ±0.6 mV, $p < 0.05$). We concluded that 1) H-R impairs EDHF-mediated function with more impact of prolonged period; 2) ST better preserves EDHF at 4°C than at 37°C.

Key words: EDHF, Cardioplegia, Hypoxia-Reoxygenation

Supported by RGC of Hong Kong (CUHK4127/01M & 4383/03M) and St. Vincent Medical Foundation, Portland, OR, U.S.A.

P110150

Mechanism of Nitric Oxide Effects On The Myocardium

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Nitric oxide (NO) modulates cardiac functions. This study examined the mechanism of how NO affects the myocardium. The activity of the isolated right atrium and left papillary muscle from the rat heart was recorded. Tissue levels of cAMP and cGMP were measured by RIA. 8-Br-cGMP (0.1-100 mM) decreased the contractions of cardiac tissues but did not affect the sinus rate. Diethylamine NONOate (DEA) (0.1-100 mM) decreased contractions and the sinus rate of right atrium but had no effect on the papillary muscle. The effect of DEA on right atrium contractions was blocked by ODQ (10 mM), TEA (5 mM) and glyburide (3 mM). The effect of DEA on the sinus rate was inhibited by SOD (25 U/ml). DEA (0.1 mM) elevated the cGMP level in the right atrium and papillary muscle. However, the cAMP level was elevated by DEA only in the papillary muscle. This study indicated that in the right atrium the negative inotropic effect of exogenous NO depends on cGMP elevation and K^+ channels activation, but its depressive effect on the heart rate is due to oxidative signals.

Key words: NO, myocardium

* This study was supported by the Ankara University Research Foundation (2001-08-09-067)

P110151

G-TYPE NATRIURETIC PEPTIDE MEDIATES ERK1/2 PHOSPHORYLATION VIA Gi-COUPLED NATRIURETIC PEPTIDE RECEPTOR-C IN RAT AORTIC SMOOTH MUSCLE CELLS

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G-Type natriuretic peptide (CNP) is an endothelium-derived hyperpolarising factor and exerts anti-atherogenic actions including inhibition of smooth muscle proliferation, leukocyte recruitment and platelet aggregation; many of these effects are mediated via the Gi-coupled natriuretic peptide receptor-C (NPR-C; Ahluwalia & Hobbs [2005] TIPS, 26, 162). Since G-protein coupled receptors are known to govern extracellular-regulated kinase (ERK1/2) activation, in this study we investigated if CNP-NPR-C signalling regulates ERK1/2 phosphorylation and cell proliferation in primary rat aortic smooth muscle cells. CNP caused concentration- and time- dependent ERK1/2 phosphorylation that was blocked by the selective NPR-C antagonist M372049 and the Gi-inhibitor pertussis toxin. The effects of CNP were mimicked by the selective NPR-C agonist cANF4-23. CNP inhibited vascular smooth muscle growth; an effect that was not altered by the ERK1/2 pathway inhibitor PD98059.

These data show that CNP evokes an NPR-C and Gi dependent ERK1/2 phosphorylation in vascular smooth muscle cells and inhibits proliferation.

P110152

Arteriogenesis and vascular reactivity in hypercholesterdaemic rabbits following bilateral hindlimb ischaemia.

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Aims: to assess 1% cholesterol (Chol), ischaemia & their interaction on vascular function in rabbits. Diet & bilateral femoral artery (Lig) or no ligation (Unlig) groups: Normal Unlig; Normal Lig; Chol Unlig; & Chol Lig. Responses to adenosine (Aden), acetylcholine (ACh) & 5-hydroxytryptamine (5-HT) were assessed on Days 0-28 post-Lig or Unlig & arteriogenesis on Day 28. Pre Lig, dilatation to all agonists was similar in the 4 groups. By Day 7, there was a large decrease in Aden & ACh responses in Chol Lig compared to Normal Lig or Chol Unlig. 5-HT-induced dilatation was markedly attenuated in all Lig rabbits. By Day 28, Aden & ACh responses were similar between groups, but 5-HT responses were still diminished in Lig animals. Lig caused a doubling in collateral vessel number, irrespective of diet. Vessel density was increased in Chol Unlig & Lig compared to Normal groups. Conclusions: Lig with Chol caused supra-additive attenuation of dilatation with Aden & additive attenuation of ACh or 5-HT responses. Lig led to increased number & density of collateral arteries; Chol caused a further density increase suggesting an enhanced arteriogenic process.

Key words: ischaemia, hypercholesterolaemia, reactivity

P110153

Relaxation mechanism of pipoxolan on isolated rat aorta

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Pipoxolan is clinically known to relax smooth muscle, especially on uterus. In our preliminary study, we also demonstrated that pipoxolan inhibited the spontaneous contraction of isolated rat uterus induced by acetylcholine (ACh), oxytocin or PGE_2, and that induced by ACh on rat urinary bladder. However, the effects of pipoxolan on vascular smooth muscle are not clear. The mechanisms of action of pipoxolan on the isolated aorta were investigated in the present study. The vasoconstraction induced by norepinephrine (NE) or high K^+ was inhibited by pipoxolan both with or without endothelium. The relaxation of pipoxolan on high K^+ induced vasocontraction was dose-dependently enhanced by sodium nitroprusside, but inhibited by thapsigargin and cyclopiazonic acid and was not affected by methylene blue and nifidipine on NE-induced vasocontraction. From the above results, the relaxation of pipoxolan on the isolated rat aorta was not selective and might partly be via the activation of Ca^{2+}-ATPase which reuptaked the cytoplasm calcium into the sacroplasmic reticulum, then reduced the contraction of calcium and produced the relaxation.

Key words: pipoxolan, isolated aorta, vasorelaxation.

P110154

Oxygen-derived free radicals mediate endothelium-derived contractions in the femoral artery from streptozotocin-treated rats

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The present experiment was designed to study the role of oxidative stress in contractions mediated by endothelium-derived contracting factor (EDCF) in the femoral artery from both control and streptozotocin (STZ)-treated rats. Rings with and without endothelium were suspended in organ chambers for isometric tension recording, in the presence of L-NAME. In arteries from the STZ- rats, endothelium-dependent contractions were augmented and potentiated by xanthine/xanthine oxidase or tetrahydrobiopterin, suggesting that oxygenderived free radicals, potentiated EDCF in arteries from STZ-treated ratsSuch potentiation by xanthine/xanthine oxidase and tetrahydrobiopterin was not observed in arteries from contrd rats. Tiron and MnTMPyP reduced EDCF-mediated contractions while SOD had no effect. Catalase, diethyldithiocarbamic acid and deferoxamine reduced endothelium-dependent contractions. These data suggest that O^{2-}, after transformation to hydroxyl radicals, is the primary source of EDCF in the femoral artery of rats with type I diabetes.

The study was supported in part by RGC grant HKU 7524.

Keyword: endothelium-derived contracting factor; oxygen-derived free radicals; streptotozotin-induced diabetes

P110155

Laser Doppler flowmetry for assessment of myocardial microperfusion in beating rat heart

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Laser Doppler flowmetry is widely used in determining microperfusion, but rarely in the field of coronary microcirculation. In this in vivo study we assessed myocardial microperfusion by means of laser Doppler flowmetry in the beating heart of SD rats, with ascending aortic flow and other hemodynamic parameters recorded simultaneously. A needle probe with a hold was placed appropriately adherent to the epicardium of the left ventricular myocardium close to the left anterior descending coronary artery in an anaesthetized open-chest rat. The epicardial microperfusion is predominately diastolic and its phase is opposite to the ascending aortic flow. Captopril (5, 10 mg/kg, iv.) exhibited an increasing effect in the initial phase of the epicardial microperfusion. A sustained elevation in the epicardial microperfusion was observed after nifedipine (75 μg/kg, iv.) administration, which nifedipine (150 μg/kg, iv.) does not exhibit. Furthermore, an increase of cardiac output was also observed after bolus injection of both drugs. In conclusion, laser Doppler flowmetry provides a means of assessing myocardial microperfusion in beating rat heart.

P110158

Reduced Up Regulation of SP-D in response to TNF-α in senescent Endothelial Cells

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To examine the role of Surfactant protein D (SP-D) in endothelial cell senescence, its expression was measured in primary cultured porcine coronary arterial endothelial cells (PCAECs). The basal expression of SP-D was dependent on nitric oxide, PI3K/Akt and Erk pathways at an early passage, P1, since it was reduced by L-NAME (NOS inhibitor), wortmannin (PI3K/Akt inhibitor) and PD 98059 (Erk1/2 inhibitor). SP-D was upregulated by exposure of the cells to TNF-α. Both the basal expression level of SPD and its sensitivity to TNF-α were reduced in senescent PCAECs (P_4). The reduction in basal level at P_4 was reversed partially by diethylenetetraamine NONOate (NO donor) and by activation of PI3K/Akt. Western blot analysis revealed a reduced expression of eNOS, but increased expression of Akt 1/2 and Erk 1/2. Thus the reduced basal expression of SP-D in senescent PCAECs is due likely to a reduced nitric oxide synthesis despite the upregulation of PI3K/Akt and Erk.

Key words: SP-D, nitric oxide, senescent endothelial cells, in vitro aging

P110159

ENDOTHELIUM-INDEPENDENT RELAXATION ENHANCED BY ISOFLAVONE METABOLITE EQUOL

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The most abundant metabolite found in the body after soy protein intake is called equol. The objective of this study is to examine the vascular effects of equol. Sprague-Dawley rats were used in the experiments. The thoracic aortae were isolated and cut into rings of 3 mm in length. Changes in isometric tension were recorded in the isolated rings. The rings contracted more than 1.5 g to phenylephrine (1 μM) and relaxed more than 90 % to acetylcholine (1 μM) were considered suitable for experiments. Relaxation response to equol was carried out on a half-log basis. Equol produced significant vasorelaxation at 30 μM and 100 μM. Physiological concentrations of equol (0.1 μM- 10 μM) were chosen to explore their effects on other vasodilators. Relaxation responses to endothelium-independent vasodlator, sodium nitroprusside (0.1 nM- 100 μM), as well as endothelium-dependent vasodlator, acetylcholine (0.1 nM- 100 μM), were then examined. Equol significantly enhanced relaxation induced by sodium nitroprusside at 1 μM and 10 μM but there was no effect on acetylcholine. This preliminary study supported that equol can enhance endothelium-independent relaxation.

Key words: Equol; vascular; rat aorta.

P110160

Cross talk between endothelial nitric oxide synthase and constitutive arginase

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Reduced Nitric Oxide (NO) synthesis contributes to endothelial dysfunction and may be related to limited availability of L-arginine (L-Arg). By using the competitive arginase inhibitor, N-hydroxy-nor-L-arginine (Nor-NOHA) our objective was to characterize the role of constitutive arginase in regulating intracellular L-Arg supply to eNOS into human umbilical vein endothelial cells (HUVECs). The NO released at the cell surface was measured by electrochemistry. In whole cells arginase and eNOS activity were measured as the formation of ^{3}H-Urea and ^{3}H-L-citrulline consequently from ^{3}H-L-Arg. The expression of arginase mRNA was detected by RT-PCR. Arginase II was constitutively expressed in HUVECs. Nor-NOHA reduced arginase activity with maximal inhibition (40 %) and increased eNOS activity and NO release with maximal effects (48 %). When internal L-Arg pools were depleted by extracellular L-lysine, NO release was partly reduced and the Nor-NOHA activator effect was maintained, suggesting the participation of 2 distinct pools in L-Arg supply to eNOS. These results demonstrate that inhibition of constitutive arginase may be of interest to increase endothelial NO availability.

Key Words: NO, Arginases, eNOS

P110161

Impact of red wine polyphenols (RWP) on the function and structure of the rat cerebral arteriole.

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We investigated the effects of red wine polyphenols (RWP, essentially catechins and anthocyanins) on the function and structure of the cerebral arteriole with the cranial window preparation. In normotensive rats, RWP superfusion (0.01 mg/ml) had no effect on endothelium-independent vasodilatation (sodium nitroprusside, SNP) but doubled endothelium-dependent vasodilatation (adenosine diphosphate, ADP). Hemorrhagic hypotension (-17 % blood volume) produced a 40 % increase in diameter; RWP had no effect. Spontaneously hypertensive rats were given RWP (100 mg/kg per day po) for 2-3 months. RWP consumption did not modify systemic arterial blood pressure, ADP-induced vasodilatation or dilatation induced by hypotensive hemorrhage. In EDTA-deactivated arterioles, RWP produced a shift to the left in the stress/strain relationship and a 19 % increase in diameter/wall thickness ratio. In summary, RWP improve endothelium-dependent vasodilatation acutely; chronic consumption produces pressure-independent changes in wall structure and mechanics. In conclusion, the beneficial acute and chronic effects of RWP underpin the concept that red wine consumption has a favorable effect of the cerebral circulation.

P110162

Responses of rat cardiovascular system to L-DOPA and dopamine following treatment with rasagiline [N-propargyl-1R(+)-aminoindan] or selegiline

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Combined treatment of Parkinsonian patients with L-dihydroxyphenylalanine (L-DOPA) and the selective inhibitor of monoamine oxidase-B (MAO-B) selegiline has been linked to an increased incidence of hypotension and other cardiovascular side-effects. Selegiline is metabolized to amphetamines whereas the new selective MAO-B inhibitor rasagiline [N-propargyl-1R(+)-aminoindan] is metabolized to aminoindan which is devoid of amphetamine-like activity. In pithed rats, selegiline (1, 5 mg.kg^{-1}) but not rasagiline (0.2, 1 mg.kg^{-1}) significantly increased heart rate, plasma levels of noradrenaline and adrenaline, and potentiated pressor response to dopamine. Inhibition of hepatic MAO-A and -B was similar by both drugs. Given orally daily for 8 days to conscious rats, selegiline (5 mg.kg^{-1}) but not rasagiline (0.2 mg.kg^{-1}) caused a hypotensive response following L-DOPA/carbidopa (50/12.5 mg.kg^{-1}) although both drugs caused a similar inhibition of brain MAO-A and B. The catecholamine-releasing effects of selegiline may explain its hypotensive action by a CNS mechanism.

Monoamine oxidase, catecholamines, blood pressure, dopamine

P110163

p38 Kinase Rescues Failing Myocardium after Myocardial Infarction: Evidence for Angiogenic and Anti-Apoptotic Mechanisms

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Objectives: Mitogen-activated protein kinases (MAPKs) regulate critical cellular processes including stress response and cell survival of the cardiomyocytes, but their effects in post-infarction remodeling are unknown. Methods: Rats were subjected to experimental myocardial infarction by ligating the left anterior descending coronary artery. Western blots and kinase assays were used to determine MAPK activities. p38 MAPK activity was modulated by local adenovirus-mediated overexpression.

Results: Myocardial infarction resulted in a sustained inactivation of p38 MAPK. Normalization of p38 MAPK activity by cardiac-specific gene transfer after myocardial infarction significantly improved ejection fraction and fractional shortening and decreased left ventricular diastolic diameter. Normalization of p38 MAPK activity increased angiogenesis in the ischemic border zone. Apoptosis, fibrosis and infarct size were reduced. Conclusions: These results indicate that reduced p38 signaling predisposes to adverse post-infarction left ventricular remodeling. The rescue of failing myocardium with p38 kinase may be a potential new therapy for ischemic heart failure.

Key words: p38 MAPK; myocardial infarction

P110164

Vascular Inflammation Modulates α1 and β1 Soluble Guanylate Cyclase Promoter Activity in Human Aortic Smooth Muscle Cells (HASMCs)

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As a principal receptor for NO, soluble guanylate cyclase (sGC) plays a fundamental role in cardiovascular homeostasis. Yet, mechanisms regulating sGC α/β heterodimer expression in the vasculature have not been fully elucidated. We investigated the transcriptional regulation of human sGC α_1 and β_1 genes in primary HASMCs. 5'flanking regions harbouring both human α_1 and β_1 sGC genes were isolated and analysed for promoter activity using luciferase-reporter constructs. Fragments of 0.3 kb and 0.5 kb exhibited maximal promoter activity for the α_1 and β_1 sGC genes, respectively. MatInspector software was used to identify putative transcription factor (TF) binding sites in both α_1/β_1 sGC promoters. The functional significance of consensus TF binding sites was investigated by site-specific deletions. Our data reveal repressors and activators for α_1/β_1 sGC transcription under basal and pro-inflammatory conditions and in the presence of NO. These data provide a systematic analysis of human sGC promoter regulation in HASMCs, a cell system relevant to cardiovascular (patho) physiology.

Acknowledgement: Supported by the Wellcome Trust.

Key words: sGC, promoter, nitric oxide, vascular smooth muscle.

P110165

Retina Derived Relaxations Are Not Mediated By KATP and KCa^{2+} Channels In Isolated Bovine Retinal Arteries

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Retinal arterial tone is controlled by several factors including the newly discovered retinal relaxing factor (RRF). In this study we aimed to evaluate possible role of potassium (K^+) channels in the relaxant effects of retina on bovine retinal arteries. Retina was placed in close proximity to the precontracted retinal arteries that mounted in a multichamber wire myograph. To evaluate possible role of K^+ channels in the effects of retina, retinal arteries and retinas were incubated with K^+ ATP channel inhibitor, glibenclamide (GLI, 10^{-5} M), K^+ Ca^{2+} channel inhibitor, tetraetilammonium (TEA, 10^{-2} M), BK^+ Ca^{2+} channel inhibitor, charibdotoxin (CTX, 10^{-7} M), SK^+ Ca^{2+} channel inhibitor, apamin ($5x10^{-7}$ M) or a combination of CTX and apamin for 30 minutes. Retinal tissue produced acute, biphasic and complete relaxations on precontracted retinal arteries. Preincubation with the inhibitors of K^+ channels did not cause any significant difference in retina induced relaxations compared to corresponding controls. The relaxing effect of retina on bovine retinal arteries seems unrelated to the activation of K_{ATP} and KCa^{2+} channels.

Key words: Retina, potassium channels, relaxation

P110167

The influence of stevioside and bile acids on the pharmacological effects of cardioactive drugs

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Interaction of aqueous solutions of stevioside (Jaja, USA) with cardioactive drugs was studied in rats by registering changes in their electrocardiograms (ECG). Wistar rats received daily doses of 20 mg/kg (i.p.) of stevioside or physiological solution (controls), then were narcotized and connected to the ECG apparatus. The jugular vein was prepared and connected to an infusion pump to introduce one of the drugs: adrenaline (0.1 mg/ml), verapamil (2.5 mg/ml) or metoprolol (1 mg/ml) to animals of both groups, while recording their ECGs. In the animals of control group, adrenaline produced a drop in heart frequency, while with stevioside-pretreated rats this effect appeared significantly earlier. No toxic effect of adrenaline was observed, either in control or stevioside-pretreated group. Infusion of stevioside to intact animals caused no significant changes in the ECG patterns. The myocardium sensitivity to metoprolol remained unchanged in animals of all groups if compared with control, except for a mild drop in heart frequency. Stevioside produced a significant increase in the myocardium sensitivity to verapamil, but no toxicity effect was observed in any of the cases.

P110168

Resveratrol enhances cytokine-induced inflammatory responses in rat vascular smooth muscle cells

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Resveratrol, a polyphenolic antioxidant abundant in grapes, has been reported to be cardioprotective. In this study, we tested the effects of resveratrol on the functional expression of inflammatory enzymes in vascular smooth muscle cells (SMC) from normoglycaemic and streptozotocin-diabetic rats. SMC were isolated from male rat aorta four weeks after diabetes induction. In SMC stimulated with a cytokine mixture for 24 h, treatment with resveratrol (0.1 100 μM) enhanced production of inducible NO synthase (iNOS) in SMC from both animal groups. This effect was observed as well after treatment with the structurally related isoflavone genistein (1-n M- μM), which however did not increase iNOS activity in contrast to resveratrol. Inhibition of estrogen receptors (ER) by the pure antiestrogen ICI 182,780 partially reversed resveratrol action on iNOS. Resveratrol failed to alter cyclooxygenase-2 protein levels and reduced the accumulation of prostaglandin E_2 in the culture medium of SMC from normoglycaemic, but not diabetic rats. These results indicate that resveratrol enhanced inflammatory responses in vascular SMC from normoglycaemic and diabetic rats via ER-mediated pathways.

P110169

The Effects of Bumetanide on Human Umbilical Artery Contractions

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Umbilical circulation is very important for normal fetal growth and viability. We have investigated in vitro effects of bumetanide, a loop diuretic and a Na-K-2Cl cotransport (NKCC1) inhibitor, on serotonin, histamine and KCl-induced contractions in human umbilical artery (HUA). Rings of HUA segments from vaginal deliveries with normal term pregnancies were suspended for isometric tension recording in organ baths. Cumulative concentration-response curves to serotonin (10^{-8} - 10^{-4} M), histamine (10^{-8} - 10^{-4} M) and KCl (5 - 80 mM) were performed in the absence (control) or in the presence of bumetanide (10^{-5} - 10^{-3} M). The contracting agents caused concentration-dependent contractions of HUA. Bumetanide pretreatment, concentration-dependently, decreased the sensitivities and maximal contractions of HUA to serotonin and histamine. The highest concentration of bumetanide, 10^{-3} M, inhibited the maximum contractions to sero-

tonin and histamine, extent to approximately 60 %. This findings raises the possibility that NKCC1 may play a role in the regulation of the feto placental vascular tone.

P110170

TM-1 attenuates the inflammatory response in ischemia-reperfusion hearts

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Myocardial ischemia-reperfusion injury is associated with an acute inflammatory process that may be beneficial in initiating tissue repair and scar formation, but it is also known to extend myocardial injury. The investigation on male SD rats subjected to myocardial ischemia (60 min) and reperfusion (120 min) treated with TM-1 (0.05 mg/kg ~ 5 mg/kg) or with vehicle at 10 min before reperfusion were performed. TM-1 at 0.5 mg/kg was found to possess maximal effects on reducing the infarct size and plasma CK-MB levels. This beneficial effect of TM-1 was associated with increase eNOS protein levels and with reduction of iNOS and ICAM protein levels in the ischemia-reperfusion area. In vitro study, TM-1 (0.3 ~ 3 uM) significantly suppressed N-formyl methionyl-leucyl phenylalanine (fMLP)-activated human neutrophil migration in a concentration-dependent manner. The results of this study suggest that TM-1 is beneficial for the treatment of reperfusion-induced myocardial damage may be particularly mediated by inhibition of the neutrophil associated inflammation.

P110171

Stable gastric pentadecapeptide BPC157 studied for IBD (PLD116, PL14736, Pliva) inhibits thrombformation following abdominal aorta anastomosis in rat

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A stable anti-ulcer gastric pentadecapeptide BPC 157 is in inflammatory bowel disease trials (PLD116, PL14736, Pliva). Rat aortal segment between the renal and common iliac arteries was clamped and cut, and a terminoterminal anastomosis performed results after 24h with thrombat the anastomotic site, and almost no blood flow in blood vessel with apparently narrowed diameter. Contrary, gastric pentadecapeptide BPC 157 (dissolved in saline, 0/ml, 2 pg/ml, 2 ng/ml, 2 u/ml, at the site of anastomosis, 1 ml bath) shows a dose dependent effect, i.e., only a thrombotic ring at the site of the anastomosis, along with preserved blood flow with much larger than in thrombotic controls and preserved blood vessel diameter, at the range of values noted in the healthy rats (ugregimen). Conclusion. Together, an inhibition of all events related to abdominal aorta anastomosis in rat is along with this pentadecapeptide BPC 157 as an agent known to protect mucosa, endothelium, and to modulate NO system (Eur J Pharm, 332, 23-33, 1997). Likewise, with respect to virtually no toxicity in clinical studies, these findings could be likely relevant for further therapy applications.

P110173

Gonadal Hormones Modulate Mitochondrial Function in Male Rats

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Mitochondrial dysfunction and reactive oxygen species (ROS) production may underlie aging and cardiovascular disease. In female rat brain blood vessels estrogen (E) increases respiratory chain proteins and mitochondrial enzyme activities but decreases ROS. Nothing is known about effects of testosterone (T). Four groups of male rats were treated (4 wk): intact, orchiectomized (O), T-treated (O+T), and E-treated (O+E). Androgen receptors were undetectable in mitochondria. In contrast to the effect of E to increase cytochrome c protein, cerebral vessels from O+T showed no significant change in cytochrome c. Mitochondrial ROS inactivates aconitase with no effect on fumarase. Therefore, the ratio of activities of aconitase to fumarase (A/F) is a functional indicator of mitochondrial ROS. T did not alter, but E increased, the A/F ratio in brain mitochondria, suggesting decreased ROS production. We are investigating the effects of another T metabolite, dihydrotestosterone, on mitochondrial function and oxidative damage. Thus modulation of mitochondrial function and ROS production by E, but not T, may contribute to neuroprotection and affect aging and agerelated diseases such as stroke. NIH HL-50775.

P110174

Role of endothelium in urotensin II-induced relaxation of the isolated human artery

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We examined pharmacological action of human urotensin II (UII) and role of endothelium in segments of human internal mammary (IMA) and radial artery (RA) from patients undergoing coronary surgery. In prostaglandin F2α-contracted IMA and RA rings, UII caused concentration-dependent relaxation only in endothelium-intact arteries. Treatment with N^G-nitro-L-arginine plus hemoglobin and indomethacin or with charybdotoxin (IK_{Ca} and BK_{Ca} blocker) plus apamin (IK_{Ca} blocker) partially attenuated the relaxation of UII in arteries with endothelium. A combination of all five inhibitors abolished the relaxation. Guanylate cyclase inhibitor inhibited relaxation to UII in IMA. Iberiotoxin (IBX, BK_{Ca} blocker) reduced relaxation to UII and sodium nitroprusside in IMA. Thus, UII produces endothelium-dependent relaxations of isolated human arteries and the relaxation is likely mediated through endothelium-derived nitric oxide [NO] and hyperpolarizing factor. NO may activate IBX-sensitive K_{Ca} channels in human arterial smooth muscle to mediate the relaxation to UII.

Urotensin; Human artery; Endothelium

Supported by St. Vincent Medical Foundation, Portland, OR, U.S.A. & RGC of HongKong (CUHK4127/01M & CUHK4383/03M).

P110175

Pharmacological evidence for the role of eNOS in the cardiovascular adaptation in a rodent model of simulated microgravity.

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The objective of this study was to investigate the role of nitric oxide (NO) in the cardiovascular adaptations that occur to following simulated microgravity. Methods: After 14 d of hindlimb unweighting (HLU), mean arterial pressure (MAP), heart rate, carotid artery conductance (C.carotid) and iliac artery conductance (C.iliac) were measured in anaesthetised rats (or controls). Dose-response curves for MAP, C.carotid and C.iliac were constructed for acetylcholine (ACh), sodium nitroprusside (SNP), L-NAME (non-selective NOS inhibitor) and 1400 W (selective iNOS inhibitor). Results: Dose-response relationships between MAP, C.carotid/C.iliac and ACh/L-NAME were altered by HLU in such a way as to suggest that eNOS-derived NO production was increased overall, and in the hindlimb vasculature, but decreased in the cerebrovasculature. No change in response to 1400 W or SNP were observed, suggesting that changes in iNOS expression/activity or guanylate cyclase activity did not account for the observations with ACh/L-NAME. Conclusions: eNOS-derived NO is altered between the hindlimb vasculature and cerebrovasculature following simulated microgravity.

Key words: microgravity; cardiovascular adaptation; nitric oxide

P110176

Ergotamine inhibits the cardiac sympathetic outflow by alpha2A/2C-adrenoceptors and dopamine D2-like receptors in pithed rat

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Continuous intravenous (i.v.) infusions of ergotamine inhibit the tachycardic responses to preganglionic sympathetic stimulation in pithed rats. This study set out to identify the pharmacological profile of this response. The cardiac sympathoinhibition to ergotamine was: (1) unaffected by saline; (2) partially blocked by rauwolscine or haloperidol; (3) abolished by the combination of rauwolscine plus haloperidol. Moreover, in animals pretreated with haloperidol, the sympathoinhibition to ergotamine was: (1) apparently not modified by BRL44408; (2) significantly blocked by MK912; (3) completely blocked by the combination of BRL44408 plus MK912; and (4) unaffected by GR127935 given alone or in combination with rauwolscine. Therefore, ergotamine-induced cardiac sympathoinhibition seems to be mediated by alpha2A/2C-adrenoceptors and dopamine D2-like receptors, but not by 5-HT1B/1D receptors.

Key words: Alpha2-adrenoceptors, sympathoinhibition, tachycardia.

Acknowledgements: We thank Conacyt (Mexico) for their financial support.

P110177

PHARMACOLOGICAL CHARACTERIZATION OF THE VASOPRESSOR RESPONSES TO CLONIDINE, MOXONIDINE AND RILMENIDINE IN PITHED RATS

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This study has pharmacologically characterized the receptors involved in the vasopressor responses induced by clonidine, moxonidine and rilmenidine in pithed rats. For this purpose, Wistar rats were anesthetised, pithed and prepared for the measurement of blood pressure. Intravenous (i.v.) bolus injections of clonidine, moxonidine, rilmenidine and B-HT933 produced dose dependent increases in blood pressure, which were unaffected by saline. The vasopressor responses to clonidine and moxonidine, but not those to rilmenidine or B-HT933, were blocked by prazosin. Interestingly, the vasopressor responses to clonidine, rilmenidine and B-HT933, but not to those to moxonidne, were antagonized by rauwolscine. In all cases, the combination of prazosin plus rauwolscine produced a blockade similar to that produced when the antagonists were given separately. These results suggest that the vasopressor responses to: (1) rilmenidine and B-HT 933 are mainly mediated by alpha2- adrenoceptors; (2) moxonidine may involve alpha1-adrenoceptors; and (3) clonidine are mediated by alpha1/2-adrenoceptors.

Acknowledgements: This study was supported by Conacyt (Mexico).

Key words: moxonidine, clonidine, rilmenidine.

P110179

Heme Oxygenase-1 Induction Modulates NADPH Oxidase Function In vitro and In vivo

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Heme oxygenase-1 (HO-1) has potent protective effects against oxidative damage. In the present study we examined the effects of HO-1 expression on NADPH oxidase, a major source of reactive oxygen species (ROS). In apolipoprotein (E)-deficient mice, hemin (25 mg kg-1) enhanced HO-1 expression by 30- and 16-fold in aorta and kidney and this reduced NADPH oxidase activity by 25% and 50% ($P < 0.05$). In situ superoxide levels were also reduced. The effects of hemin were blocked by the HO-1 inhibitor tin-protoporphyrin (SnPP, 15 mg kg-1). In human endothelial cells, the NO donor DETA-NONOate (NO, 1 mM for 6 h) induced HO-1 expression and reduced NADPH oxidase activity. The effect of NO on NADPH oxidase was blocked by SnPP and the transcription inhibitor actinomycin D, and mimicked by bilirubin, the end product of HO-1. In the presence of NO, blockade of HO-1 expression with siRNA enhanced TNFalpha-induced ROS production. The expression of major subunits of NADPH oxidase was not altered either in vitro or in vivo. We suggest that HO-1 induction suppresses NADPH oxidase activity, and this highlights the cardiovascular protective effects of bilirubin.

P110180

The effect of thaliporphine on cardiovascular response to serotonin

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Serotonin is believed to aggravate the ischemia-reperfusion (I/R) injury by ways of inducing platelet aggregation, coronary artery constriction, and increasing the oxidative stress in cardiomyocytes. Inhibiting reuptake of serotonin and blocking serotonin receptors have been proved to protect heart from I/R injury in several studies. In the past years, thaliporphine has been proved to protect rat hearts from post-I/R injury in vitro and increases survival rate and attenuates multiple organ injury in LPS-induced endotoxaemia in vivo. In the present study, we found tha 10 nM thaliporphine attenuate the positive inotropic effect of serotonin on left atria and right atria derived from rats and guinea pigs. In langendoff perfused rat heats, 10 nM thaliporphine attenuate the vasodilating response to 10 μM serotonin but did not affect the vasoconstractile response to serotonin in rat deendothelium thoracic aorta. Thaliporphine at 1 μM was found to increase coronary blood flow of langendoff perfused rat hearts. Whether the antagonist of cardiovascular effect of serotonin contribute to the cardioprotective effect of thaliporphine in I/R animal remains to be clarified.

P110181

Regulation of Angiotensin II on Na^+, K^+-ATPase in Guinea-Pig Ventricular Myocytes

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OBJECTIVE: This investigation used freshly isolated guinea-pig ventricular myocytes to examine the regulation of angiotensin II (Ang II) on Na^+, K^+-ATPase activity and its molecular basis. METHODS: The Na^+, K^+-ATPase activity was measured by using a coupled enzyme assay method. The expressions of α_1 and α_2 isoforms and their mRNA were evaluated by RT-PCR and Western blot. RESULTS: The Na^+, K^+-ATPase activity was stimulated by acute (10 min) treatement and inhibited by prolonged (24 h) treatment with Ang II. The expression of α_1 isoform was affected by neither acute nor prolonged treatment. The expression of α_2 isoform mRNA was decreased when incubated with Ang II for 24 hours, which was abolished when preincubating with Ang II receptors 1 (AT1) blocker Valsartan, but not affected by AT2 blocker PD123,319. CONCLUSIONS: These results suggested that Ang II regulates the Na^+, K^+-ATPase activity by α_2 isoform through the AT1 receptor.

Key words: Na^+, K^+-ATPase; Angiotensin II; RT-PCR; Western blot

P110182

Age, hypertension and nitric oxide synthase (NOS) inhibition augment endothelium-derived contracting factor (EDCF) in the rat renal artery

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NO inhibits EDCF in the rat aorta. The hypothesis was tested that a chronic treatment with a NOS inhibitor increases EDCF in isolated arteries of young rats. Rats (SD and WKY, 12-16 weeks) were treated with L-NAME (60 mg/kg/d, 4 weeks). Old WKY and SHR (7-8 months) were also used. Acetylcholine (ACh, 10^{-10} to 10^{-6} M) induced concentration-dependent relaxations during contraction to phenylephrine, which were similar in SD and WKY and reduced in L-NAME-treated rats as in old WKY and SHR. At 10^{-6} to 10^{-4} M, ACh evoked a secondary increase in tension which was augmented in L-NAME-treated and old WKY compared to WKY, and in SHR compared to old WKY. In the presence of L-NAME, ACh (10^{-8} to 10^{-4} M) caused a concentration and endothelium-dependent contraction in quiescent rings, which was inhibited by indomethacin and S18886. These contractions were comparable in SD and WKY augmented in L-NAME-treated WKY and old WKY, and greater in SHR than in old WKY. These findings demonstrate the occurrence of EDCF-mediated responses in the rat renal artery. EDCF is augmented by ageing, hypertension and chronic treatment with a NOS inhibitor.

Key word: EDCF, renal artery, L-NAME, hypertension. This work is financed by FRM (France).

P110183

Polycystin-1 participates in flow-induced Ca^{2+} influx in vascular endothelial cells

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Previous studies have demonstrated that flow-induced vasodilation in rat small mesenteric arteries is Ca^{2+}-dependent. When shear stress is applied to the lumen of small mesenteric arteries, it induces an intracellular Ca^{2+} influx into the endothelial cells. However, the identity of mechanosensitive channel through which the Ca^{2+} enters the cells is not known. One of the possible candidates for the mechanosensitive channel is polycystin-1 (PC1). PC1 is encoded by Pkd1. It was reported to mediate the mechanosensation in the primary cilium of kidney cells. We hypothesized that PC1 is the mechanosensitive channel that is involved in flow-induced Ca^{2+} influx. Antibody against PC1 was raised. Its effect on flow-induced Ca^{2+} influx in H5V cells (mouse microvessel endothelial cells) as well as in rat mesenteric arteries was investigated. The endothelial $[Ca^{2+}]_i$ changes in both H5V cells and small mesenteric arteries were measured by the fluorescent indicator fura-2 AM. After incubation with Anti-PC1 but not pre-immunized serum, the flow-induced Ca^{2+} influx in H5V cells, and rat small mesenteric arteries were all abolished. These suggest that PC1 plays roles in flow-induced Ca^{2+} influx in endothelial cells.

P110184

Evidence that clonidine-like drugs peripherally inhibit the vasopressor sympathetic outflow in pithed rats

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This study has investigated the potential capability of clonidine-like drugs to inhibit the sympathetically induced vasopressor responses in pithed rats. For this purpose, male Wistar rats were pithed and prepared for measurement of blood pressure and heart rate. Then, the effects of i.v. continuous infusions of saline, clonidine, moxonidine, cirazoline, B-HT 933 or methoxamine were determined on the vasopressor responses induced by either selective electrical stimulation (2 ms, 60 V; 0.03-3.0 Hz) of the vascular sympathetic outflow (T7-T9) or i.v. bolus injections of exogenous noradrenaline (0.03-3 μg/kg). Electrical stimulation elicited frequency dependent increases in diastolic blood pressure, which remained unaffected by saline, but were significantly inhibited by clonidine, moxonidine, cirazoline, B-HT 933 and methoxamine. Interestingly, the vasopressor responses to noradrenaline, which remained unaffected by saline, moxonidine, cirazoline or B-HT 933, were significantly blocked by clonidine and methoxamine. These results suggest that the above inhibition elicited by moxonidine, cirazoline and B-HT 933, but not by clonidine, involves a prejunctional sympathoinhibitory mechanism.

P110185

Interaction between hydrogen sulfide (H_2S) and nitric oxide (NO) during the process of myocardial ischemia in rats

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In the current study, we investigated the relationship between H_2S and NO in myocardial infarction (MI). Animals were randomly sorted and received either 5 mg/kg L-NAME or 12 mg/kg Sildenafil or saline for 1 week before MI surgery and the treatment continued for a further 2 days. Mortality was 50% in L-NAME, 37.5% in Sildenafil and 45% in saline treated groups. Plasma H_2S level of Sildenafil group was significantly increased after MI compared to saline group and L-NAME group. The findings were further confirmed by the Real-Time PCR for the expression of cystathionine-gamma-lyase (CSE) which is responsible for endogenous H_2S production. We showed NO production inhibited by L-NAME could secondary down-regulate CSE gene expression and cause reduction of endogenous H_2S production after MI. On the contrary, Sildenafil was found to induce endogenous H_2S production and up-regulate CSE gene expression level. We concluded that NO-NOS and H_2S-CSE system have synergistic cardioprotective effects in MI experimental rats.

Key words: H_2S, NO, myocardial infarction. Acknowledgement: The study is supported by a research grant of Fudan University.

P110186

Molecular basis for the cardioprotective effect of KR-32568 in a rat heart model of ischemia and reperfusion (I/R) injury

Jung In-Sang*, Shin Hwa-Sup*. Depart. Applied Biochem., Coll. Biomed. & Health Sci., Konkuk Univ., Chungju, Korea The cardioprotective effects of KR-32560, a new NHE-1 inhibitor, were investigated in a rat model of I/R heart injury with special emphasis on the delineation of possible mechanisms. In isolated rat hearts subjected to 30-min global ischemia/30-min reperfusion, KR-32560 (3 and 10 μM) significantly improved reperfusion left ventricular developed pressure, end-distolic pressure and double product. These effects were accompanied by a significant decrease in malondialdehyde and an increase in activities of both glutathione peroxidase and catalase. According to SDS-PAGE/western blotting, KR-32560 significantly increased phosphorylation of both Akt and GSK3β in left ventricle reperfused for 10 min, together with a slight increase in phosphorylation of p70S6K and no effect on eNOS and p-Bad. These results indicate that KR-32560 exert protective effects against I/R heart injury by enhancing activities of antioxidant enzymes and recruiting proteins involved in RISK pathway.

Key words: KR-32560, cardioprotection, reperfusion, RISK

(Supported by the Ministry of Science and Technology through the Bio-Food and Drug Research Center at Konkuk University, Chungju, Korea)

P110187

LIPOPOLYSACCHARIDE-INDUCED VASCULAR DYSFUNCTION IN RESISTANCE ARTERIES: INTERACTION BETWEEN NOS AND COX.

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A consequence of lipopolysaccharide (LPS)-induced endotoxaemia is vascular dysfunction characterised by hyporesponsiveness to NO donors due to iNOS-derived NO-induced desensitisation of guanylate cyclase (Chauhan et al, 2003, Faseb J, 17:773). In conduit arteries of eNOS knockout (KO) mice this LPS-induced desensitisation is absent and associated with a suppression of iNOS expression and NO production. Herein we examine whether resistance arteries behave similarly. Male (B6129SV) WT and eNOS KO mice were treated with saline or LPS (12.5 mg/kg, iv, 15 h). Unlike conduit vessels, LPS-induced desensitisation of relaxation responses to the NO donor, spermine-NONOate (SPER-NO; 0.001-3 μM) was still evident in isolated mesenteric resistance arteries of eNOS KO mice ($P < 0.001$, $n > 5$). COX2 protein expression was significantly elevated in resistance arteries of LPS-treated eNOS KO mice ($P < 0.05$ vs WT; $n = 4$) and this was associated with elevation of plasma 6-keto $PGF1_{1a}$ levels ($P < 0.05$, $n = 4$). Our data suggests a regulatory role for COX2-derived PGI_2 in the control of guanylate cyclase expression/activity during endotoxaemia in resistance but not conduit arteries.

Key Words: LPS, NO, COX2.

SF is supported by The MRC, UK.

P110188

Dimethyl pyrazine ethylamine hydrochloride disaggregates platelets in vivo by stimulating prostacyclin synthesis.

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Dimethyl ethylamine hydrochloride (DPEH) induces vascular smooth muscle contractility by acting as a calcium agonist.[1] This study investigated the effect of DPEH on in vivo platelet reactivity. Platelet aggregation was monitored in pentobarbital anaesthetized wistar rats using the Technicon Autocounter. Plasma levels and in vitro synthesis of PGI2 were measured by radioimmunoassay. A bolus dose of DPEH (5 mg·kg^{-1}) i.v. caused a rapid rise in circulating platelet count with a peak increase of 20.7 ±3.2% in 5 to 7 min. Indomethacin significantly reduced but did not abolish DPEH-induced rise in platelet count. DPEH reduced collagen-induced fall in platelet count from 17.8 ±2.5% to 12.5 ±3.2%. The basal plasma level of 6-keto-$PGF_{1\alpha}$ of 26.2 ±5.1 fg·ml^{-1} was significantly increased by DPEH (5 mg·kg^{-1}) to 102.7 ±7.3 pg·ml^{-1}. The basal level of 6-keto-$PGF_{1\alpha}$ synthesis by rat aortic rings was 12.5 ±1.5 ng·mg^{-1}·hr^{-1}. DPEH caused a dose-dependent increase in aortic ring PGI_2 synthesis with E_{max50} of 4.5×10^{-5} M and 68.1 ±2.3 ng·mg^{-1}·hr^{-1} at 10^{-4} M. DPEH stimulates PGI_2 synthesis and increases the number of circulating platelets without its direct pressor effect being abolished. Supported by British Technology Group grant.

P110189

Inhibitory Effect of Epigallocatechin-3-gallate on Angiotensin II-Induced Expression of Adhesion Molecules in Vascular Endothelial Cells.

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Attachment of leukocytes to the vascular endothelium and the subsequent migration of cells into the vessel wall are early events in atherogenesis. Expression of endothelial adhesion molecules play an important role in this process. In the present study, we tested the effect of Epigllocatechin-3-gallate (EGCG) on proatherogenic agent, angiotensin II (Ang II)-induced expression of adhesion molecules in vascular endothelial cells. We showed that EGCG inhibits Ang II-stimulated VCAM-1 and ICAM-1 expression in HUVECs. Inhibition of Ang II-induced adhesion molecules expression was manifested already on the transcriptional level. EGCG pretreatment inhibited Ang II-stimulated activation of p38 MAPK and ERK 1/2, while EGCG did not exert any significant changes in activation of c-Jun N-terminal kinase (JNK). In addition, a specific p38 MAPK inhibitor, SB202190 or ERK 1/2 inhibitor, PD98059, suppressed Ang II-stimulated VCAM-1 and ICAM-1 expression. Conclusion: These results suggest that EGCG inhibits Ang II-induced adhesion molecules expression, which is regulated by p38 MAPK and ERK 1/2 signaling pathways

P110190

Neuroprotective effects of daidzein in cerebral ischemia and reperfusion e in gerbils

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AIM: To study the protective effect of daidaein (DZ) on acute ischemic and

reperfusion brain damage in gerbils. METHODS: The gerbil cerebral ischemia model was prepared by the left carotid artery occlusion and ischemic-reperfusion injury was produced by recirculating after the ligation of bilateral carotid artery for 10 min. Stroke index score was got by observing the stroke symptom during ischemia. The contents of water, calcium and sodium remained in gerbils brain were measured at 24h ischemia and reperfusion. RESULTS: Stroke index score of the ischemic gerbils was diminished and the neuronal damage was markedly improved by DZ (70 mg·kg^{-1}, ip). After 24h ischemia and reperfusion, the brain water, calcium and sodum contents in DZ group were significantly lower than that in vehicle group ($P < 0.05$ and $P < 0.01$). CONCLUSION: DZ exhibited protective effects on cerebral ischemia and ischemic-reperfusion injuries in gerbils, and its mechanism might be related to reducing the intracellular calcium, sodium and water accumulation.

Key words: daidzein; cerebral ischemia reperfusion; calcium

P110191

Nitrative Inactivation of Thioredoxin-1 and Its Role in Post-Ischemic Myocardial Apoptosis

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Intracellular proteins involved in oxidative stress and apoptosis have been demonstrated to be nitrated in diseased but not normal tissues. The aims of the present study were to determine whether thioredoxin (Trx), a novel anti-oxidant and anti-apoptotic molecule, is susceptible to nitrative inactivation and to establish a causative link between Trx nitration and myocardial apoptosis after ischemia/reperfusion (I/R). Utilizing both in vitro and in vivo models, we have demonstrated that Trx is susceptible to nitrative modification and its anti-oxidant, ASK1 binding ability and anti-apoptotic effects were inhibited after nitration. Moreover, we have demonstrated that in vivo MI/R caused significant Trx nitration and inactivation. Treatment with a novel peroxynitrite decomposition catalyst before R blocked nitrative Trx inactivation, attenuated ASK1 activation and reduced myocardial apoptosis. These results strongly suggest that nitrative inactivation of Trx plays a pro-apoptotic role under those pathologic conditions where production of RNS is increased, and that anti-nitrating treatment may have therapeutic value in MI/R injury.

P110192

Differential Activation of Ras/Raf/MAPK Pathway between Heart and Cerebral Artery in Isoproterenol-induced Cardiac Hypertrophy

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Cardiac hypertrophy contributes an increased risk to major cerebrovascular events. However, the molecular mechanisms underlying cerebrovascular dysfunction during cardiac hypertrophy have not yet been characterized. In the present study, we examined the molecular mechanism of isoproterenol (ISO)-evoked activation of Ras/Raf/MAPK pathways in cerebral artery of rabbits, and we also studied whether the activations of these signaling pathways were altered in cerebral artery, during ISO-induced cardiac hypertrophy compared to heart itself. The results show that the mRNA level of c-fos in heart and these genes in cerebral artery were considerably increased during cardiac hypertrophy. These results that the PKA activity and activations of Ras/Raf/ERK cascade as well as c-fos expression in rabbit heart during cardiac hypertrophy were consistent with previous reports. Interestingly, however, we also showed a novel finding that the decreased PKA activity might have differential effects on Ras and Raf expression in cerebral artery during cardiac hypertrophy.

P110193

Fluvastatin decreases the inflammatory status in diabetic patients

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We studied whether Fluvastatin (FLU) treatment has an effect on the inflammatory status of diabetic patients (DP). Through a cross-sectional design, 28 patients undergoing coronary artery bypass graft surgery were recruited at the Cardiac Surgery Service. Interleukin-6 (IL6), C-reactive protein (CRP) and fibrinogen were measured by ELISA. Cultured vascular smooth muscle cells (VSMC) from DP were treated with IL6 (1 ng/ml, 18h) in the presence or absence of FLU 1 mcM and COX-2 expression was analyzed (Western blotting). DPs showed high levels of CRP (0.4 mg/dl), fibrinogen (416 ±122 mg/dl) and IL6 (9.6 ±2.4 pg/ml). FLU treatment (40 mg daily) decreased serum levels of IL6 (Spearman correlation $R = 0.35$, $p < 0.05$) and fibrinogen (Spearman correlation $R = 0.44$, $p < 0.05$), but not C-RP. FLU pretreatment of hVSMC diminished IL6-induced COX-2 expression. Treatment with FLU decreases serum inflammatory markers in DP as well as the expression of COX-2 in vitro.

P110194

Pioglitazone induces apoptosis in human vascular smooth muscle cells from diabetics by involving the TGF-b pathway

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We aimed to study whether the PPAR-g agonist pioglitazone (PIO) induces apoptosis in vascular smooth muscle cells (VSMC) from diabetic patients and its relationship with TGF-b. Methods: VSMC were isolated by explants from internal mammary arteries. Apoptosis induced by PIO 100 mcM was analyzed by DNA fragmentation ELISA and Bcl-2 degradation (Western blot) in the presence or absence of SB-431542 (blocker of TGF-b receptor ALK-4/5/7). Phosphorylation of Smad2 analyzed by confocal microscopy. PIO induced apoptosis in human VSMC in 15 mM glucose-containing medium but not in 5 mM glucose-containing one. PIO also induced the phosphorylation of the TGF-b-related protein Smad2. Both effects were inhibited in the presence of SB-431542 (10 mcM). PIO induces apoptosis in human VSMC by involving the TGF-b, ALK-4/5/7 and Smad2 pathway.

P110195

Platelet adhesion to von Willebrand Factor under fluctuating flow conditions

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The central role of von Willebrand Factor (vWF) in mediating blood platelet adhesion is well established. This study was designed to investigate platelet adhesion to von Willebrand Factor (vWF) under fluctuating flow conditions. Fluctuating flow was performed at a alternate shear rate between 300 s^{-1} and 1000 s^{-1} respectively. Flowing blood at shear rates of 300 s^{-1} and 1000 s^{-1} was control as steady flow. After vWF was coated on glass coverslips as adhere surface, perfusion studies were performed in a parallel- plate perfusion chamber, and surface coverage and morphology of the platelets adhering to surface-coated vWF were observed. The results showed that, when perfusions were performed for 5 minutes, the percentage coverage of platelets in fluctuating flow was more than that at the shear rates of both 300 s^{-1} and 1000s^{-1}. Moreover, The surface consisted of mostly spread platelets under fluctuating flow, whereas the dendritic platelets were dominant at both 300 s^{-1} and 1000 s^{-1}. It is concluded that fluctuating flow can enhance platelets adhesion to vWF, which may be involved in platelet spreading.

Key Words: platelet adhesion, von Willebrand Factor, blood flow

P110197

DISCREPANCY IN THE EFFECT OF ADRENOMEDULLARY TYROSINE HYDROXYLASE INCREASE ON CONTRACTILE RESPONSES TO PHENYLEPHRINE IN RAT AORTA: A STRESS STUDY

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Tyrosine Hydroxylase (TH) is the rate limiting enzyme in catecholamine biosynthesis in sympathoadrenal system. Angiotensin II (Ang-II) and stress alter adrenomedullary TH level. We aimed to elucidate the association of the stress- or

Ang-II-induced alterations in adrenomedullary TH level with the peripheral vascular contractile responses to phenylephrine (PE). 24 male Sprague-Dawley rats were assigned into Control (C), Stress (CS; restraint stress, 2h/dx5d), Ang-II (A; 100 Ug/kg/dx5d, ip) and Ang-II + Stress (AS) groups. Thorasic aorta rings and adrenal medullae were isolated for isometric contractility and Western Blot experiments, respectively. Both stress and Ang-II increased the adrenomedullary TH level. Isolated organ experiments revealed that efficacy of PE (10^{-8} - 10^{-4} M) was not different among groups, whereas PE was more potent in A and AS, but less in CS group compared to the controls. Antagonistic potency of prazosin on PE contractions was not affected by the protocol. There is discrepancy between the effects of Ang-II and stress on adrenomedullary TH level and peripheral vascular responses to PE.

Key words: angiotensin, stress, tyrosine hydroxylase

Funded by B. U. Research Grant (DA03/28).

P110198

The protection of oxyphenamone on myocardium against ischemia-reperfusion injury

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Objective: To investigate the protective effects of oxyphenamone (oxy), a calcium sensitizer, on myocardium against ischemia-reperfusion injury (I-R). Methods: The regional I-R was established by ligaton of the left anterior descending coronary artery (LAD) followed by reperfusion (10/15 min in rats, 30/60 min in cats) and the global I-R in rat hearts was created by stopping the perfusion (40 min) followed by reperfusion (30 min). Results: Administration of oxy (infusion 1～10 μmol·L-1, iv 0.1～8 mg·kg-1) ameliorated the ventricular arrhythmia, antagonized the changes in myocardial CPK, LDH, MDA, SOD, GSH, GSH-px, ATP, PCr and mitochondrial [Ca^{2+}], improved cardiac hemodynamics and preserved the integrity of myocardial ultrastructure dose-dependently. Conclusion: Oxy could protect myocardium against I-R remarkably.

Key words: Oxyphenamone; Myocardial ischemia-reperfusion

P110199

Protection of GBE50 on cardiovascular system in rat model with hyperlipemia

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The protection of GBE50 on the cardiovascular system was investigated on the rat model with hyperlipemia. Young male Wistar rats were fed with high lipid food for 4 months before injected with VD3, then divided into several groups treated with GBE50. The serum was measured for the blood lipid and lipoprotein level by electrophoresis. The aorta and heart were checked for their pathological change and the caspase-3 expression. The anti-oxidatase activities in rat hearts were determined. The effects of GBE50 were checked on cultured endothelial cell line (bEnd.3) against the damage by lysophosphatidylcholine (LPC). The expression of caspase-3 proved that GBE50 could effectively inhibit this apoptosis induced by hyperlipemia and VD3. The anti-oxidative enzymes' activities decreased by hyperlipemia were enhanced by GBE50 in a dose-dependent manner. The damage by LPC was obviously reversed by GBE50 dose-dependently. GBE50 can inhibit the cardiovascular injury induced by hyperlipemia and produced its protective effects on the endothelia cells.

Key words: GBE50, hyperlipemia, caspase-3 expression, endothelial cell culture.

Acknowledgement: This research was funded by Chinese "863" Project (No. 2003AA2Z2032).

P110200

Estrogen stimulates the activity of nitric oxide synthase-1 and calcium-activated K+ channels in human coronary artery smooth muscle cells.

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Sex steroids exert controversial effects on cardiovascular function, but the molecular basis for acute, nongenomic effects is unclear. We have combined molecular and cellular functional studies to identify a novel target of estrogen action in human coronary artery smooth muscle cells (HCASMC): Type 1 (neuronal) NOS. Fluorescence studies demonstrated that 17beta;-estradiol (E_2) increased NO production in HCASMC, and patch-clamp experiments revealed that E_2 opens calcium-activated potassium (BKCa) channels via the cGMP/NO pathway. Expression of only the nNOS isoform was detected in HCASMC. Furthermore, coimmunoprecipation studies revealed that E_2 stimulates association of HSP90 with nNOS, whereas HSP90 inhibitors reversed the stimulatory effect of E_2 on BKCa channels. Overexpression of nNOS increased BKCa channel activity, and augmented the effect of E_2 on these channels. We conclude that estrogen opens BKCa channels in HCASMC by stimulating nNOS activity. These findings provide a mechanism to help explain how E_2 enhances coronary blood flow in patients with diseased coronary arteries.

Key words: Estrogen, coronary, BKCa channel, nNOS.

Supported by the American Heart Association and NHLBI.

P110201

HDL DECREASED ESTROGEN-INDUCED RELAXATION IN RAT AORTA

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Estrogen and HDL (High-Density Lipoprotein) have previously been reported to exert both endothelium-dependent and independent relaxations in various animal arteries. The present study examined the effect of HDL preincubation on the endothelial relaxation to 17beta-estradiol in aorta from male rats. Isometric tension was recorded in isolated aortic rings. Superoxide production was measured by lucigenin-enhanced chemiluminescence. 17beta-estradiol produced marked relaxation starting physiologically relevant concentrations (1 nM) in aortic rings with endothelium. HDL (0.001-0.3 ug/ml) also induced concentration-dependent relaxations in aortic rings. Preincubation with HDL (0.01, 0.03 ug/ml) selectively inhibited endothelium-dependent relaxation to 17beta-estradiol because endothelium-independent relaxations to 17beta-estradiol and sodium nitroprusside were not decreased in the presence of HDL. Parallelly, HDL preincubation provoked vascular superoxide production in the presence of 17beta-estradiol in aortic rings with endothelium. HDL can inhibit endothelium-dependent relaxation to 17beta-estradiol by increasing endothelial superoxide production in rat aorta.

Key words: Estrogen, HDL, relaxation, superoxide

P110202

HYPERTENSION-INDUCED VENTRICULAR REMODELING IS ASSOCIATED WITH UPREGULATION OF INTERMEDIN AND REDUCED CAPACITY OF THE DEGRADATIVE PATHWAY THROUGH NEUTRAL ENDOPEPTIDASE

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Intermedin (IMD), a recently discovered vasodilator peptide, has potential to attenuate ventricular remodeling in response to pressure overload. Its actions are mediated by the calcitonin receptor-like receptor in association with receptor activity modifying proteins (RAMPs 1-3) and a receptor component protein (RCP). Using the spontaneously hypertensive rat (SHR) model at 20 weeks of age and the normotensive WKY control, the aim was to examine expression of: (i) IMD and receptor components; (ii) neutral endopeptidase (NEP), probably an important mediator of IMD degradation. In SHR vs. WKY rats: myocyte width was greater in both left (LV) and right ventricle (RV), but in RV there were no large changes in mRNA expression; in contrast, there were significant (fold) increases in IMD (6.8) and RAMP-1 (2.5) and a 64% decrease in NEP in LV myocytes. Similarly in non-myocytes, IMD increased 8.7-fold and RCP by 98%. Increased expression of IMD and receptor components in myocytes and non-myocytes indicates an important paracrine role for the peptide in SHR myocardium. The local concentration and action of IMD may be enhanced by downregulation of NEP.

P110203

Endothelial NO regulates mitochondrial oxygen consumption in vessels and increases O_2 availability in the surrounding tissues

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OBJECTIVE: to analyse the role that endothelial NO plays in the regulation of mitochondrial O_2 consumption and O_2 availability in isolated vessels. METHODS: Mitochondrial O_2 consumption was analysed as previously described (1) in arteries from human umbilical cord (HUC) and aorta from rats (R), eNOS-KO mice (KO) and their controls (WT). O_2 was visualised in the isolated mesenteric arterial tree by confocal miscroscopy using Ruthenium Red (O_2) and Hoechst 33342 (vital cell nuclei) as markers. RESULTS: The apparent Km (10^{-6} M) for O_2

was diminished in vessels from eNOS-KO mice, without endothelium (E-) in the presence of the NOS inhibitor L-NNA 10^{-4} M or the guanylate cyclase inhibitor ODQ 10^{-4} M. An increase in O_2 concentration was observed by confocal microscopy in the tissues surrounding the isolated mesenteric arterial tree when acetylcholine or DETA-NO were added. CONCLUSIONS: Endothelial NO controls mitochondrial oxygen consumption, by not only decreasing the apparent affinity of the cytochrome c oxidase for O_2, but also increasing O_2 availability in the surrounding tissue.

P110204

Nucleobase and Nucleoside Uptake in Human Cardiac Microvascular Endothelial Cells (hMVECs): Evidence of a Novel Transporter

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The equilibrative nucleoside transporters 1 and 2 (ENT1, ENT2) are responsible for movement of nucleosides across cell membranes. ENT2 can also transport nucleobases such as hypoxanthine (HX). Regulation of HX levels by ENT2 may be important in reducing oxidative stress. We assessed the characteristics of sodium-independent nucleoside and nucleobase uptake by cardiac hMVECs. Measurement of [3H]2-chloroadenosine uptake showed these cells have ENT1 but not ENT2. Despite the lack of ENT2, [3H]HX entered the cells at a rate greater than that expected for passive diffusion. [3H]HX accumulation was dipyridamole-insensitive, but was inhibited by the purine nucleobases adenine (IC50 = 20 ±7 uM) and guanine (17 ±4 % at 1uM). In contrast, pyrimidine bases thymine and uracil had no effect on [3H]HX uptake. Under ATP-depleted conditions (to reduce metabolism), saturable [3H]HX uptake displayed a Km of 86 ±30 uM and a Vmax of 1.4 ±0.3 pmol/ul/s. These data suggest that the major route of sodium-independent HX uptake in hMVECs is through a novel dipyridamole-insensitive, purine-selective transporter.

Key words: hypoxanthine, transport, cardiac, endothelial
Supported by the Heart and Stroke Foundation of Canada.

P110205

Integrative Cardio-vascular Pharmacology and Agenda 21 of UNO

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Effective pharmacological research needs multidimensional and holistic observations. On example of MEGeffects (mercapto-ethyl-guanidine: 1-400x10-6g/ml) are demonstrated recent and earlier results (rat, chicken, etc.). 1. Circulatory system. Blood pressure reactions to hormones, (non-/nicotinic) ganglionstimulating drugs, (central/peripheral) vagal ElectroStimulation (cvES): biphasic depressor/pressor ACH/cvES and inversion of 5-HT/nicotine depr. responses, etc. 2. Organ preparations. Pos. ino-/chronotropic (frog, fish heart), inhibitory (ACH, 5-HT contractions in portal vein), but augmentory effects of neurogenic ES (10-100 Hz, 0.3s, 3s) in vas def. 3. Myocytes. Electropharmacological analysis of MEG-influence (inhibitor of NO-synthetase, cyclooxygenase, cytochrome C) on ionic channels (MP/AP, etc.; intracell. rec.) and cellular regulation (cAMP/cGMP, etc.). An effective integrative pharmacology could be realized by foundation of intern. institutes, e.g. for pharmacology (network of national inst.), promoting common research/educ. programmes, personnel, students in context of UNO-Agenda 21, leading to better health, economy, etc. in all countries.

P110206

PEROXYNITRITE MODULATION OF 72KD MATRIX METALLOPROTEASE-2 ACTIVITY THROUGH S-NITROSYLATION

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Matrix metalloprotease-2 (MMP-2) is ubiquitously expressed in the heart and its activation leads to degradation of a variety of intra- and extracellular targets. Since reactive nitrogen species activate certain MMPs through S-nitrosylation or S-glutathiolation, we tested whether MMP-2 activity is also modulated by these reactions. Low concentrations of peroxynitrite (0.3-3 μM) and SNAP (10 μM) significantly increased MMP-2 activity, whereas high concentrations (100 μM) significantly decreased its activity. GSH did not potentiate ONOOeffect, but prevented the loss of MMP-2 activity induced by high concentrations of $ONOO^-$. MMP-2 challenge with $ONOO^-$ resulted in its S-nitrosylation, as detected by biotin-switch and confirmed by mass spectrometry (Cys102 in the propeptide and Cys363 in the collagen-binding domain). DTT-sensitive S-glutathiolation of Cys102 was detected when GSH was added. In conlcusion, low $ONOO^-$ and SNAP enhance MMP-2 activity by S-nitrosylation of critical cysteine residue(s). Thus an imbalance between $ONOO^-$ and GSH in the heart can lead either to MMP-2 activation or inactivation, with consequences in the development of disease caused by oxidative stress.

P110207

Lipoxygenase-mediated generation of mitochondrial reactive oxygen species by 4-hydroxynonenal leads to vascular smooth muscle cell apoptosis

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4-Hydroxynonenal (HNE), generated by lipid peroxidation, is implicated in numerous pathological states including vascular disorders through oxidative stress, however, little is known about the signals involved in HNE-induced reactive oxygen species (ROS) generation. Thus, we determined the possibility that lipoxygenase plays a role in HNE-induced ROS generation in vascular smooth muscle cells (VSMC). The results showed that HNE (10 μM) induced ROS formation and alteration of mitochondrial membrane potential ($\delta\Psi m$), ultimately leading to VSMC apoptosis. Pretreatment with lipoxygenase (LOX) inhibitor, nordihydroguaiaretic acid (NDGA) prevented HNE-induced ROS generation in a dose-dependent manner. NDGA also blocked loss of $\delta\Psi m$ and VSMC apoptosis by HNE, indicating that LOX is closely involved in mitochondria-derived ROS production. Furthermore, we used confocal laser microscopy to estimate the ability of DNGA to attenuate HNEinduced ROS formation in mitochondria, thus, confirming the LOX-mediated ROS generation in mitochondria. These findings suggest that LOX mediates HNE-induced VSMC apoptosis by inducing mitochondrial dysfunction leading to generation of ROS in mitochondria.

P110208

Different expression character of CYP2J3, 2E1 mRNA during myocardial ischemic/reperfused in rats

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In order to understand whether cytochrome P-450(CYP) 2J3, 2E1 are involved in myocardial ischemic/reperfused(I/R) damage in vivo, rats were subjected to 40 min myocardial ischemia, followed by 0, 15, 30 min and 1 h, 3 h reperfusion. RT-PCR analysis indicated that CYP2J3 mRNA expression in left ventricles increased markedly, positively correlated with superoxide generation and the increment of serum creatine kinase(CK) activity. The localization character of CYP2J3 gene switched from appeared higher in the right ventricle physiologically to the left, the major injury region, during myocardial I/R. Nevertheless, CYP2E1 mRNA expression in the heart decreased persistently during the whole period of reperfusion. In rat livers, CYP2J3 as well as CYP2E1 gene level declined in this pathological situation. The results demonstrate that CYP2J3, 2E1 mRNA have diverse expression character during myocardial I/R in rats. The correlation analysis implied that CYP2J3 expressed in hearts may involved in reactive oxygen species (ROS) production, if possible, mediate the tissue damage during myocardial I/R.

Key words: CYP2J3; CYP2E1; myocardial reperfusion; reactive oxygen species

P110209

Dexmedetomidine-induced contraction in human internal mammary artery: involvement of α2-adrenoceptor subtypes

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Dexmedetomidine (DEX), an α_2-adrenoceptor agonist, is used for its sedative and analgesic actions in anesthesia. There are conflicting reports about its hemodynamic effects. We investigated the direct effects of DEX on isolated human internal mammary artery (IMA). DEX (10^{-9} M- $3x10^{-5}$ M) caused biphasic contraction in the endothelium denuded IMA segments in tissue baths. First phase of contraction (10^{-9} M- $3x10^{-7}$ M) was attenuated by α_2-adrenoceptor antagonist yohimbine (10^{-7} M), while second phase of contraction (10^{-6} M- $3x10^{-5}$ M) was attenuated by α_1-adrenoceptor antagonist prazosin (10^{-8} M). Incubation of segments with larger concentrations of DEX (10^{-6} M, 10^{-5} M) caused inhibition of phenylephrine (10^{-9} M- $3x10^{-4}$ M) induced contraction. In view of these findings, we conclude that DEX causes contraction by activating α_2-adrenoceptors

at lower concentrations and it may also activate α1-adrenoceptors at higher concentrations. The action od DEX on phenylephrine-induced contraction may be related to a α1-adrenoceptor antagonistic effect produced via partial α2-adrenoceptor agonistic action.

Key words: Dexmedetomidine, contraction, α2adrenoceptors, internal mammary artery

P110210

Effects of five stilbene compounds on the NO mediated vasodilation and their structure-activity relationship

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Objective: To study the effects of five stilbene compounds, that is resveratrol (RES), diethylstilbestrol (DES), tetrahydroxystilbene-glucoside (THSG), trans-stilbene (TS) and stilbene water addition (SWA), on nitric oxide (NO)-mediate vasodilation and explore the structure-activity relationship. Methods: In the rat thoracic aorta with and without endothelium, the vascular tension was observed. Results: RES, DES and THSG(1～100 μmol·L^{-1}) could dose-dependently antagonize vessel contraction induced by phenylephrine (10 μmol·L^{-1}) with the potency of THSG > DES > RES. But TS and SWA (1～100 μmol·L^{-1}) could not markedly dlate vessel. The vasodilational effect of RES, DES and THSG could be strengthened by L-arginine (1 μmol·L^{-1}), while attenuated by methylene blue (1 μmol·L^{-1}). In addition, the vascular total NO content and NOS activity were increased by RES, DES and THSG. Conclusion: These indicate that diphenyl ethylene structure and existence of hydroxyl group in diphenyl are essential for vasodilational effect and the quantity and situation of hydroxyl group is important for their potencies.

Key words: stilbene, structure-activity relationship, NO

P110211

Effects of repeated antigen exposure on endothelin-1-induced bronchial smooth musde contraction and activation of RhoA in rats

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It has been revealed that the acetylcholine (ACh)-induced, RhoA-mediated Ca^{2+} sensitization of bronchial smooth musde contraction is augmented in rat bronchial asthma which exhibits a marked airway hyperresponsiveness (AHR). However, it is not known whether or not the phenomenon is specific to ACh. In the current study, the changes in endothelin-1 (ET-1)-induced contraction and activation of RhoA in bronchial smooth muscle of repeatedly antigen-challenged rats were examined. The ET-1-induced contraction of bronchial smooth musde was significantly enhanced in the repeatedly antigen-challenged group. In normal control animals, ET-1 induced a time- and concentration-dependent translocation of RhoA to the plasma membrane, indicating an activation of RhoA by ET-1 in rat bronchial smooth musd. The level of ET-1-induced RhoA translocation was increased much more markedly in the AHR group than in the control animals. It is suggested that the augmented activation of RhoA observed in the hyperresponsive bronchial smooth musde might be responsible for the enhanced ET-1-induced contraction of bronchial smooth musde in AHR rats, as in the case of ACh-induced one.

Key words: airway hyperresponsiveness; Ca^{2+} sensitization; RhoA; endothelin-1

P110212

Nicotine-induced contraction in rat basilar artery: invdvement of endothdlial arachidonic acid metabolites

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The mechanism(s) of nicotine-induced contraction in rat basilar artery was not well analyzed. The aim of this study was to investigate the pharmacological nature of nicotine-induced contraction in rat basilar artery. The rat basilar artery was isolated from the brain and cut into a spiral strip. In the presence of endothelium-remover (saponin), the contraction-induced by nicotine was significantly attenuated. PLC inhibitors (NCDC and U-73122), iPLA2 inhibitor (BEL), COX-2 inhibitors (nimesulide, L-745337 and celecoxib) and 5-LOX inhibitor (ZM230487) attenuated the concentration-dependent nicotine-induced contraction. COX-1 inhibitors (flurbiprofen and ketoprofen), sPLA2 inhibitor (indoxam) and cPLA2 inhibitor (AACOCF3) did not affect the nicotine-induced contraction. These results clearly indicate that the nicotine-induced contraction in rat basilar artery is endothelium-dependent and the contraction is due to endothelial arachidonic acid metabolites. The endothelial arachidonic acid metabolism may play an important role in the cerebrovascular pathophysiology.

key words: nicotine, contraction, endothelium, rat basilar artery

P110213

The Effect of Synephrine, An Active Ingredient of Citrus Aurantium, on L-type Calcium Channel Currents in Single Guinea Pig Ventricular Myocytes

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Aim: To investigate the effects of synephrine on the L-type calcium currents (ICa-L) in ventricular myocytes. Methods: The effect of synephrine on ICa-L in enzymatically dispersed single guinea pig ventricular myocytes was investigated by using whole-cell patch-clamp technique. Results: synephrine significantly enhanced systolic blood pressure of rats in vivo. In myocytes, synephrine concentration-dependently increased ICa-L, with the EC50 at 22.2 µM. Synephrine didn't alter the shape of the I-V curve, reversal potential and the steadystate activation curve of ICa-L. But it markedly shifted the steady-state inactivation curve of ICa-L towards more positive potential from -17.46 ±0.44 mV to -5.51 ±0.09 mV, and accelerated the recovery of ICa-L from inactivation state, with time constant of 109.32 ±16 ms and 86.44 ±14 ms in control and synephrine, respectively. Conclusions: synephrine positively modulates the L-type Ca2+ channels in ventricular myocytes, which may contribute to the anti-shock mechanism of citrus aurantium extract.

Key words: Synephrine; Citrus Aurantium; Ventricular myocyte; L-type calcium current

Acknowledgment: This work was supported by the "85" Project Foundation. Grant No 85-919-0302

P110214

Protective effects of preischemic treatment with rosiglitazone on cerebral ischemia-reperfusion injury in rats

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AIM: To explore the protective effect of rosiglitazone (RSG) on cerebral ischemia-reperfusion injury. METHODS: The model of cerebral ischemia-reperfusion was induced by MCAO in rats for 2 h, followed by 24 h reperfusion. RSG (1 and 4 mg/kg) was administered by oral gavage daily for 1 week. The infarct volume and histopathology were determined to evaluate the brain injury. Tissue MDA, NO levels and SOD, MPO activities were determined by biochemistry method. The mRNA expressions of PPARγ, iNOS, COX-2 were measured by RT-PCR. Expression of ICAM-1, NF-κB and JNK were determined by histochemistry and western blot, respectively. TUNEL staining was employed to detect cell apoptosis. RESULTS: RT-PCR showed significant increase in PPARγ mRNA in ipsilateral cortex after reperfusion. Pretreatment with RSG corrected the disorders in morphology, reduced infarct volume, the rise of MPO, NO and MDA levels, increased SOD activity, reduced mRNA expression of COX-2 and iNOS and protein expression of NF-κB p65 and phosphorylated JNK. However, RSG had no effect on neuronal apoptosis. CONCLUSIONS: RSG might attenuate cerebral ischemia reperfusion injury by activating PPARγ/NF-κB or JNK signal transduction pathway.

P110215

Effects of serum contained Xinshu oral liquid on rat aorta smooth musde cell proliferation and rabbit platdet aggregation in vitro

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OBJECTIVE: To study the anti-ischemia mechanism of Xinshu oral liquid. METHODS: By the serum pharmacology method, added the serums contained Xinshu Oral Liquid at different time points on rat aorta smooth muscle cell, cultured for 48 hours and used the MTT methods to investigate the effects of cell proliferation. By the serum pharmacology method, the effects of serum contained Xinshu oral liquid on rabbit platelet aggregation caused by both arachidonic acid (AA) and adenosine diphosphate (ADP). RESULTS: The results showed that serum contained Xinshu oral liquid at different time points could obviously inhibit

the proliferation of aorta smooth muscle cell ($P < 0.05$) and the platelet aggregation rate induced by arachidonic acid and ADP ($P < 0.05$) compared with control and positive control. CONCLUSIONS: The anti-ischemia mechanism of Xinshu oral liquid was concerned with the inhibition of the aorta smooth muscle cell proliferation and platelet aggregation.

P110216

Protective effect of DAXXK on the experimental acute cerebral ischemia

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AIM: To reconstruct the experimental acute cerebral ischemia model of mice, study protective effect of DAXXK on the experimental acute cerebral ischemia. METHODS: Divide the mice into five groups randomly: normal contrasting group, cerebral ischemia group, nimodipine treating group, DAXXK treating groups. The mice of DAXXK group were given gavages by DAXXK once a day, 7 days after, determine cerebral homogenate SOD, MDA, GSH-PX and cerebral index of mice. RESULTS: Cerebral index of mice, SOD and GSH-PX of preconditioning groups of mice was increased obviously compared with that of cerebral ischemia model group, there was a significant difference ($p < 0.01$); Cerebral homogenate MDA significantly lower, the difference had remarkable significance ($p < 0.01$), the difference had remarkable significance. CONCLUSION DAXXK might have a protective effect on the damage of cerebral ischemia.

Key words: DAXXK; Cerebral ischemia; SOD; MDA; GSH-PX

P110217

Involvement of EDHF in relaxing peripheral resistant vessels of the rat hind limb

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The involvement of NO and EDHF in the endothelium-dependent relaxation (EDR) was examined in rat hind limb perfusion model. The CCh-induced relaxation was abolished after denudation, but resistant to either LNA or indomethacin. However, the relaxation was significantly inhibited by a K^+ channel inhibitor and under the depolarization with KCl. Furthermore, charybdotoxin (CTX) in combination with apamin (APM) diminished the CCh-induced relaxation. The SNP-induced relaxation was accompanied by the increase in the cyclic GMP production, but not CCh. Low concentrations of KCl produced a relaxation. An activator of the K^+ca channels also produced relaxation, which was inhibited by CTX and under the depolarization with KCl. Catalase did not inhibit CCh-induced relaxation and H2O2-indused relaxation was different from CCh-induced one. Inhibitors of cytochrome P450 monooxygenase inhibited the CCh-induced relaxation. These results suggest that CCh produces an endothelium-dependent, EDHF-dependent and NO-cyclic GMP independent relaxation and that K^+ ion and metabolites of P450 monooxygenase play an important role for this relaxation.

P110218

Recovery of the down-regulated FKBP12.6 and SERCA2a and acute heart failure in sepsis by a novel endothelin receptor antagonist CPU0213 in rats

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The acute heart failure (AHF) crucially affects the morbidty and mortality in patients of septic shock, which could be mediated by an activated ET system. The aim of study was to test the effectiveness of CPU0213 in attenuating the septic AHF by up-regulating the FKBP12.6 and SERCA2a. The septic AHF was caused by acute peritonitis by puncturing the cecum for 72h. CPU0213 (30 mg/kg/d, q12h, sc ×3d) was administered in rats at 8h after operation. In the untreated model group, survival rate decreased markedly ($P < 0.01$), the hemodynamics were compromised seriously ($P < 0.01$). The mRNA and protein expressions of FKBP12.6, SERCA2a and PLB were down-regulated significantly ($P < 0.01$&$P < 0.05$) in accompanied with the elevated ET-1 concentration and the mRNA levels of the preproET-1, ECE and ETAR and ETBR ($P < 0.01$) in the LV tissue. All of the abnormalities were reversed significantly after CPU0213 administration. CPU0213 improves significantly the cardiac insufficiency associated with up-regulating expression of FKBP12.6, SERCA2a and PLB by blocking both the ETAR and ETBR.

Key words: CPU0213; septic shock; AHF; gene and protein expression.
Acknowledgement: The research is supported by NSFC (No: 30572193).

P110219

Antithrombotic Effects of Polydatin and its Possible Mechanisms

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Antithrombotic effects of polydatin (PD) and its mechanisms were investigated. Injection of arachidonic acid (AA), electrically stimulated carotid thrombosis and inferior vena ligation were used to evaluate PD's antithrombosis; platelet aggregation was tested by Born's method; platelet cytosolic calcium was determined by fluorometry; thromboxane B_2 (TXB_2) and 6-keto-$PGF_{1\alpha}$ level was monitored by immuno-assay; rosette assay and Born's method were used to observe platelet-neutrophil interactions. PD protected against thrombosis in above models. In vitro and vivo PD inhibited platelet aggregation induced by AA and ADP. PD lowered both the influx of extracellular calcium and the mobilization of calcium from intracellular stores. PD decreased TXB_2 and increased 6-keto-$PGF_{1\alpha}$ level. PD also decreased the binding of platelets to neutrophils and suppressed platelet aggregation stimulated by activated neutrophil suspension. It is suggested that PD have evident antithrombotic effects and the mechanisms may be related to its anti-platelet aggregation, decrease of platelet cytosolic calcium, decrease of plasma TXB_2 while increase of plasma 6-keto-$PGF_{1\alpha}$ Level and suppression of platelet-neutrophil interactions.

Key words: polydatin; thrombosis; platelet; neutrophils
Acknowledgement: The project supported by Natural Science Foundation of Yunnan Province (2004C0019Q), the United Cultivation Base of Yunnan Province for Innovative Talents of Medicine & Biotechnology and Pharmacological Innovative Group Foundation of Kunming Medical College (KMC2005DG04).

P110220

Pharmacodynamic Studies of Thrombolytic Properties of HTU-PA

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Objectives: To study the therapeutic effect of HTU-PA on hamsters with pulmonary embolism and rabbits with jugular vein thrombosis. Methods: pulmonary embolism model of hamsters was induced by injecting a clot from the jugular vein catheter. A rabbit model with jugular vein thrombosis was induced by infusing fresh human plasma of 0.3 ml with 10 μl 125I-labeled human fibrinogen into the vein segment followed immediately by addition of 100 μl of a mixture containing bovine thrombin (50 NIH U/ml) and $CaCl_2$ (25 mg/ml). Results: HTU-PA displayed an obvious thrombolysis in hamsters with pulmonary embolism and rabbits with jugular vein thrombosis and the values of which were higher than rt-PA at the same doses. Conclusions: The results indicate that HTU-PA has a dose-response thrombolysis in hamsters with pulmonary embolism and rabbits with jugular vein thrombosis, the thrombolytic rate of which is higher than that of rt-PA at the same dose.

Key Words: HTU-PA, Thrombolysis, pulmonary embolism, jugular vein thrombosis

P110221

Pharmacodynamic Studies of HTU-PA

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Objectives: To investigate the therapeutic effect of HTU-PA on focal cerebral ischemic injury and coronary artery thrombosis Methods: The canine model of coronary artery thrombosis and the focal cerebral ischemic model were induced by electrical stimulation and photothrombotic middle cerebral artery occlusion respectively. Results: HTU-PA displayed a dose correlation therapeutic effect in the dogs with coronary artery thrombosis. The activity of plasmin, α2-AP and PAI and the quality of FDP, plasminogen and fibrinogen showed a dose-response increase. However, the extents of decrease of PAI, Fg and increase of FDP were less than those of rt-PA, showing the probability of the side effect about bleeding may less than that of rt-PA. HTU-PA also decreased the brain infarct size, improved the neurobehavioral deficit in rats and the therapeutic effect was better than that of rt-PA. Conclusions: The results indicate that HTU-PA had a obvious dose-response therapeutic effect on dog coronary thrombosis and focal cerebral ischemic injury by intravenous bolus injection and HTU-PA are more effective than rt-PA at the same dose.

Key Words: HTU-PA, Focal cerebral ischemia, Coronary artery thrombosis,

P110222

Therapeutic Effects of FNS on Focal Cerebral Ischemia/Reperfusion Injury in Rats

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Objective: To investigate the neuroprotective effect of FNS on focal cerebral ischemia/reperfusion injuries in rats. Methods: Transient focal cerebral ischemia was induced in rats by 240 min occlusion of middle cerebral artery, followed by 20hr reperfusion. Vehicle (saline), FNS (5,10,20 mg/kg) or nimodipine (2 mg/kg) was administered iv. at 120 min after the onset of ischemia. At the end of reperfusion period, neurological deficit score (NDS) test was performed, then under deep anesthesia the brain was removed and prepared for the evaluation of cortical infarct volumes using triphenyltetrazolium chloride staining and cerebral histopathological change. Results: Postischemic intravenous administration of FNS 5-20 mg/kg significantly reduced infarct volumes ($P < 0.05$ or 0.01, and also effectively improved NDS ($P < 0.05$ or 0.01). Conclusion: FNS possessed neuroprotective effects against focal cerebral ischemia/reperfusion injuries.

Key Words: focal cerebral ischemia, FNS, thread occlusion

P110223

Influence of Hydroxysafflor yellow A on contraction of isolated ileac lognitudinal muscle and rings of vascular

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Objective: To observe the influence of Hydroxysafflor yellow A (HYSA) on KCl-induced contraction of rings of vascular and glutamic acid-induced contraction of isolated ileac longitudinal muscle. Methods: Take the thorax-aorta of rats, cut into 4 to 5 mm long rings of vascular and take the guinea pigs after fasting for 24 hours to take 40 cm length of ileum and split out ileac lognitudinal muscle carefully. Then observe and record the contraction curves of HYSA on KCl-induced contraction of rings of vascular and glutamic acid induced contraction of isolated ileac longitudinal muscle. Conclusion: HYSA with different concentration has no suppression effect on the KCl-induced contraction of rings of isolated thorax-aorta while it has suppression effect on the glutamic acid-induced contraction of isolated ileac lognitudinal muscle in positive correlation with its dose.

Key Words: Hydroxysafflor yellow A, ileac lognitudinal muscle, rings of vascular

P110224

Effects of L-carnitine on hemodynamic functions in ischemic-reperfused isolated rat hearts

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Effects of L-carnitine (L-Car) on cardiac hemodynamic functions were investigated during 30 min regional ischemia followed by 120 min reperfusion in isolated rat hearts. The hearts were perfused by drug free or L-Car enriched Krebs-Henseleit solution during ischemia and reperfusion (Protocol 1), 10 min before and after ischemia (Protocol 2) or reperfusion (Protocol 3). Perfusion of L-Car in protocol 1 significantly reduced left ventricular end diastolic pressure, increased left ventricular developed pressure and rate pressure product ($p < 0.05$ for all). Short time pre-ischemic application of L-Car (Protocol 2) improved some cardiac functions; however, its pre-reperfusion usage had lower effects compared to the other protocols. Beneficial effects of L-Car were reversed by Etomoxir (a CPT-I inhibitor) or Ranolazine, suggesting intramitochondrial action of L-Car. Among the potential cardioprotective mechanisms for L-Car, activation of pyruvate dehydrogenase (PDH), increase in glucose oxidation and fatty acid metabolism, reduction of fatty acid metabolites and oxygen free radicals are more relevant.

Key words: L-carnitine, hemodynamic factors, ischemia reperfusion, isolated rat heart

P110225

Anti-remodeling Effect of Berberine on the Cardiac Hypertrophy Model Induced by Pressure-Overload in Rats

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Our previous studies showed that Berberine could improve abnormal cardiac function. In this study, we intend to explore its influence on left ventricular remolding. Cardiac hypertrophy was induced in male SD rats by suprarenal abdominal aorta constriction and the sham-operated rats were used. The chemicals were orally administered for 10 weeks starting from 2 weeks after surgery at dosage of Berberine 5,10,20 mg/kg and Captopril 50 mg/kg. Cardiac index, left ventricular front wall thickness and hydroxyproline (Hyp) content in left ventricular tissue were measured. Compared with the sham-operated rats, the cardiac index, left ventricular front wall thickness and Hyp content of the model rats increased significantly, which indicated that left ventricular remodeling occurred after suprarenal abdominal aorta banding. With treatment of Berberine, all the indcators above were improved in close-dependent manner. It suggested that Berberine had beneficial effect on alleviating left ventricular remodeling by decreasing collagen volume in left ventricular tissue.

Key words: Berberine, left ventricular remodeling.
The study was supported by the NSFC Grant of China.

P110226

Angiotensin II stimulates the expression of vascular cell adhesion molecule-1 and E-selectin by AT1 receptor in brain microvascular endothelial cells

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The objective of study was to investigate the effect and mechanism of angiotensin (Ang) II on vascular cell adhesion molecule-1 (VCAM-1) and E-selectin expression in brain microvascular endothelial cells (BMEC). The experiment was performed in cultured rat BMEC. The mRNA and protein expression of VCAM-1 and Eselectin in BMEC was analyzed by RT-PCR and western blotting respectively. The result showed Ang II stimulated mRNA and protein expression of VCAM-1 and E-seletin in BMEC significantly. These effects were abolished by pretreatment with the selective AT1 receptor antagonists losartan and EXP-2528, or losartan plus the AT2 receptor antagonist PD123319, but not by PD123319 alone. Moreover, there were no significant differences between the losartan and losartan plus PD123319 groups. These findings indicate that Ang II upregulated VCAM-1 and E-seletin in BMEC by activating AT1 receptor and then involved in the development of cerebrovascular disease.

Key Words: brain microvascular endothelial cells; angiotensin II; vascular cell adhesion molecule-1; E-selectin.
Acknowledgements: This work was financially supported by the national Natural Science Foundation of China, No 30572187.

P110227

The protective effect of ischemic post-conditioning on long-term heart preservation

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The purpose was to assess whether ischemic post-conditioning as same as mitochondrial KATP channel as an additive to cardioplegia solution could enhance myocardial portection during long-term hypothermic preservation of the rat heart. Langendorff model isolated rat heart was used. After 30 min stabilization of perfusion, the hearts were stored in Celsior cardioplegia solution at 4 °C with or without diazoxide, a mitoKATP channel opener, for 8h followed by 1h reperfusion. Ischemic post-conditioning was done before reperfusion. (1) Ischemic post-conditioning treatment improved the recovery of left wentricular developed pressure and $\pm dp/dt_{max}$ dose-dependently. Left ventricular end-diastolic pressure was lower in Ischemic post-conditioningtreated hearts than in Celsior solution (2) The leakage of myocardial enzymes in the coronary effluent was significantly reduced in ischemic post-conditioning-treated hearts. (3) The cardiac effects of ischemic postconditioning were attenuated by a mitoKATPblocker 5-hydroxydecanoate. These results indcate that ischemic post-conditioning could enhance myocardial protection during long-term hypothermic heart preservation via opening of mitachondrial KATP channel.

P110228

Characterisation of RAMP2 transgenic mice in a LPS model of sepsis

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The vasodilatory peptide adrenomedullin (AM), acts on receptors composed of calcitonin receptor-like receptor (CL) and a receptor activity modifying protein (RAMP1, 2 or 3)1. The role of CL/RAMP2, the AM1 receptor, is unclear. We have evaluated RAMP2 transgenic (RAMP2-TG) mice, which have en-

hanced responses to AM2, in a lipopolysaccharide (LPS) model of sepsis. LPS-induced hypotension, as assessed by tail cuff plethysmography, was significant in RAMP2-TG ($p < 0.05$ compared to vehicle), but not in WT, mice 1.5h following LPS. After 4h, both groups had significant and comparable hypotension. However, decreases in temperature were attenuated in RAMP2-TG mice ($p < 0.05$) at both times. Elevations in nitric oxide (NO) in peritoneal exudate fluid (Greiss assay) and in the lungs (L-citrulline assay), were similar in WT and RAMP2-TG mice. These results suggest that the AM1 receptor can influence events in sepsis, but there's little evidence for an effect on NO.

P110229

NEUTRALIZATION OF IL-18 INHIBITS INJURY-INDUCED NEOINTIMA FORMATION

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We investigated the effective role of IL-18 in neointima formation after balloon injury in rats. IL-18 and IL-18Ralpha/beta mRNA and the active form of IL-18 were highly expressed in injured arteries from day 2 to 14 after angioplasty. Strong immunoreactivity for IL-18 was detected in the medial smooth muscle cells (SMC) at day 2 and 7 after balloon injury and in SMC in neointima at day 14. Moreover, serum concentrations of IL-18 significantly increased after vascular injury. Rats treatment with neutralizing rabbit anti-rat IL-18 IgG significantly reduced by 27% ($P < 0.01$) neointima formation 14 days following angioplasty. In addition, IL-18 neutralization reduced number of proliferating cells, inhibited IFN-gamma, IL-6, IL-8 mRNA expression and nuclear factor-kB activation in injured arteries. These results identify for the first time a critical role for IL-18 in neointima formation after balloon injury in rats suggesting a potential therapeutic role for IL-18 neutralization in vascular injury.

Key words: neointima formation, interleukin-18.
Supported by the Italian PRIN 2003.

P110230

Potential modulation by angiotensin II of pressor responses mediated by alpha-1D and alpha-1A adrenoceptor subtypes in pithed rats.

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This work analyzed the effect of the angiotensin-converting enzyme inhibitor, captopril (CAP), on pressor responses mediated by alpha-1D and alpha-1A adrenoceptors in pithed rats. Male Wistar rats were pithed under pentobarbital anesthesia and prepared for blood pressure recording and intravenous (i.v.) drug administration. A dose-response curve to the alpha-1D and alpha-1A adrenoceptor agonists, buspirone (BUS) and oxymetazoline (OXY), respectively, was built in animals that had received either saline (1 ml/kg, i.v.) or an antagonist (1 mg/kg, i.v.) for alpha-1D (BMY7378; BMY) and alpha-1A (5-methyl-urapidil; 5-MU) adrenoceptors; this protocol was performed in rats pretreated with saline (1 ml/kg, i.v.) or CAP (5 mg/kg, i.v.). CAP significantly decreased pressor responses to BUS but increased those to OXY; also, CAP strongly increased the inhibitory effect of 5-MU and slightly increased that of BMY against BUS- and OXY-induced effects. Taken together, these data suggest that angiotensin II may modulate pressor responses mediated by alpha-1 adrenoceptor subtypes in opposite ways, namely, promoting facilitation and depression of alpha-1D and alpha-1A adrenoceptor-mediated effects, respectively.

P110231

Relationship between the changes in systemic blood pressure and autonomic nervous activities in posture change.

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A high sympathetic activity during underperfusion on the cardiac function is deleterious, particularly in diabetic hearts. Then, an assessment of the autonomic nervous activity may be significant in individual care. In the present study, we investigated relationship between the changes in systemic blood pressure and autonomic nervous activities in posture change, because the sympathetic acceleration is individual. Autonomic nervous activities and heart rate were assessed by power spectral analysis of heart rate variability during posture change from supine to standing positions in healthy young volunteers. The continuous noninvasive tonometric blood pressure was measured on the radial artery. Many subjects showed temporal hypotension immediately after standing and fast recovery along with increased sympathetic activity, and some subjects showed slow recovery from the hypotension with delayed markedly high sympathetic activity. Some other subjects showed temporal hypertension rather than hypotension with lower sympathetic activity. The results indicate that higher sympathetic activity in standing is related to slow recovery from the hypotension.

P110232

Cardiovascular characterization of the adenosine A1 receptor knock-out mouse

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Aim: To investigate the role of adenosine A_1 receptor (A_1R) in cardiovascular system. Methods and results: Awake A_1R knock out (A_1R-/-) mice had a normal heart rate (HR) and body temperature. Administration of the adenosine receptor agonist R-PIA resulted in a decrease of HR and body temperature which was less pronounced in A_1R-/- mice than in A_1R+/+. After addition of the βadrenergic receptor blocker timolol, HR was less reduced in the A_1R-/- mice than in A_1R+/+. HR was higher in Langendorff-perfused A_1R-/- hearts compared to A_1R+/+ hearts. There was no evidence for major structural changes using echocardiography. In aortic rings an adenosine analogue caused contractile response, which was eliminated in aortas from A_1R-/- mice. In mesenteric arteries no contractile response was seen and adenosine mediated relaxation was identical between genotypes. Conclusion: Adenosine A_1 receptor appears to play only rather minor role in cardiovascular system under basal conditions, but may be essential in pathophysiologic processes.

Key words: Adenosine, blood pressure, heart rate, blood vessel

P110233

EGCG inhibits cardiac apoptosis, telomere erosion and TRF2 loss in pressure overload induced cardiac hypertrophy in rats

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Aim: To investigate the effect of epigallocatechin gallate (EGCG) on telomere dysfunction mediated apoptotic signal in cardiac hypertrophy. Methods: Cardiac hypertrophy was induced by abdominal aortic constriction in rats and monitored at 3, 5, 7 weeks postoperation. Cardiac apoptosis was evaluated by TUNEL. Telomere length was measured by southern blot. TERT mRNA expression was detected by in situ hybridization. Western blot was used to determine telomere repeat binding protein2 (TRF2), bcl-2, c-myc and p53 protein. Results: Progressive cardiac apoptosis and telomere attrition was found in hypertrophic myocardium, whereas EGCG 50, 100 mg/kg administered for 6 w markedly reduced apoptotic cardiomyocyte and prevented telomere erosion. No significant alteration of TERT mRNA was found, whereas progressive TRF2 attrition was revealed and the level reduced to 17.3% of control at 7w. Progressive upregulation of p53, c-myc and downregulation of bcl-2 were also found, while EGCG 50, 100 mg/kg inhibited all these alterations remarkably. Conclusion: EGCG attenuates cardiac apoptosis in hypertrophic myocardium through inhibiting telomere erosion and TRF2 loss.

Key words: EGCG; cardiac hypertrophy; telomere; TRF2

P110234

Optimization of G protein inhibitory polypeptide and its activities on cardiac hypertrophy

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G protein inhibitory peptide (GCIP) which was cloned in our lab previously could inhibit myocardiocyte hypertrophy in vitro. Bioinformatics methods were used to analyze the physicochemical properties and structure of GCIP and designed polypeptides. Several cardiac hypertrophy models in vitro and in vivo were prepared to evaluate effects of selected peptides. The results showed there were 2 hydrophilicity peak, 3 hydrophobic clusters, 2 αhelixes and 3 βturns in GCIP. The highest solvent accessibility area located between position 14 and 18, while highest flexibility located 15 and 30. Base on analyses, 51 polypeptides were designed and two (GCIP-27 and -31) were selected. GCIP-27 decreased the diameter, protein content and synthesis rate of myocardiocytes markedly compared with norepinephrine (NE) or Ang II group, while GCIP-31 could not. Compared with model groups (NE or abdominal aortic stenosis group), GCIP-27 decreased heart weight, left ventricular weight, heart index, left ventricular index significantly in

mice and rats. In conclusion, GCIP-27 was the most optimized peptide of GCIP, could improve cardiac hypertrophy in vitro and in vivo.

Key words: cardiac hypertrophy; bioinformatics; polypeptide

P110235

Pharmacological characterization of potassium channels regulating arteriolar myogenic tone in vitro **and** in vivo

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The aim of this study was to establish the functional role of the various K^+-channel subtypes contributing to myogenic tone of skeletal muscle arterioles both in vitro and in vivo. For in vitro studies, arterioles (1A) isolated from the rat cremaster muscle were maintained at an intraluminal pressure of 70 mmHg. Measurements of intraluminal diameter were made using video microscopy. In vivo studies were performed using video microscopy of the exteriorized rat cremaster muscle (1A, 2A and 3A arterioles). The BKCa inhibitors TEA (1 mM) and iberiotoxin (0.1 μM) and the Kv blocker 4-AP (1 mM) each constricted arterioles in vitro by approximately 15%. Neither the K_{IR} inhibitor Ba^{2+} (50 μM) nor the K_{ATP} blocker glibenclamide (10 μM) caused constriction of arterioles in vitro. In the in vivo preparation, TEA constricted all arterioles (1A, 2A, 3A) by approximately 15%; 4-AP had no effect on 1A arterioles but did constrict 2A and 3A and Ba^{2+} caused a transient constriction of all arterioles. These studies suggest BKCa and Kv channels are active in vessels with myogenic tone in vitro and in vivo, although their role in regulation of tone is unclear; K_{IR} and K_{ATP} channels are not active in iv vitro preparations, but K_{IR} are active in vivo.

Key words: microcirculation; arteriole; myogenic tone; potassium channel.

This work was supported by the National Health and Medical Research Council of Australia.

P110236

Leukemia inhibitory factor induces endothelial differentiation in cardiac Sca-1 + stem cells

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The importance of interleukin-6 (IL-6)-related cytokines in cardiac homeostasis has been extensively studied, however, little is known about their biological significances in cardiac stem cells. Here, we demonstrated that leukemia inhibitory factor (LIF), a member of IL-6-related cytokines, activated signal transducer and activator of transcription 3 (STAT3) and extracellular signal regulated kinase 1/2 (ERK1/2) in cardiac Sca-1 + stem cells. Moreover, LIF induced endothelial specific genes, including VE-cadherin, Flk-1 and CD31, in cardiac Sca-1 + cells. Immunocytochemical analyses showed that Sca-1 + cells were expressed CD31 14 days after LIF stimulation. In cardiac Sca-1 + cells, transduction with dominant negative STAT3 abrogated the LIF-induced endothelial differentiation, and the inhibition of ERK1/2 also prevented endothelial differentiation. Thus, both STAT3 and ERK1/2 are required for LIF-mediated endothelial differentiation in cardiac stem cells. Collectively, it is proposed that LIF regulates the commitment of cardiac stem cells into the endothelial cell lineage, contributing to neovascularization in the process of tissue remodeling and/or regeneration.

Key words: cytokine, endothelial, heart

P110237

Collagen XII is regulated by shear stress and nifedipine in cultured endothelial cells

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Fluid shear stress induced by blood flow may play a important role in the prevention of atherosclerosis by changing endothelial functions. We select a shear stress-specific clone, identified as collagen XII, from a bovine aortic endothelial cells (BAECs) cDNA library. ECs were cultured and exposed to laminar shear stress, collagen XII mRNA and expression were observed by Northern blotting analysis, RT-PCR and Western blotting analysis respectively. Collagen XII mRNA expression in both BAECs and human umbilical vein ECs (HUVECs) were found increased from 1 to n 12 hours at 20 dyne/cm2 of shear stress. Collagen XII protein expression increased after exposure to shear stress for 12 and 24 hours. Calcium antagonist nifedipine increased collagen XII mRNA and protein expression induced by shear stress. These results suggest that collagen XII expression induced by shear stress and nifedipine may play a role in stabilizing the vascular structure and preventing atherosclerosis.

Key words: Collagen XII; shear stress; nifedipine; atherosclerosis

P110238

Neuroprotective effects of Hydroxyethylpuerarin against focal cerebral ischemia-reperfusion in rats

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Objective: To investigate the neuroprotective effects of hydroxyethylpuerarin (HEP) against 1 hour of ischemia followed by 48 hours of reperfusion by middle cerebral artery occlusion (MCAO) in male Wistar rats. Methods: Rats were divided into sham-operate group, cerebral ischemia-reperfusion group, nimodipine 0.2 mg/kg/d group and HEP15, 30, 60 mg/kg/d groups randomly. 48 hours after reperfusion, animals were scored to estimate the degree of neurological deficit, and brains were removed then homogenized to determine LDH level using spectrophotometic assay methods. Pathologic histological changes were observed by HE stain and the occurrence of apoptosis was determined by flow cytometry. Results: Compared with ischemia-reperfusion group, treatment with HEP exhibited significant neuroprotective effects on rats against focal cerebral ischmia-reperfusion injury by markedly decreasing neurological deficit scores and the release of LDH, reducing necrosis and apoptosis of neurons. Conclusion: Hydroxyethylpuerarin might provide neuroprotective effects against the cerebral ischemia-reperfusion injury in rats.

Key words: Cerebral ischemia-reperfusion, Hydroxyethylpuerarin, Neuroprotection

P110239

Protective effects of Baicalin against focal cerebral ischemia-reperfusion in rats

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Objective: To investigate the neuroprotective effects of baicalin against 1 hour of ischemia followed by 24 hours of reperfusion by middle cerebral artery occlusion (MCAO) in male Wistar rats. Methods: Baicalin 25, 50, 100 mg/kg were administrated intravenous injection at the very beginning of both ischemia and reperfusion. 24 hours after reperfusion, rats were scored to estimate the degree of neurological deficit, then brains were removed to measure the brain infarct volume by TTC staining as well as to determine the histologic lesion of pyramidal cells in the CA1 region of hippocampus by HE staining. Results: The results showed that after focal brain ischemia-reperfusion, neurological scores, infarct volume and lesion levels were all significantly increased, while treatment with baicalin at the doses of 25, 50, 100 mg/kg can reduce all the indexes of neural injury. Conclusion: These data indicate that baicalin can protect cerebral tissue from focal ischemia-reperfusion insult.

P110240

Differential Role of Cytoplasmic and Nuclear Isoforms of CaMKII in the Heart

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Heart contains both the nuclear targeted deltaB and the cytoplasmic deltaC splice variants of Ca/calmodulin-dependent protein kinase II (CaMKII). Transgenic (TG) expression of the nuclear CaMKII induces cardiac hypertrophy while TG mice expressing the cytoplasmic CaMKII develop dilated cardiomyopathy and heart failure. We hypothesize that cytoplasmic and nuclear CaMKII play distinct roles in Ca handling and transcriptional responses. We find that phosphorylation of the CaMKII site on the ryanodine receptor and phospholamban are significantly increased, in association with increased Ca spark frequency, when cytoplasmic CaMKII is expressed in TG mice. In contrast, phosphorylation and spark frequency are unaltered in TG mice expressing nuclear CaMKII. Conversely, both nuclear and cytoplasmic isoforms of CaMKII can induce HDAC translocation and enhance MEF2-dependent gene expression in vitro (by luciferase assays) and in vivo (by MEF2 indicator mice). In conclusion, CaMKII isoforms have distinct ef-

fects on Ca handling but similar effects on MEF2 gene expression, suggesting that differential patterns of isoform activation may play distinct roles in the pathogenesis of cardiac hypertrophy and heart failure.

P110241

Involvement of Cydic Nudeotides and Potassium Channels in Hypoxic Vasodilatation in Pig Coronary Arteries

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We hypothesized that cyclic nucleotide-dependent pathways are pivotal for hypoxia-induced coronary relaxation. Large pig coronary artery segments were mounted in myographs for isometric tension recording. Arteries were contracted with either K^+ or PGF2α and in the absence and presence of a protein kinase A (PKA) inhibitor or a K-channel-blocker, respectively, oxygen was gradually reduced (95%-0%). Intracellular calcium ($[Ca^{2+}]ic$) was measured using Fura-2-AM. Following PGF2α (10^{-5} M) contraction, arteries relaxed in proportion to the level of hypoxia. An inhibitor of PKA, Rp-CPT-cAMPS (100 μM), reduced hypoxic relaxation. Hypoxic relaxation was diminished by a blocker of $BKCa^{2+}$-channels, Iberiotoxin (100 nM), a blocker of KV-channels, 4-aminopyridine (0.5 mM) and in arteries contracted with 30 mM K^+, respectively. Only a minor reduction was found with a blocker of KATP-channels, Glibenclamide ($3x10^{-6}$ M). Hypoxia induced relaxation was associated with reduced $[Ca^{2+}]ic$ in PGF2α contracted arteries but not in 30 mM K^+ contracted arteries. Hypoxia relaxes coronary arteries by activation of PKA and by opening of potassium channels following lowering of $[Ca^{2+}]ic$. Desensitization contributes to hypoxic relaxation.

P110242

A mouse knock-in model of dilated cardiomyopathy associated with deltaK210 mutation in cardiac troponin T and its potential pharmacotherapy

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Dilated cardiomyopathy (DCM) is characterized by cardiac dilation and systolic dysfunction, which often leads to severe heart failure and sudden death. However, little is known about its pathogenic mechanism, and no therapeutical methods have been established except for cardiac transplantation. We created a knock-in mouse model of DCM caused by a mutation deltaK210 in cardiac troponin T and explored its pathogenic process and potential pharmacotherapy. Mutant mice developed enlarged hearts with ventricular dilation and systolic dysfunction and suffered sudden death frequently. Skinned cardiac muscle fibers showed a decreased Ca^{2+} sensitivity of force generation. Surprisingly, however, intact cardiac muscle fibers showed no significant reduction in isometric force per cross-sectional area. Fura-2 loaded cardiomyocytes revealed that this was due to an increase in the intracellular Ca^{2+} transient. Biochemical analyses strongly suggested that Ca^{2+} transient was increased through down-regulation of PDE4B and associated increase in cAMP in cardiomyocytes, which could compensate for the decreased myofilament Ca^{2+} sensitivity but would increase the risk for arrhythmia and sudden death due to SR Ca^{2+} overload.

P110243

Laminar shear stress induces CYP1A1 through the aryl hydrocarbon receptor-xenobiotic response element signaling pathway in vascular endothelial cells

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Although CYP1A1 plays an important role in the detoxification of polycyclic aromatic hydrocarbons, its regulation mechanism by blood flow has not been well studied. In this study, we examined the effect of laminar shear stress (SS) on CYP1A1 expression including mechanisms using human umbilical vein endothelial cells (HUVECs). Physiological level laminar SS (15 dynes/cm2) enhanced the expression and enzymatic activity of CYP1A1. SS stimulated the CYP1A1 promoter activity, whereas did not influence the protein degradation. However, SS-induced CYP1A1 transactivation was markedly suppressed by deletion or mutations of upstream two xenobiotic response elements (XREs) activated by aryl hydrocarbon receptor (AhR) binding. SS also enhanced the AhR expression and furthermore, an AhR antagonist, alpha-naphthoflavone and small interfering RNA of AhR significantly suppressed the laminar SS-induced CYP1A1 expression. SS-induced AhR and CYP1A1 expressions were reduced by co-treatment with c-Jun N-terminal kinase (JNK) inhibitor SP600125 or p38 inhibitor, SB203580. Our results suggest that laminar SS transcriptionally activates CYP1A1 through XRE probably by JNK/p38-mediated AhR induction in HUVECs.

P110244

Effect of Penehydidine Hydrochloride on Rat's Disfunction of Microcirculation

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Objective: To observe effect of Penehydidine Hydrochloride (PHC) improving hemorrheology and microcirculatary disturbance on rat's mesentery. Methods: Normal adult SD rats were induced acute disfunction of microcirculation by lingual vein injecting 10% high molecular dextran (HMD) 3.5 ml/kg, then observed variation of bloodstream on mesentery under the stereo microscope, after that, divided into 5 groups, respectively injecting PHC 0.023 mg/kg、0.07 mg/kg、0.2 mg/kg, anisodamine (Ani) 2 mg/kg, NS. 2 ml/kg, and continued to observe variation of bloodstream. 40 min later, drew blood doing hemorrheology detection, also measuring contents of TXA_2 and PGI_2. Results: There is significant difference on way of bloodstream, blood viscosity, plasma viscosity, volume of packed red blood cell, erythrocyte electrophoretic time, K value of blood sedimentation equation and contents of TXA_2 and TXA_2/PGI_2 in model group, compared with the normal group. PHC 0.2 mg/kg group can obviously decrease plasma viscosity. The else indexes above all have greatly improved in groups of PHC 0.023 mg/kg、0.07 mg/kg、0.2 mg/kg, Ani 2 mg/kg, compared with the model group. Conclusions: Penehyclidine Hydrochloride can improve acute disfunction of microcirculation induced by HMD.

Key words: microcirculation, hemorrhedology, TXA_2/PGI_2

Acknowledgement: Greatly appreciate department of blood and department of isotope in affiliated hospital of Xuzhou Medical College.

P12. ion Channel Pharmacology

P120001

Kv1.3 channels located in smooth muscle mediated the relaxation of rat renal artery induced by resveratrol

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Resveratrol, a stilbene polyphenol found in grapes and red wine, has recently been found to produce vasorelaxation in endothelium-dependent and endothelium-independent manner. The aim of this study is to define the mechanism(s) of relaxation produced by resveratrol in the isolated rat renal artery (RA) precontracted by phenylephrine. Resveratrol produced concentration-dependent relaxation of RA rings without endothelium (EC_{50} = 15 micro M). To analyse the contribution of different types of K channels in resveratrol-induced relaxation in the RA, various K channel blockers were used. The relaxation of RA was not blocked by glibenclamide, a selective ATP-sensitive K channel blocker, and tetraethylammonium, a non selective blocker of calcium-dependent K channels. 4-aminopyridine blockers of voltage-dependent K (Kv) channels, antagonized resveratrol-induced relaxation of RA. Caribdotoxin and margatoxin, blockers of Kv1.3 channels antagonized the resveratrol effect on RA. Kv1.3 channels were detected in smooth muscle of RA using peptide-specific antibodies in immunoperoxidase. It is likely, that Kv1.3 channels are involved in relaxation of RA produced by resveratrol.

P120002

Effects of isodiensinine on BK_{Ca} and $[Ca^{2+}]i$ of cultured porcine coronary arterial smooth muscle cells

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Objective: To investigate the effects of isodiensinine (ID) on large conductance Ca^{2+} activated K^+ channel (BK_{Ca}) and $[Ca^{2+}]i$ of cultured porcine coronary arterial smooth muscle cells (CASMCs). Methods: Whole-cell patch clamp techniques were used to record K^+ outward current. Fura-2/AM labeled the cells and $[Ca^{2+}]i$ was analyzed by Calcium imaging system. Results: ID 1 μmol·L^{-1} could significantly increase K^+ outward current (10 mV depolarizing steps from -40 to +80 mV, 400 ms, 0.1 Hz, holding -80 mV), which vanished by iberiotoxin 100 nmol·L^{-1}. ID 10 μmol·L^{-1} did not affect it. ID 100 μmol·L^{-1} could significantly decrease K^+ outward current, which reversed by NS1619 10 μmol·L^{-1}. ID 0.1～10 μmol·L^{-1} did not influence rest $[Ca^{2+}]i$. ID 0.1～100 μmol·L^{-1} pretreatment for 5 min could concentrationdependently inhibit $[Ca^{2+}]i$ enhanced by K^+ 60 mmol·L^{-1}, angiotensinII 0.1 μmol·L^{-1} or phenylephrine 1 μmol·L^{-1} respec-

tively. Conclusions : IL possesses the biphasic effect on BK_{Ca} and the inhibitory effect on $[Ca^{2+}]i$ increase. IL < 10 μmol·L^{-1} maybe direct open BK_{Ca}. While IL > 10 μmol·L^{-1} maybe significantly decrease $[Ca^{2+}]i$, resulting in inhibiting BK_{Ca}.

Key words : isoliensinine, coronary arterial smooth muscle cells, BK_{Ca}, $[Ca^{2+}]i$

P120003

Thyrotropin-releasing Hormone (TRH) Increases GABA Release by Inhibiting a Resting K^+ Conductance in Hippocampal Interneurons

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The hippocampus expresses both TRH and TRH receptors. However, the functions of TRH in the hippocampus have not been determined. In the present study, we have examined the effects of TRH on GABAergic transmission by recording GABAA receptor-mediated synaptic currents in hippocampal slices. Our results demonstrate that TRH increases GABA release by facilitating the excitability of GABAergic interneurons. TRH increased the action potential firing frequency recorded from hippocampal interneurons and induced membrane depolarization of interneurons. TRH-induced depolarizing current had a reversal potential close to the K^+ reversal potential suggesting that TRH inhibits K^+ channels to generate membrane depolarization. The TRH-sensitive K^+ channels were sensitive to Ba^{2+} but resistant to other classical K^+ channel blockers (TEA, 4-AP, Cs^+) suggesting TRH acts on the two pore domain K^+ channels. The effects of TRH were independent of intracellular second messengers suggesting a direct coupling of G-proteins and K^+ channels. Our results demonstrate a novel mechanism to explain the physiological functions of TRH in the brain.

Key wards : synapse, GABA, G-proteins, ion channels;
(supported by NIH)

P120004

The impact of the disruption of cellular localization of IK1 and SK3 potassium channels on EDHF-mediated response in rat mesenteric arteries.

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We have studied the effects of caveolae disruption using methyl-beta-cyclodextrin (MCD) on the endothelium-derived hyperpolarizing factor (EDHF) pathway in rat mesenteric arteries. In pressurized vessels pre-contracted with U46619 in the presence of 100 nM apamin, 1 and 3 X10-6 M ACh increased vessel diameter (51 ± 2 % and 79 ± 2 %, respectively; n = 4). After 5 mM MCD pre-treatment, ACh-mediated dilatations were unchanged. However, in the presence of 10 X10-6 M TRAM-34 dilatations to 1 and 3 X10-6 M ACh were reduced by MCD (control; 48 ± 2. 2 % and 61 ± 2. 1 %; MCD 7. 5 ± 0. 2 % and 19 ± 1. 9 %; P < 0. 001, n = 4). In sucrose-density gradient studies, MCD reduced the SK3 protein in caveolin-rich fractions but had no effect on IK1 protein located in caveolin-poor samples. Immunofluorescence methods showed that MCD shifted SK3 from the cell surface to the cytoplasm. These studies show that SK3 but not IK1 protein is present in endothelial caveolae. MCD selectively reduces the role of SK3 channels in EDHF-mediated relaxations generated by ACh in rat mesenteric arteries.

Key words : Caveolae, EDHF, calcium-activated potassium channels
Supported by the University of Aleppo (MA) and the British Heart Foundation (GE, AHW).

P120005

Modulation of BK_{Ca} channels via cAMP- and cGMP-dependent protein kinases by Eugenosedin-A in cerebral myocytes

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The study investigated whether eugenosedin-A, a serotonin antagonist, enhances the delayed-rectifier K^+ (K_{DR})- or large-conductance Ca^{2+}-activated K^+ (BK_{Ca})-channel activity in basilar artery myocytes through cAMP/cGMP-dependent protein kinases. Cerebral myocytes were dissociated from rat basilar arteries. Conventional whole cell, perforated and inside-out patch-clamp was used to monitor K^+-channel activities. Eugenosedin-A (1 μM) had no effect on the K_{DR} current but dramatically augmented BK_{Ca} channel activity in a concentration-dependent manner. Increased BK_{Ca} current activity was abolished by charybdotoxin (100 nM) or iberiotoxin (100 nM), but not affected by apamin (100 μM). BK_{Ca} current activation by eugenosedin-A was inhibited by an adenylate cyclase inhibitor (SQ22536, 10 μM), a soluble guanylate cyclase inhibitor (ODQ, 10 μM), competitive antagonists of cAMP and cGMP (Rp-cAMP, 100 μM and Rp-cGMP, 100 μM), or cAMP- and cGMP-dependent protein kinase inhibitors (KT5720, 300 nM and KT5823, 300 nM). Eugenosedin-A reversed the PKC activator (PMA, 100 nM)-induced BK_{Ca} currents inhibition. Eugenosedin-A enhances BK_{Ca} currents by stimulating the activity of cyclic nuclectide-dependent protein kinases.

P120006

Role of K^+ channels in prostanoid EP3 and TP receptors-mediated inhibition of noradrenaline release from the rat stomach

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We investigated a role of K^+ channels in prostanoid EP3 and TP receptor-mediated inhibitions of electrically evoked noradrenaline (NA) release using the isolated, vascularly perfused rat stomach. The gastric postganglionic sympathetic nerves were electrically stimulated twice at 1 Hz for 1 min. Test reagents were added during the second stimulation. Prostaglandin E_2 (PGE_2) and U-46619 (an agonist of TP receptor) dose-dependently inhibited the evoked NA release. Tetraethylammonium (TEA), 4-aminopyridine (4-AP) (blockers of voltage-dependent K^+ channel) and charybdotoxin (ChTX) (a blocker of BK channel) augmented the NA release in dose-dependent manner. In the presence of TEA (1.0 mM) or 4-AP (0.1 mM) throughout the experiment, the U-46619-induced inhibition was attenuated, while PGE_2-induced inhibition was not influenced. ChTX (0.01 micro M) had no effect on neither of these inhibitions. These results suggest the involvement of different mechanisms in the TP receptor (PTX-sensitive)- and EP3 receptor (PTX-insensitive)-mediated inhibitions of NA release. Voltage-dependent K^+ channels are probably involved in the TP receptor-mediated inhibition.

Key words : Noradrenaline, Stomach, TP receptor, K^+ channel

P120007

Effects of Salvianolic acid B on L-type calcium channel in isolated rat ventricular myocytes

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Objective : To observe the effect of Salvianolic acid B (Sal B) on L-type calcium channel in isolated adult rat ventricular myocytes. Methods : The single rat ventricular myocytes were obtained by enzymatic dissociation. When the holding potential was 40 mV, cells were depolarized to 60 mV for 250 ms with steps of 10 mV at frequency of 0.5 Hz by whole cell patch clamp technique. Results : 0.1 μmol·L-1 Sal B did not affect the L-type calcium current (ICa L) (P > 0.05), 5, 10, 20 μmol·L-1 Sal B inhibited ICa L by 17.2 %, 38.1 %, and 52.5 % (P all < 0.01), respectively, without altering the shape of the current-voltage (I-V) curve, reversal potential and the steady-state activation curve of ICa L.. Conclusion : The Sal B can inhibit ICa L in a concentration dependently manner and has calcium antagonistic effect.

Key words : Salvianolic acid B; L-type calcium channel; path clamp; myocardium

P120008

Adenosine inhibits epithelial Na channels (ENaC) by cytochrome-P450 (CYP)-epoxygenase-dependent metabolites of arachidonic acid

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We used the patch-clamp technique to examine the effect of adenosine on ENaC activity in the rat cortical collecting duct (CCD). Application of adenosine inhibits ENaC activity and the effect of adenosine was mimicked by cyclohexyladenosine (CHA) and cyclopentyladenosine (CPA) which inhibits A1 adenosine receptor. In contrast, application of CGS21680, an A2a adenosine receptor agonist, had no effect on ENaC. Inhibition of phospholipase C failed to abolish the effect of CHA on ENaC. The effect of CHA on ENaC was absent in the presence of the phospholipase A2 inhibitor. To determine the metabolic pathway of arachidonic acid (AA) responsible for the effect of adenosine, we examined the effect of CHA in the presence of indomethacin or MS-PPOH. Inhibition of CYP450 epoxygenase blocked the effect of CHA on ENaC. In contrast, CHA reduced the ENaC activity in the presence of indomethacin. Moreover, addition of 11,12-EET inhibited the ENaC channels in the CCD. We conclude that adenosine inhibits ENaC activity by stimulation of the A1 adenosine receptor in the CCD and that the effect of adenosine is mediated by 11,12-EET.

P120009

Volume-sensitive outwardly rectifying chloride channels are involved in oxidative stress-induced apoptosis of mesangial cells

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The purpose of the present study was to explore the role of volume-sensitive outwardly rectifying (VSOR) Cl- channels in oxidative stress-induced apoptosis of mesangial cells. Whole cell patch clamp was employed to record VSOR Cl- currents. Here, we demonstrated that the exogenous application of 150 μM H_2O_2 led to activation of VSOR Cl- conductance in mesangial cells. Moreover, blockage of VSOR Cl- by DIDS(100μM), NPPB(10μM) or niflumic acid(10μM) rescued mesangial cells from H_2O_2-induced apoptotic cell death. Treatment for 2h with 150 μM H_2O_2 resulted in significant reduction in cell volume (vs. control, $p < 0.01$, $n = 6$). However, the early-phase alterations in cell volume were markedly abolished by pretreatment with VSOR Cl- channel blockers. We concluded that VSOR Cl- channels are involved in H_2O_2-induce apoptosis in cultured mesangial cells and its mechanism is associated with apoptotic volume decrease (AVD) processes.

Key words: apoptosis; mesangial cells; volume-sensitive chloride channels; apoptotic volume decrease.

P120010

Synchronized oscillations of $[Ca^{2+}]_i$ in endothelial and smooth muscle cells in rat mesenteric small arteries exposed to cyclopiazonic acid (CPA)

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The mechanisms leading to vasomotion in the presence of inhibitors of the SERCA pump were investigated in isolated rat mesenteric small arteries. Isometric force, membrane potential and confocal images of Ca^{2+} were obtained in smooth muscle (SM) and endothelial (ED) cells. During stimulation with noradrenaline, CPA induced oscillations of tone with a low frequency and high amplitude. The oscillations were unaffected by ryanodine but the amplitude was reduced by indomethacin and increased with L-NAME. The oscillations were inhibited by nifedipine, and the frequency increased about 3 times by removal of the ED, by charybdotoxin plus apamin. The oscillation of tone was associated with oscillations of membrane potential in ED and SM cells which were in phase and oscillations of Ca^{2+} which were in antiphase. The data suggest that inhibition of SERCA causes synchronization between ED and SM which leads to antiphase oscillations of Ca^{2+} in two cell types and thus oscillation in tone.

Key words: CPA, oscillation, membrane potential, artery.
Acknowledgments: K. Skaarup and J. Andresen for technical assistance, the Danish Heart Foundation for financial support.

P120011

Tubulin as a possible binding partner of the heag2 potassium channel

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We have previously cloned the human potassium channel, heag2, and we have identified tubulin as a likely protein binding partner of this channel, using pull-down assays with a GST fusion protein of a heag2 fragment, followed by mass spectrometry. Here we have investigated the functional effect of tubulin on heag2. Using the oocyte expression vector pGem-He-Juel, Xenopus laevis oocytes were injected with RNA for heag2, or coinjected with RNA for heag2 and human α tubulin. Potassium currents were then recorded using two-electrode voltage clamping 1-2 days later. Recordings from cells injected with RNA for tubulin alone gave currents that were indistinguishable from those in uninjected cells. Cells coinjected with RNA for both tubulin and heag2 displayed currents that were significantly reduced ($P < 0.05$) as compared with currents for heag2 alone. The shape of the current-voltage relationship was otherwise unaffected by tubulin. The data show that tubulin binds to the heag2 channel and affects its function. This may be due to a direct effect of tubulin on the channel, or due to an effect on trafficking of the channel to the membrane. Supported by BBSRC.

Key words: Potassium channel, tubulin, electrophysiology

P120012

Characterization of Ca^{2+} influx by dimethylphytosphingosine and lysophosphatidylcholine in U937 human monocytes

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Elevations in intracellular Ca^{2+} concentration can modulate cell growth and apoptosis. In this study, Ca^{2+} influx induced by dimethylphytosphingosine (DMPH) and lysophosphatidylcholine (LPC) was characterized by fluorescence spectrophotometer using Fura-2. L-type voltage-gated Ca^{2+} channel blockers, verapamil and nifedipine, significantly reduced LPG-induced Ca^{2+} influx, but not significantly DMPH-induced one. Nonspecific Ca^{2+} channel blockers, gaddinium and lanthanum, considerably reduced DMPH- and LPG-induced Ca^{2+} influx. Preincubation of forskolin increased DMPH-induced Ca^{2+} influx, however, LPG-induced Ca^{2+} influx was not affected by the treatment. Taken together, LPC might induce Ca^{2+} influx through modulation of L-type voltage-gated Ca^{2+} channels. However, DMPH utilized Ca^{2+} channels that are modulated by forskolin treatment, and TRPM7 is supposed to be a candidate for this event.

Key words; dimethylphytosphingosine; lysophosphatidylcholine; Ca^{2+} influx
This work was supported by the Korea Science and Engineering Foundation Grant. (R01-2005-000-10011-02005)

P120013

Interfering Expression of HERG Channels Depresses Existence and Proliferation of Neuroblastoma Cells

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The experiment was carried out to explore the potential therapeutic effects on neuroblastoma cells (SH-SY5Y) by using RNA interference technology and targeting the human ether-a-go-go-related gene (HERG). Vectors of small hairpin interfering RNA targeting HERG were constructed (shRNA-HERG). The interfering effects on the expression of HERG mRNA and protein of potassium channels were determined by RT-PCR and Western blot. The growth and proliferation of SH-SY5Y cells were examined by cell growth curve and colony forming experiment. It was found that transcription and expression of HERG channels were suppressed remarkably when shRNA-HERG vectors were transfected into SH-SY5Y cells. The growth doubling time of SH-SY5Y cells was prolonged by 165.3%, and the ability of colony forming was depressed by 45.0%. The in vivo experiment displayed that shRNA-HERG could retard the growth of tumor formed by SH-SY5Y cell injection into nude mice. The results suggested that the shRNA-HERG vectors might be a promising antineoplastic agent for neuroblastoma.

Key words: RNAi; HERG; neuroblastoma.
Acknowledgement: The work was supported by National Natural Science Foundation of China (No. 30472019 and 30500620).

P120014

Involvement of ASIC1a in apoptosis and cell death induced by extracellular acidosis

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Acid-sensing ion channels (ASICs) are gated by extracellular protons and six ASIC subunits have been cloned, which are encoded by four genes (ASIC1-ASIC4). In the experiment, the effect of ASICs in apoptosis and cell death induced by acidosis was explored. The results revealed that neuroglioma cells (C6) expressed nearly all the ASICs subunits, except for ASIC4. An interfering vector for silencing ASIC1a expression was constructed and transfected into C6 cells. It was found that low pH value induced apoptosis and cell death were distinctly alleviated when ASIC1a expression was retarded in C6 cells. In the sustained acid-stimulating situation, the intracellular Ca^{2+} concentration in wide-type C6 cells increased remarkably. However, the acid induced Ca^{2+} increase in the ASIC1a expression interfered C6 cells was depressed. The results suggested that ASIC1a subunit might been involved in the facilitation of proton-induced apoptosis and cell death by increasing the intracellular Ca^{2+} concentration.

Key words: Acid-sensing ion channels; neuroglioma cells; apoptosis; calcium
Acknowledgement: The work was supported by National Natural Science Foundation of China (No. 30472019).

P120015

4-Amino-Piperidine Derivatives Block N-type Calcium Channels

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Numerous studies implicate N-type Ca^{2+} channels as key mediators of nociceptive signaling in dorsal root ganglion neurons, and as potential targets for the development of analgesic drugs. A series of 4-Aminopiperidines have been shown analgesic effect by blocking N-type Ca^{2+} channels. To fin more potential analgesic drugs, we synthesized some new compounds based on the structure of 4-aminopiperidine. To evaluate these compounds, N-type Ca^{2+} channels($\alpha_{1B}/\beta_{3b}/\alpha_{2}\delta$) were expressed in HEK-293 cells and Xenopus oocytes. Calcium currents were recorded by whole-cell recording and two-electrode voltage clamp recording technique, respectively. It was found 13 compounds could depress Ca^{2+} currents at a lower concentration (50 mM, inhibitory rate > 80%). Among them, compound #88 depressed Ca^{2+} currents with IC50 0.45 ±0.09 μM. The results suggested some new compounds displayed potent blocking effect on N-type Ca^{2+} channel, and might become promising leading compounds for analgesic drug development.

Key words: N-type calcium channel, antagonists, electrophysiology

Acknowledgement: The work was supported by National Key Basic Research Program (No. 2003CB515406)

P120016

A Cytoplasmic C-Terminal Coiled-Coil Domain Mediates TRPM2 Subunit Interactions

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TRPM2 is a melastatin-related transient receptor potential channel thought to be a tetramer formed by four subunits surrounding a central aqueous pore. To study the potential role in subunit interaction of a cytoplasmic coiled-coil domain in the proximal C-terminus of human TRPM2 subunit, we constructed deletion and point mutants, expressed in HEK293 cells and performed co-immunoprecipitation. Deletion of the coiled-coil domain (L1167 to S1201) dramatically attenuated its interaction with the co-expressed wild type subunit. Substitution by glutamine of individual predicted interacting hydrophobic residues identified four key residues in two microdomains (L1177 and L1180, and I1194 and L1198). Double mutants displayed weaker interaction with wild type subunit than single mutants, and mutants containing three or all four mutations attenuated the subunit interaction to a degree similar as the deletion mutant. Together our results demonstrate that this coiled-coil domain is an important molecular determinant mediating the subunit interaction needed to form functional TRPM2 channels.

Key words: TRPM2, coiled-coil domain, subunit interaction

Acknowledgement: This work is supported by the Wellcome Trust

P120017

Effects of Okadaic Acid, a protein phosphatase inhibitor, on potassium channel currents in cultured rat trigeminal ganglion neurons

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To investigate the effects of okadaic acid on IA and IK in cultured rat trigeminal ganglion neurons. Whole-cell patch clamp technique was used to record the IA and IK before and after perfusion of 1 μmol. L^{-1} okadaic acid. we found that 1 μmol. L^{-1} okadaic acid inhibited IA by 28.6 ±8.5 % (+50 mv, n = 8, P < 0.05), but increased IK by 22.7 ±10.7 % (+50 mv, n = 6, P < 0.05). 1 μmol. L^{-1} okadaic acid reduced inactivation time course of IA (n = 8, P < 0.01), and produced significant hyperpolarizing shift in the G-V curve and H-infinity curve. 1 μmol. L^{-1} okadaic acid also produced significant hyperpolarizing shift in G-V curve of IK. These indicate that Okadaic acid has effects on K^+ channel currents in cultured trigeminal ganglion neurons of the rat, possibly partly by the inhibition of protein phosphatases.

Key words: okadaic acid; potassium channel currents; trigeminal ganglion neuron

The project supported by the National Natural Science Foundation of China (30271500)

P120018

Mechanism of Diazoxide-mediated Cardioprotection

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Ischemic preconditioning (IPC) can be mimicked by K^+ channel openers such as diazoxide. Diazoxide has multiple ATP-sensitive K^+ channel (KATP)-independent actions, and the mechanism underlying diazoxide mediated cardioprotection remains inconclusive. Giving that the KATP pore-forming subunit Kir6.2-knockout mice have shown no cardioprotection of IPC, we tested the hypothesis that diazoxide protects the heart by promoting import of Kir6.2-containing KATP into mitochondria from cytosol where they are synthesized. The effect of diazoxide on mitochondrial localization of Kir6.2 was examined in KATP deficient COS-7 cells transfected with HA-tagged Kir6.2 and SUR2A by laser confocal microscopy. We found that the percentage of cells showing mitochondrial localization of Kir6.2 was significantly higher in diazoxide (100 micro M)-treated group than that in control group (68.0% vs. 11.0%). The effect was almost completely prevented by the KATP channel inhibitors 5-hydroxydecanoate or glibenclamide, or a selective protein kinase C (PKC) inhibitor chelerythrine. We conclude that diazoxide increases Kir6.2-containing KATP channels in mitochondria by activation of PKC.

Key word: preconditioning, diazoxide, PKC

P120019

Chloride Channel Inhibition Blocks the Protection of Ischemic Preconditioning and Pharmacological Ischemic Preconditioning of Sasanquasaponin in Rat Cardiomyocytes

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This study was to examine the role of chloride (Cl-) channels in myocardial protection of ischemic preconditioning (IP) and pharmacological ischemic preconditioning(PIP) of sasanquasaponin(SQS). Prior to anoxia-reoxygenation (A/R) injury, Cultured neonatal rat cardiomyocytes were pretreated with SQS(3.75 × 10-4 mmol·L^{-1}) followed by 5-nitro-2-(3-phenyl propylamino) benzoic acid (NPPB, 1 ×10-3 mmol·L^{-1}) or 4-acetamido-4'-isothiocyanato-stibene-2,2'-disulfonic acid(SITS, 0.1 mmol·L^{-1}) for 10 min to inhibit Cl- channels. Viability and ultrastructure of myocytes, LDH activity in medium were examined. Compared with A/R, IP and SQS pretreatment significantly decreased the LDH activity, increased cell viability ($p < 0.01$), and kept cardiomyocyte ultrastructure. NPPB and SITS, however, abolished the protection of IP and SQS pretreatment. Our results suggest that Cl- channels may be involved in the IP or SQS's PIP protection of the myocardium against A/R injury.

Key words: ischemic preconditioning; chloride channel; sasanquasaponin

Acknowledgement This work was supported by a grant from Natural Science Foundation of China (No. 30560049).

P120020

Comparison of two alpha1-blockers bunazosin and doxazosin on electrophysiologic effects

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In Langendorff-perfused adult rat heart with constant pressure at 80 mmHg, we found pretreating 10 micro M alpha1-blocker doxazosin caused occasional arrhythmia in form of premature ventricular contraction or polymorphic ventricular tachyarrhythmia, whereas another alpha1-blocker bunazosin at same concentration did not. In isolated right atria muscle strips, doxazosin but not bunazosin was found to decrease heart rate without alternating contractile force. Therefore we used whole cell patch clamp method to investigate the electrophysiologic effects of these two agents. The results showed that doxazosin inhibited INa, ICa, and Ito, without changing IK1 but bunazosin only inhibited ICa about 30%. Doxazosin also shifted the inactivation curve of INa left. Moreover, doxazosin prolonged action potential duration and suppressed action potential amplitude and upstroke velocity in single cell, whereas bunazosin did not. With right atrium excised, the heart was stimulated by external stimulator and doxazosin no longer caused arrhythmia. We suppose doxazosin-induced arrhythmia may be resulted from atrium rather than ventricle, but the underlying mechanism remains to be further determined.

P120021

Ranolazine Does Not Affect Ventricular Activation Pattern in Guinea Pig Isolated Hearts, Whereas Flecainide Does

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Background : Ranolazine has clinical anti-anginal activity and is a selective blocker of late relative to peak sodium current. Objectives : The purpose of this study was to determine the effect of ranolazine on cardiac activation. Methods : Langendorff-perfused guinea pig isolated hearts were stained with the potentiometric fluorescent dye di-4-ANNEPS, and treated with increasing concentrations (1 to 30 μM) of either flecainide or ranolazine. Action potentials were simultaneously recorded from 256 anterior right and left ventricular epicardial sites using a high-resolution photodiode-array-based optical mapping system. Total activation time was measured. Results : Flecainide (10 μM) caused a significant delay in activation from 12.1 ±1.7 msec (control) to 35.3 ±3.5 msec ($n = 7$, $p < 0.001$) and changed the activation pattern. In addition, 4 of 7 hearts developed conduction alternans. Increasing the flecainide dose to 30 μM resulted in complete activation block in all hearts. In contrast, ranolazine (30 μM; $n = 7$) did not significantly alter either the activation time (12.6 ±1.1 msec in control and 13.5 ±1.0 msec) or pattern. Conclusion : Ranolazine (up to 30 μM) does not affect cardiac activation.

P120022

Calmodulin kinase II phosphorylation and Calmodulin binding produced no run-down L-type Ca^{2+} channel in guinea-pig ventricular myocytes

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We previously reported that the basal activity of L-type Ca^{2+} channel was suppressed by calmodulin (CaM)- dependent protein kinase II (CaMKII) inhibitors, and that CaM reprimed the channel after the basal activity run down. However, the effect of calmodulin was time-dependent. This study was to investigate the relations of CaMKII and CaM in maintaining the Ca^{2+} channel basal activity. Patch clamp technique (single channel recording) was used. Three GST-fusion peptides, CT-1, CT-2 and CT-3 of the guinea-pig Cav1.2 C-terminal tail, were prepared. After run down, CaMKIIαT286D, a constitutively active CaMKII reprimed the Ca^{2+} channel activity to only 1.85 to 10.1 % of the basal activity, respectively. However, in the presence of CaMKIIαT286D, the effect of CaM became time-independent. In pull-down assay, CT-1 treated with CaMKII showed a higher affinity for CaM than that treated with phosphatase. Conclusion : Both of CaMKII and CaM are required in maintaining the Ca^{2+} channel basal activity. CaMKII phosphorylation and CaM binding may produce no run-down L-type Ca^{2+} channel.

Key Words : calmodulin, calmodulin kinase II, calcium channel, run-down.

P120023

THE EFFECT OF GINKGOLIDEB ON POTASSIUM CHANNELS OF HIPPOCAMPI IN RATS

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Objective : The effects of ginkgolide B on K^+ channel in hippocampal neurons in SD rats. Methods : SD rats brains hippocampal neurons were acutely dissociated by a combination of mechanical and enzymatic means. The effects of 10 nmol/μl GingkolideB on K^+ channel currents, current-voltage curves, activation curve and inactivation curve were studied using whole-cell patch-clamp techniques. The current signals were filtered at 1 kHz and digitized at 20kHz using Bessel and analyzed using pClamp software HEKA Pulse 8.5. The pipettes had resistance of 3-5 MΩ for whole cell recording when filled with electrolyte solution. Results : GingkolideB could reduce 54.7% the K^+ channel currents of hippocampal neurons; GingkolideB caused about 15 mv depolarizing shift of the activation curve but no obviously effect on the steady-state inactivation curve of K^+ channel; Conclusion : GingkdideB could reduce the K^+ channel currents; GingolideB could affect the process of the activation of K^+ channel, but no obviously effect on the process of th inactivation.

Key words : GingolideB; hippocampi; K^+ channel; whole cell patch-clam

P120024

Effects of resveratrol and 3,5,4'-trimethoxystilbene on sodium current in guinea pig ventricular myocytes

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Aim: To investigate the effects of trans-resveratrol (Res) and its methylated derivate 3,5,4'-trimethoxystilbene (trans - 3,5,4' - trimethyl-resveratrol, TMR) on sodium current (I_{Na}) in guinea pig ventricular myocytes. Methods : Single cardiac myocytes were isolated by enzyme, and the effects were assessed by applying whole-cell patch clamp technique. Results : Res (10, 30, 100 μmol·L^{-1}) was shown to inhibit I_{Na} of guinea pig ventricular myocytes in a concentration dependent manner, and the inhibition ratio of 30, 100 μmol·L^{-1} was 14.5 ± 1.5 % ($n = 5$, $P < 0.005$) and 56.6 ±7.9 % ($n = 5$, $P < 0.001$), respectively. TMR (10 μmol·L^{-1}) was also shown to inhibit I_{Na} of guinea pig ventricular myocytes by 47.3 ±13.7 % ($n = 8$, $P < 0.05$). The maximal activating voltage of I_{Na} was not changed. The two drugs acted quickly (about 3 min) and their effects were reversible completely after a 10 min washout. Conclusion : Res and TMR can exhibit direct inhibitory effects on I_{Na} in guinea pig ventricular myocytes and act rapidly. The effect of TMR is stronger than Res.

P120026

Analysis of ventricular arrhythmias in Andersen's syndrome (LQT7) : In vitro and in silico studies.

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Andersen syndrome (LQT7) is a inherited disorder characterized by periodic paralysis and ventricular tachyarrhythmias. The mutation of Kir2.1 reduces a function of inwardly rectifying potassium current (IK1) and results in this syndrome. The relationship between the inhibition of IK1 and the triggered activity was examined. Action potentials and IK1 were recorded from guinea pig isolated ventricular myocytes using the voltage or current-clamp method. IK1 current was dose-dependently inhibited by $BaCl_2$, however, triggered activities similar to ventricular arrhythmias in the heart, were induced in an all-or-none manner. The computer-simulated study revealed critical (threshold) reduction of IK1 was essential for the triggered activity in cardiac action potential and indicated that this threshold is the result of the balance between the inward and outward current in early repolarizing phase. These results indicate that arrhythmogenic mechanism in LQT7 is unstability of the resting membrane potential and the threshold may also be important for the initiation of arrhythmias.

P120028

Role of 5-HT_2 antagonist on the regulation of ATP-sensitive potassium channel activity in the mouse ventricular cardiomyocytes

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Effects of 5-HT_2 antagonists on the ATP-sensitive K+ (KATP) channels were studied in mouse ventricular cardiomyocytes. Under whole-cell voltage-clamp conditions, ketanserin (KT; 1-100 μM), a 5HT_2 antagonist, reversibly inhibited pinacidil-induced KATP current with a Ki value of 9.36 μM and the Hill coefficient was 0.67. This inhibition was developed even in the presence of 5-HT (100 μM). Prazosin, a selective alpha1- antagonist, failed to mimic the effect of KT. KT did not affect the channel activity in inside-out patches under ATP-free condition. KT, applied to external solution, did not affect the pinacidil-induced channel activity in cell-attached patches, but did inhibit it when applied into the pipette. Pirenperone (PP; 100 μM), another 5-HT_2 antagonist, also decreased the pinacidil-induced current in whole-cell voltage-clamp condition, but less potent than those of KT. In inside-out patches, PP also did not affect the channel activity. These results indicate that 5-HT_2 antagonists used in the present study inhibited KATP channel activities, and this action was not mediated through 5-HT_2 or alpha1-adrenoceptor, rather a direct one on the cardiac KATP channels.

P120029

Interactions between calpastatin and calmodulin in activation of L-type Ca^{2+} channels

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Activity of L-type Ca^{2+} channel is known to be abolished in cell-free conditions (run-down). We have hypothesized that some cytoplasmic factors are required to maintain channel activity, and found that both calpastatin (CS) and calmodulin (CaM) can restore channel activity after run-down in inside-out patches of guinea-pig cardiac myocytes. CaM (0.03-3 μM) + ATP (3 mM) dose-dependently produces channel activity of up to 200-300 % of that seen in the control cell-attached condition, and 100 % activity is observed at 0.3-0.5 μM CaM. On the other hand, CS + ATP produces only 20-30 % activity in run-down chan-

nels. Although the effect of CS is weak, its action can be mimicked by L-domain, a region in the N-terminal side of CS. L-domain of CS does not potentiate but rather suppresses channel activity when applied to the channel pre-activated by CaM + ATP, implying that there is a complicated interactions among CaM, CS and the channel. Conclusion: Activity of L-type Ca^{2+} channels is maintained by cytoplasmic factors, in which CaM rather than CS plays a major role, and that CS affects the interaction between CaM and the Ca^{2+} channel.

Key words: calcium channel, run-down, calmodulin, calpastatin.

P120030

Effect of valsartan on cardiac myocytes contraction function and calcium transient in heart failure rats

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Objective: To determine the effects of valsartan on calcium channel and sodium-calcium exchanger in isolated ventricular myocytes of congestive heart failure (CHF) rats. Methods: The rats with heart failure were divided randomly into the group treating with valsartan (CHF-T) and placebo (CHF-C). Sham-operated group rats served as negative controls (PS). Each group rats were selected randomly for the study of ion channel, single cardiac myocyte contractile and calcium transient were measured simultaneously with confocal imaging technique. Results: Compared CHF-C with PS group, LVEDP increased($p < 0.01$), BP, LVSP and $\pm dp/dt$ max decreased ($p < 0.05$). Compared CHF-T group with CHF-C group, LVEDP decreased($p < 0.01$), LVSP and $\pm dp/dt$ max increased ($p < 0.05$). Compared CHF-C group with PS group myocyte areas, diastolic cell length increased significantly ($p < 0.05$) and fractional cell shortening decreased significantly ($p < 0.05$), Compared CHF-C group with PS group ,the amplitude of $[Ca^{2+}]i$ transients decreased significantly ($p < 0.05$), End-diastolic $[Ca^{2+}]i$ and time to 50% decline in $[Ca^{2+}]i$ increased significantly ($p < 0.01$). Treatment with valsartan showed that those parameters were significantly improved. Conclusion: Administration of valsartan was effective in preventing from cardiac function deterioration and improving cardiac myocytes contractile function, it may be relative to calcium regulation.

Key words: Valsartan, Cardiomyocyte, Calcium channel
Acknowledgments: This work was supported by GDNSF (015015)

P120031

Voltage-dependent block of NMDA receptors by dopamine and D1 receptor ligands

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Using whole-cell patch-clamp recording of HEK cells and hippocampal neurons, we characterized direct channel blocking effects of dopamine and D1 ligands on NMDA receptor mediated currents. D1 ligands blocked NMDA receptors as open channel blockers, regardless of whether they are agonists or antagonists for D1 receptors. These ligands exhibited the typical voltage-dependent property of channel pore blockers with a significant block at hyperpolarizing potentials. In addition, they only blocked NMDA receptors when channels were activated while they had no effects when channels were closed. Furthermore, this channel blocking effect was independent of dopamine D1 receptors and the PKA or PKC pathway. These results suggest, in addition to D1 receptor dependent pathways, dopamine and D1 ligands can directly modulate NMDA receptors through a D1 receptor independent pathway, which is blocking NMDA receptors as open channel blockers.

Key words: NMDA receptor, dopamine, ligands, channel blocker

P120032

Calcium activated potassium channels: dynamic regulation by the actin cytoskeleton

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Dynamic changes of the actin cytoskeleton are fundamental to a wide range of cellular events including cell motility, adhesion, cytokinesis and ion transport. In the present study, we have examined how actin depolymerization affects large conductance voltage- and calcium-activated potassium channels (BK). Using an inside-out patch clamp technique BK channels were recorded from transfected human embryonic kidney (HEK293) cells. Cytochalasin D (CD), an actin filament disrupter, markedly enhanced activity of BK channel and this action persisted even after CD washout. Biochemical studies indicated that actin co-immunoprecipitates with the BK channels and confocal microscopy demonstrated cytoskeleton was disrupted after CD was applied to HEK293 cells. Phalloidin (Phal), the actin filament stabilizer, pre-treatment prevented the CD-induced facilitatory action on BK channel activity. Furthermore, BK channel with mutations in the C-terminal domain of the channel were insensitive to changes in actin cytoskeletal dynamics. Our data suggest an important C-terminal domain linking BK channels to actin cytoskeleton allowing channel activity to be regulated by the dynamic assembly or disassembly of actin.

P120033

A novel fluorine-containing analogue of pinacidil.

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The effects of the novel fluorine-containing analogues of pinacidil on bladder contractile function and on vascular tone were examined in vitro. The most selective compound for detrusor tissue was investigated in vivo. Female rat bladder strips and thoracic aorta rings were analyzed by method of organ baths. Experiments in vivo were conducted on an adult female rats anesthetized with urethane. The intravesical pressure was recorded via a catheter passed through the urethra. All new analogues of pinacidil concentration-dependently decreased contractions evoked by 60 mM K^+ and 15 mM K^+. The tested compounds inhibited KCl- and phenylephrine - induced contraction of the rat aortic rings in a concentration-dependent manner. Compound PF-5 (1 mg/kg) inhibites the micturition reflex in the rat but does not alter arterial pressure. Preincubation of preparations with glibenclamide depressed the relaxant effect of compound PF-5. Thus, we have demonstrated that structural modfications to prototipical potassium channel opener pinacidil have provided novel compound with potential utility in urological therapeutic areas.

Key words: pinacidil, potassium channel opener, urinary bladder, overactive bladder.

P120035

The mitogenic role of K^+ currents in rat UMR 106-01 osteoblastic cells

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We investigated the role of K^+ currents in the mechanisms regulating the proliferation of UMR 106-01 osteoblastic osteosarcoma cells. Specific inhibitors of K^+ channels, tetraethylammonium (TEA) and the class III antiarrhythmic methanesulfonanilide E-4031, affected cell proliferation in opposite ways: TEA inhibited proliferation by 65 % whereas E-4031 enhanced it by 83 %. Electrophysiological analysis showed that UMR 106-01 cells produce robust K^+ currents that are selectively inhibited by the two drugs. Application of TEA or E-4031 in the bath solution did not induce instantaneous changes in the level of cytosolic calcium, however, the calcium content was increased upon prolonged incubation with E-4031. Taken together these data indicate that distinct K^+ currents can exert opposite effects on the proliferation rate of bone osteoblast cells through distinct mechanisms

P120036

Activation of PAR1 increases cardiac action potential duration through stimulation of the late Na current.

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Thrombin constitue the main activator of the protease activated receptor PAR1, however, to date only few informations are available on the pathophysiological relevance of PAR1 in the heart. The main goal of the present study was to investigate the affect of PAR1 activation on the action potential duration of guinea-pig papillary. Firstly, an immunochemistry study using monoclonal mouse antibody to thrombin demonstrate the presence of PAR1 with a great density on the endocardium. Using the patch-clamp technique on freshly isolated cardiomyocytes, we demonstrate activation of PAR1 with thrombin 32 UI/ml increase the amplitude of the late sodium current (2.2 ± 0.5 pA/pF). In addition, the action potential duration were significantly increase APD50 in a concentration dependent manner both by thrombin 32 UI/ml (max var: 14,80 %) and by SFLLR 100 µM (max var: 13, 09 %). In each case, these PAR1 activations were fully block by TTX 1 µM confirming the involvement of the late sodium current in the action potential prolongation. Similarly, the PAR1 induced an increase of APD50 was concentration dependently block by two PAR1 antagonist compounds, SCH 203099 and ER 112787.

Key words: PAR1, APD, Late Sodium Current.

P120037

Regulation of Cl-/HCO3-exchange activities by CFTR and calcium signaling

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Pancreatic bicarbonate secretion is important to conserve optimal pH for digestion and to maintain the patency of intrapancreatic ductal trees. A significant proportion of pancreatic bicarbonate secretion is mediated by Cl-/HCO3- exchange in the luminal membrane of duct cells. We previously showed that the mechanism is CFTR dependent, cAMP-activated, and calcium-activated. The aim of this study was to identify which Cl-/HCO3- exchanger subtypes which are activated by CFTR and intracellular calcium signaling. Cl-/HCO3- exchange activity was measured in HEK293 cells, which were transiently transfected candidate Cl-/HCO3- exchangers with or without CFTR. Among the tested Cl-/HCO3- exchangers, the activities of AE4, SLC26A4, and SLC26A6 were increased by ATP-induced intracellular calcium signaling and CFTR co-transfection. However, the activities of AE1, AE_2, and AE_3 were not activated by the above treatments. Interestingly, the activity of SLC26A3 was not activated by calcium signaling, which was known to be activated by cAMP. These data suggest that the molecular targets of pancreatic bicarbonate secretion induced by calcium signaling and those by cAMP are segregated.

P120038

Amplitude and kinetics of action potential evoked Ca^{2+} current and its efficacy in triggering transmitter release at the calyx of Held synapse

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Action potentials (APs) play a crucial role in evoking the presynaptic Ca^{2+} current (ICa) through voltage-gated calcium channels (VGCCs) and transmitter release. During development and neuromodulation, the AP depolarization and repolarization phases change, but how such changes affect the characteristics of ICa and its efficacy at central synapses is not clear. By paired voltage-clamp recordings of ICa and excitatory postsynaptic currents (IEPSC) with pseudo-APs, we found that speeding the AP depolarization phase primarily reduced the number of activated VGCCs, while shortening the AP repolarization phase decreased the number of activated VGCCs and accelerated their kinetics. Both the number of activated VGCCs and their kinetics affect the total ICa integral, with each component underlying about 50 % of the maximal IEPSC (IEPSG-MAX). Crosscorrelation analyses of ICa and IEPSC evoked by real-APs and pseudo-APs demonstrated that developmental AP shortening significantly decreased the ICa integral and IEPSC. These results suggest that AP narrowing is a critical adaptation for achieving high-fidelity and high-frequency neurotransmission required for sound localization at the calyx of Held synapse.

P120039

Angiotensin II Inhibits Kir Channels in Rabbit Coronary Arterial Smooth Muscle Cells through Protein Kinase C alpha

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We investigated the effects of the vasoconstrictor angiotensin (Ang) II on the whole-cell inward rectifier K^+ (Kir) current enzymatically isolated from small-diameter coronary arterial smooth muscle cells (CASMCs). Ang II inhibited the Kir current in a dose-dependent manner. Pretreatment with PLC inhibitor and PKC inhibitors prevented the Ang II-induced inhibition of the Kir current. The PKC activator reduced the Kir currents. The inhibitory effect of Ang II was reduced by intracellular and extracellular Ca^{2+} free condtion and by Go 6976, which inhibits Ca^{2+}-dependent PKC isoforms alpha and beta. However, the inhibitory effect of Ang II was unaffected by inhibitor of PKC epsilon. Western blot analysis confirmed that PKC alpha, and not PKC beta, was expressed in small-diameter CASMCs. The Ang II type 1 (AT1)-receptor antagonist CV-11974 prevented the Ang II-induced inhibition of Kir the current. From these results, we conclude that Ang II inhibits Kir channels through AT1 receptors by the activation of PKC alpha.

Key words: angiotensin II, inward rectifier K^+ channel, protein kinase C, coronary artery

P120040

Role of 5-HT_2 antagonist on the regulation of ATP-sensitive potassium channel activity in the mouse ventricular cardiomyocytes

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Effects of 5-HT_2 antagonists on the ATP-sensitive K^+ (KATP) channels were studied in mouse ventricular cardiomyocytes. Under whole-cell voltage-clamp conditions, ketanserin (KT; 1-100 μM), a 5-HT_2 antagonist, reversibly inhibited pinacidil-induced KATP current with a Ki value of 9.36 μM and the Hill coefficient was 0.67. This inhibition was developed even in the presence of 5-HT (100 μM). Prazosin, a selective alpha1- antagonist, failed to mimic the effect of KT. KT did not affect the channel activity in inside-out patches under ATP-free condition. KT, applied to external solution, did not affect the pinacidil-induced channel activity in cell-attached patches, but did inhibit it when applied into the pipette. Pirenperone (PP; 100 μM), another 5-HT_2 antagonist, also decreased the pinacidil-induced current in whole-cell voltage-clamp condition, but less potent than those of KT. In inside-out patches, PP also did not affect the channel activity. These results indicate that 5-HT_2 antagonists used in the present study inhibited KATP channel activities, and this action was not mediated through 5-HT_2 or alpha1-adrenoceptor, rather a direct one on the cardiac KATP channels.

P120041

Adenosine-dependent regulation mechanism for inward rectifier K^+ channels in rabbit coronary arterial myocytes

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We studied the effect of adenosine on the Ba^{2+}-sensitive KIR channels in the smooth muscle cells isolated from the small-diameter (< 100 um) coronary arteries of rabbits. Adenosine increased KIR currents in concentration-dependent manner (EC_{50} = 9.4 ± 1.4 uM, maximum increase of 153 %). The adenosine-induced stimulation of KIR current was blocked by adenylate cyclase inhibitor, SQ22536 and was mimicked by adenylate cyclase activator, forskolin. The adenosine-induced increase of current was blocked by PKA inhibitors, KT5720 and Rp-8-CPT-cAMPs. The adenosine-induced stimulation was blocked by an A3 selective antagonist MRS1334, while the antagonists of other subtypes (DPCPX for A1, ZM241385 for A2A, and alloxazine for A2B) were all ineffective. Furthermore, an A3 selective agonist, 2-Cl-IB-MECA induced increase of KIR current. We also examined the effect of adenosine on coronary blood flow (CBF) rate. In the presence of glibenclamide to exclude the effects of KATP channels, CBF was increased by adenosine (10 uM), which was blocked by the addition of Ba^{2+} (50 uM). Above results suggest that in rabbit coronary arteries, adenosine increases KIR current via A3 subtype in a PKA-dependent manner.

P120042

Effects of cloperastine on the 8-OH-DPAT-induced single K(+) channel currents

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Several kinds of medicines affecting the central nervous system inhibit the currents passing through G protein-coupled inwardly rectifying K^+ (GIRK) channels. We found that cloperastine (CP), a centrally acting antitussive, is the most potent among them. Therefore, we further analyzed the effect of CP on the single GIRK channel activity. Method: Dorsal raphe neurons were acutely dissociated from 7- to 18-day-old Wistar rats and outside-out mode of patch clamp was applied. Result: The histograms of open and closed states of 8-OH-DPAT (3 nM)-activated single GIRK channel were fitted with two and three exponential functions, respectively. CP (1 μM) shortened two mean open times and prolonged the longest mean closed time. These effects of CP on the open and close kinetics were different from those of spiperon, a 5-HT(1A) receptor antagonist, Ba^{2+}, and tertiapin, a peptide GIRK channel blocker. In addition, internally applied CP at 10 μM abolished the opening by 8-OH-DPAT. The effect of internal CP at 1 μM was similar to that applied from outside. These results suggest that CP might inhibit single GIRK channel activities in a different way from other substances studied.

P120043

A glass pipette based automated patch clamp system for drug screening.

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An automated patch clamp system is presented based on standard glass patch electrodes. In principle, a few hundred cells of a cell suspension are deployed inside the patch pipette and suction is applied from the tip. A single cell is then drawn towards the very end of the tip and establishes a GigaSeal and subsequent whole-cell or perforated patch configuration, similar to manual operation. Compound application uses quartz needles for perfusion of the pipette. Cell handling, exchange of recording pipettes, called the FlipTip, and compound application are all automated, giving rise to several hours of recording without user intervention. Data will be shown for a variety of ion channels, including Kv1.5 and hERG potassium channels, Na^+, Ca^{2+}, and TRP channels. The robotic platform is equipped with either 3 or 6 channels, and achieves a daily throughput of 100-500 data points. Since standard patch clamp electrodes are used the cost for consumables are low. Moreover, cell-type specific FlipTips can be made. Hence, the Flyscreen is an elegant yet affordable APC-system for expression studies, secondary screening, safety pharmacology, and can be used in academia and in the pharmaceutical industry.

P120044

Na^+/Ca^{2+} Exchanger Contributes to Sarcoplasmic Reticulum Ca^{2+} Refilling

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In skeletal muscles, Ca^{2+} efflux is carried out via Ca^{2+}-ATPase and Na^+-Ca^{2+} exchanger (NCX). Ca^{2+} entry pathway is still not well understood, but store operated calcium entry (SOCE) is thought to play an important role. NCX expels Ca^{2+} in exchange of Na^+ in normal mode and accumulates Ca^{2+} in reverse mode. We investigated contribution of NCX to SOCE. Mechanical recordings were obtained from rat diaphragm strips. Ca^{2+} was depleted by incubating in Ca^{2+}-free media. SOCE was induced by reintroduction of 2 mM Ca^{2+}. Basal tone increase was used as a marker of Ca^{2+} entry. Area under caffeine contracture curve was used as a measure of sarcoplasmic reticulum (SR) Ca^{2+} content. In Ca^{2+} depleted muscles, Ca^{2+} administration induced SOCE and increased the basal tone. Selective NCX inhibitors, KB-R7943 and benzamil reduced basal tone increase and KB-R7943 decreased initial part of SR reloading. In Ca^{2+} depleted muscles, loading with Ca^{2+} for 30 min, yielded similar refilling status in both KB-R7943 and controls. These data suggest that NCX, operating in reverse mode, contributes to SOCE during early refilling phase and alters SR Ca^{2+} refilling kinetics.

Key words: Calcium, sodium, depletion, exchanger

P120045

Two types of K^+ channels regulate proliferation and death of bovine brain endothelial cells

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Brain capillary endothelial cells (BCECs) contribute to brain homeostasis by forming blood brain barrier. Here we report functional analyses of ion channels and receptors in the regulation of cell proliferation and cell death in t-BBEC 117, an immortalized cell line derived from bovine brain endothelial cells. In t-BBEC 117, metabotropic P2Y receptors (P2YRs), Ca^{2+}-activated K^+ channel (SK2), transient receptor potential (TRP) channel and inwardly rectifier K^+ channel (Kir2.x) were functionally expressed. We found a positive feedback mechanism for the regulation of $[Ca^{2+}]i$ following the stimulation of P2YRs. The initial rise of $[Ca^{2+}]i$ enhanced SK2 current, which hyperpolarized the cells and further increased Ca^{2+} entry through TRP channels. This mechanism enhanced cell proliferation. Furthermore, in approximately 20% of cells, where Kir2.x channels were highly expressed, the excess hyperpolarization was induced by the activation of Kir2.x following the SK2 activation. This resulted in cell death. Thus, P2Y stimulation in BCECs enhances cell proliferation via SK2 activation, and, in a portion of cells, induces cell death by switching Kir2.x channels on.

P120046

Reversal of the stimulatory effect of insulin on KATP channels by H2S preconditioning

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H_2S is endogenously produced in many mammalian cells. H_2S-induced activation of KATP channels in and suppressed insulin secretion from insulin-secreting (INS-1E) cells have been reported. Using the patch-clamp technique, it was found in the present study that either H_2S (100 uM, n=4) or insulin (100 nM, n=5) alone, independent of PI3 kinase pathway, significantly increased open probability of a 78 pS KATP channel in INS-1E cells ($p<0.05$). The stimulatory effect of insulin on KATP channels was 60+-19.2% greater in outside-out patch (n=5) than in inside-out patch (n=5, $p<0.001$). In the presence of H_2S (100 uM), insulin (100 nM) significantly reduced the open probability of single KATP channels by 2.75-fold in inside-out patches (n=4) but 4.63-fold in outside-out patches (n=4). Our results indicate that insulin predominantly acts on the exuracellular mouth of KATP channels. In the face of a high endogenous H_2S in pancreatic beta cells, insulin factually inhibits KATP channel opening, leading to beta cell membrane depolarization and potentially increased insulin release. (Supported by CIHR and NSERC).

Key words: H_2S, insulin, KATP channel, pancreatic beta-cells

P120047

3,4-Methylenedioxyamphetamine elicits action potential bursts in a central snail neuron

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The effects of 3,4-methylenedioxyamphetamine (MDA) were studied in an identifiable RP4 neuron of the African snail, Achatina fulica Ferussac, using the two-electrode voltage-clamp method. The RP4 neuron generated spontaneous action potentials and bath application of MDA reversibly elicited action potential bursts of the central RP4 neuron. The action potential bursts elicited by MDA were not blocked when neurons were immersed in high-Mg^{2+} solution, Ca^{2+}-free solution, nor after continuous perfusion with propranolol, prazosin, haloperidol, sulpiride, or methiothepin. Notably, the induction of action potential bursts was blocked by pretreatment with protein kinase C inhibitors, chelerythrine or Ro 31-8220, while not by protein kinase A inhibitors, H89 or KT-5720. Voltage-clamp studies conducted on the RP4 neuron revealed that MDA decreased the delayed rectifying potassium current. Both chelerythrine and Ro 31-8220 decreased the inhibitory effect of MDA on the delayed rectifying potassium current. It is concluded that MDA elicits action potential bursts in the central snail RP4 neuron and that the effect is closely related to the protein kinase C and the delayed rectifying potassium current.

P120048

Effects of N-n-butyl Haloperidol Iodide on Transient Outward Potassium Current in Rat Ventricular Myocytes

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Objective: N-n-butyl haloperidol iodide (NBH) was screened from a series of quaternary ammonium salt derivative of haloperidol, which maintains the effect of relaxation of coronary artery but has no extrapyramidal adverse reaction of haloperidol owing to its high polarity not to pass the blood-brain barrier. Our advanced studies have shown that it can block the calcium channel of rat vascular smooth muscle cells and ventricular myocytes. The effects of NBH on transient outward potassium current (Ito) were investigated in the present study. Methods: Ito from enzymatic dissociation of rat ventricular myocytes was examined using the whole cell voltage-clamp technique. Results: NBH decreased Ito ($IC_{50}=2.80\times10^{-4}$ M) with a negative-shift of the steady-state inactivation curve. But the steady-state activation curve of Ito was unaffected. In addition, NBH slightly slowed the rate of recovery of Ito from inactivation. Conclusions: NBH blocks the Ito channels of ventricular myocytes. Combining the effect of NBH blocking calcium channels of ventricular myocytes, these effects lead to a modification of the electromechanical function and may likely contribute to the termination of ventricular arrhythmias. These results provide an opportunity to develop an effective vasodilator and antiarrhythmic agent.

Key words: N-n-butyl haloperidol iodide; transient outward potassium current; whole cell voltage-clamp; antiarrhythmic agent

This work was supported by the National Nature Science Foundation (No. 30070304) and the National New Drugs Research Foundation of the People's Republic of China (No. 9690105231).

P120049

BRAIN ADRENERGIC RECEPTORS IN DOPAMINE-β-HYDROXYLASE KNOCKOUT MICE

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We examined CNS expression of adrenergic receptors in the postnatal absence of NE using mice with a homozygotic deletion of dopamine-beta-hydroxylase (Dbh-/-) compared to Dbh heterozygotes (Dbh+/-), which have normal NE levels. α1-AR, α2-AR and β-AR were assayed autoradiographically with [^{3}H]prazosin, [^{3}H]RX21002, and 125I-pindolol respectively; α2-AR agonist high affinity state with [125I]-piodoclonidine; and α2-AR functionality with α2-AR agonist-stimulated [35S]GTPγS. α1-AR in Dbh-/- mice were similar to Dbh+/- mice except for up-regulation (75%) in hippocampus. Decreases in α2-AR were found in septum(-15%), hippocampus (-35%) and amygdala (-15%); density of α2-AR agonist high affinity state was decreased only in septum(-20%). Neither of these were reflected in α2-AR functionality (α2-AR agonist-stimulated [35S]GTPγS binding). Density of β-AR was upregulated 30-50% in all regions examined in Dbh-/- mice compared to Dbh+/-. These findings indicate that postnatal regulation of adrenergic receptors by endogenous NE depends on receptor type and neuroanatomical region.

Key words: Adrenoceptors, dopamine-beta-hydroxylase, development, norepinephrine

Support: NS33194 (LCM), MH64772 (DBB)

P120050

Therapeutic characterization of new Na^+/H^+ exchanger inhibitor for ischemic heart disease

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The effects of Na^+/H^+ Exchanger-1 (NHE1) inhibitors in the ischemic and reperfused heart disease have been one of the most widely studied areas. Ischemia promotes NHE1 activation, and activation of NHE1 increases intracellular Na^+. Increased $[Na^+]i$ leads to the influx of $[Ca^{2+}]i$ by Na^+/Ca^{2+} exchanger (NCX). This increased $[Ca^{2+}]i$ levels cause very serious cell damage and dysfunction during reperfusion. Thus, it is important that inhibiting NHE1 before reperfusion is key in preventing heart injury. Among new NHE1 inhibitiors (KR-32511, 32570, and 330281), we screened more effective inhibitors than existing NHE1 chemicals for their efficacy in blocking NHE1 activity as well as in primary rat cardiomyocytes and specificity for NHE1. In result, except KR-32511, two inhibitors blocked NHE1 better than control inhibitors and showed better specificity towards NHE1 when tested in rat submandibular gland for NHE2 and PS120/NHE3 cells for NHE3. Furthermore, these inhibitors did not alter the function of Epithelial Na^+ Channel (ENaC) in normal human nose epithelial cells. In conclusion, KR-32570 and KR-330281 can be very potent new NHE1 inhibitors as a therapeutic target for ischemic heart disease.

P120051

K^+ Channel Regulation of Slow Wave Activity in the Guinea-pig Prostate

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In the guinea-pig prostate, the spontaneous slow waves underlie the spontaneous contractions which contribute to the prostatic resting tone. In this study, the contribution of K^+ channels in regulating slow wave activity was investigated. Prostate glands were removed from guinea-pigs (300-500g) killed humanely by stunning and exsanguination. Electrical activity from the guinea-pig prostate stroma was recorded using intracellular microelectrodes. In the presence of TEA (1-3mM) slow wave frequency was increased by 15% ($n=10$, $p<0.05$) while 4AP (1mM) increased the frequency of slow waves from 4 to 7 min-1 and duration by 15% ($n=10$, $p<0.05$). Glibenclamide (1μM) ($n=8$) and apamin (1-200nM) ($n=8$) had little effect on the slow wave activity. In the presence of SNP (10μM), a nitric oxide donor, the slow wave activity was completely abolished ($n=18$, $p<0.05$), which was reversed by the TEA (1-3mM), 4AP (1mM) and Glibenclamide (1μM). Our results indicate that slow wave frequency is regulated by BK, 4AP-sensitive and KATP channels and that the inhibitory effects of SNP on slow waves occur partially from the opening of these channels.

Key words: Prostate, K^+ channel, electrophysiology

Supported by the NHMRC (Australia).

P120052

Identification of AQP5 in lipid rafts and its translocation in rat parotid interlobular ducts

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Aquaporin-5 (AQP5), an apical plasma membrane (APM) water channel in salivary glands, has an important role in fluid secretion. M3 muscarinic acetylcholine receptor (mAChR)-induced changes in AQP5 localization was investigated in parotid interlobular ducts. Confocal microscopy revealed AQP5 localization in rafts and AQP5 trafficking to the APM 10 min after injection of cevimeline. Conversely, 60 min after injection, there was a diffuse pattern of AQP5 staining. The calcium ionophore A23187 mimicked the effects of cevimeline. Under control conditions, the majority of AQP5 localized in the Triton X-100 (TX100)-insoluble fraction and floated to light-density fraction on discontinuous density gradients. After 10-min incubation of parotid tissue slices with cevimeline or A23187, the AQP5 levels decreased in TX100-insoluble fraction and increased in TX100-soluble fraction. Thus, AQP5 localizes in the intracellular rafts and M3 mAChR activation induces AQP5 trafficking to the APM with rafts via calcium signaling and induces AQP5 dissociation from rafts to non-rafts on the APM in interlobular duct cells of rat parotid glands.

P120053

The Oxidative regulation of Ion Channels

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In this study, we investigated the redox-induced regulation of ion channels using different models. With combination of patch-clamp techniques and adenovirus-mediated NOS gene expression, we compared the different effects between NO and H2O2, NO and NOS on P/Q-type Ca^{2+} channel expressed in BHK cells. The results showed that NO can enhance the Ca^{2+} currents by direct oxidation of cysteine in ion channel proteins. The increased intracellular Ca^{2+} can activate NOS, and then produces NO, which may form a positive feedback loop to regulate neurotransmitter release. Meanwhile, β3 subunit can facilitate the methionine-specific oxidant Ch-T induced up-regulation of BK channel. The M117 in β3 subunit are critical for this facilitation of redox induced ion channel regulation. In conclusion, ROS may play a role in modifying ion channel functions via redox of amino acids. The enzyme-controlled oxidative-reductive reaction of amino acids may be one of important mechanisms for anti-oxidation in the body.

Key words: ROS; Ca^{2+} channel; BK channel

This work was supported by National Natural Science Foundation of China (30270351) and National Distinguished Young Scientists of China (30425024) to Dr. Chen J.

P120054

Effect of Cyclovirobuxine D(CVB-D) on Oddi's sphincter contraction

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AIM: To study the effect of CVB-D on contraction of guinea pig Oddi's sphincter in vitro. METHODS: Within the range from 1×10^{-7}M to 3×10^{-6}M, CVB-D's effects on the smooth muscle under the following conditions: 1) the two phases of the Oddi's sphincter contractive curve caused by 80mM K^+ and 5×10^{-6}M Ach; 2) together with Ver, the contraction of Odd's sphincter induced by 80mM K^+ and 5×10^{-6}M Ach respectively. RESULTS: The relation between the amount of CVB-D and rapid contraction of inhibitory response was in a dose-effect one as well as the plateau/peak value. Contrary to CVB-D, Ach caused rapid, continuous phase and plateau/peak value to fall as CVB-D de-

creased. Combination of Ver and CVB-D led peak value induced by K^+ to lessen, but did not influence Ver plateau used only and vanished plateau of rapid phase induced by Ach. CONCLUSION: CVB-D's effects on the contraction of Oddi's sphincter are related to the different agonists and contractive phases, which reflect its effects on Ca^{2+} channels.

Key words: Cyclovirobuxine D (CVB-D) Verapamil (Ver) Ca^{2+} channel

Acknowledgement: Thanks for the support from School of Pharmacy, Fudan University.

P120055

Electrical Responses Of Aortic Smooth Muscle In Streptozotocin-Induced Diabetes Rats

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To compare the electrical responses of isolated thoracic aorta-smooth muscle in diabetic and healthy rats. Diabete was induced by a single tail vein injection of streptozotocin 45 mg/kg. The endothelium-dependent hyperpolarization evoked by acetylcholine (ACh) using convertional microelectrode technique. Depolarization responses of aortic smooth muscle from control and 8 Wk streptozotocin-diabetic rats were compared in the presence and absence of endothelium. In the presence of endothelium, responses of aorta from diabetic animals to phenylephrine or noradrenaline were enhanced the depolarization. Following endothelium removal, no significant differences were found between control and diabetic arteries in the depolarization responses to phenylephrine or noradrenaline. Acetylcholine induced endothelium-dependent hyperpolarization that was mediated by nitric oxide (NO). NO-mediated hyperpolarization was impaired in diabetic arteries. The results of the present study indicate that enhanced responsiveness of arteries from diabetic animals to alfa adrenoceptor stimulation. In addition, there is a reduced influence of nitric oxide.

Key words: Diabet, Thoracic aorta, Electrical response

P120056

Effect of Acidic Polysaccharides CA4-3 on Ion Channels in Human Lymphocytes

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CA4-3, an acidic polysaccharide isolated from Liuwei Dihuang decoction, a famous traditional Chinese medicine prescription, possess the function to enhance immune response and lymphocyte proliferation. In present studies, the effect of CA4-3 on ion channel was investigated in human peripheral blood monocular cell (PBMC) by patch clamp technique. The results showed that CA4-3 dose-dependently enhanced voltage-gated potassium channel (Kv1.3) current in PBMC. Meanwhile it did not affect calcium activated potassium channel (IKCa1) current. Further studies on purified human T and B cell indicated that CA4-3 selectively enhances peak Kv1.3 current in B, not T, lymphocyte and causes the shift of steady-state activation toward to hyperpolarization, without influence on inactivation and other kinetics. Those results strongly proved that B lymphocyte is the main target of CA4-3 and activation of Kv1.3 channel in B lymphocyte was the early step of immunomodulating effect of CA4-3.

Key words: polysaccharides; B lymphocytes; patch clamp

Acknowledgement: This work was supported by the National Natural Science Foundation of China (No.30300453).

P120057

Effects of New Conotoxin SO-3 on Voltage-sensitive Calcium Channels Transiently Expressed in HEK293 Cells

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L-, N-, P/Q or R-type calcium channels were respectively expressed in HEK293 cells to determine whether specific types of calcium channels were affected by SO-3, a new 25-aminoacid conotoxin derived from the venom of Conus striatus. SO-3 selectively and reversibly inhibited the N-type whole-cell Ba^{2+} currents (I_{Ba}) in a concentration-dependent manner; at 0.01~0.1 μM, its inhibition effects on N-type I_{Ba} were more obvious than those of ω-conotoxin MVIIA, a selective N-type channel blocker. Akinetic analysis of the SO-3 effects on N-type channels showed that SO-3 blocked resting, open, and inactivated channels. At higher concentrations (30 and 100 μM), SO-3 could reversibly and partly inhibit the L-, P/Q-, and R-type I_{Ba}, but these effects were less than those of MVIIA. Considering the significance of N-type channels for pain transduction, SO-3, as our results showed, is a potential new N-type calcium channel blocker, may have therapeutic potential as a novel analgesic candidate.

Key words: SO-3; conotoxins; N-type calcium channel blockers.

Acknowledgment: This work was supported by 863 Program (2001AA624150) and National Natural Science Foundation (30100240, 30572175) of China.

P120058

KCNQ potassium channels in pulmonary artery smooth muscle

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Potassium channels are important regulators of pulmonary vascular tone, controlling the membrane potential and excitability of pulmonary artery smooth muscle cells (PASMCs). The finding that the KCNQ-channel blockers, linopirdine and XE991, are potent pulmonary vasoconstrictors suggests that these channels might contribute. We investigated the involvement of KCNQ channels in the resting K^+ conductance and potential of PASMCs. Intrapulmonary arteries were dissected from rats sacrificed by cervical dislocation and PASMCs isolated enzymatically. Membrane potential and currents were recorded using patch-clamp. KCNQ expression was assessed using RT-PCR, western blotting and immunostaining. Both linopirdine (10 uM) and XE991 (5 uM) reduced the background K^+ conductance by ~40% at 0 mV and caused significant depolarisation. RT-PCR revealed mRNA expression for several KCNQ subunits while immunostaining suggested protein expression for KCNQ1, KCNQ3, KCNQ4 and KCNQ5. Western blots confirmed KCNQ5 expression. This provides strong evidence for functional KCNQ channels in pulmonary artery that regulate resting potential.

Key words: Pulmonary, smooth muscle, KCNQ

Funded by BBSRC and British Heart Foundation

P120059

Regulation of Store-operated Calcium Influx by Phospholipase A2 in Dystrophic Skeletal Muscle

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The muscle degeneration occurring in Duchenne muscular dystrophy (DMD) is thought to be caused by enhanced activity of non-selective cationic channels activated either by calcium store depletion (Store-operated channels) or by stretch of the plasma membrane (Stretch-activated channels). Using both cytosolic calcium measurements with Fura-2 and the manganese quench method, we show here that store-operated calcium entry is greatly enhanced in dystrophic skeletal Flexor Digitorum Brevis (FDB) fibers isolated from mdx5cv mice, a mouse model of DMD. More interestingly we show that capacitative calcium entry in intact FDB fibers from dystrophic mice is under the control of calcium-independent phospholipase A2 (iPLA2) and that exaggerated calcium influx occurring in dystrophic fibers can be attenuated by iPLA2 inhibitors to a value close to normal fibers. The iPLA2 pathway therefore appears as an interesting potential target to reduce excessive calcium influx and subsequent degeneration occurring in dystrophic fibers.

P120060

Mechanism of the positive inotropic effect of dofetilide on isolated rat ventricular cells

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To study the effect of dofetilide in isolated rat ventricular cells and the mechanism, whole-cell patch clamp and ionic imaging techniques were used. Results showed dofetilide increased Na^+/Ca^{2+} exchange current ($I_{Na/Ca}$) in a concentration-dependent manner from 0.03 to 1.0 μmol/L on both inward and outward transport directions in rat myocytes. The EC_{50} of outward and inward $I_{Na/Ca}$ were 0.183 μmol/L (95% confidence interval (CI95) was 0.058~0.520 μmol/L) and 0.178 μmol/L (CI95 was 0.024~1.296 μmol/L), respectively. 0.2 μmol/L dofetilide significantly enhanced Ca^{2+} transient by 57±21 ($P < 0.01$) and cell shortening by 3.6±1.2 μm ($P < 0.01$), increased the calcium sensitivity, short-

ened the Ca^{2+} transient and diastolic durations in rat myocytes. When tested with patch clamp and ionic imaging simultaneously, it showed no active effect on ICa, but increased Ca^{2+} transient by 87 ±38 ($P < 0.01$) and cell shortening by 2.1 ± 0.6 μm ($P < 0.01$), respectively. In conclusion, dofetilide had positive inotropic and positive lusitropic effects on rat ventricular cells. The enhancement of $I_{Na/Ca}$ might be involved in these effects.

Key words: dofetilide, Na^+/Ca^{2+} exchange, ventricular myocytes, calcium transient

P120061

The Influence of Berberine on Cardiac Function of L-Thyroxine Induced Cardiac Hypertrophy Rat Model

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Berberine is the basic chemical component of a Chinese herb, Coptis chinensis Franch(coptis), considered to be useful in treating some diseases of the cardiovascular system, such as hypertension and chronic heart failure(CHF). In this study, we intend to assess the effects of Berberine on cardiac function of cardiac hypertrophy rats induced by L-Thyroxine. The cardiac hypertrophy model was produced by subcutaneous injection of L-thyroxine, the drugs were administrated by gastrogavage for 4 weeks at dosage of Berberine 10 mg/kg and Metoprolol 10 mg/kg. Then the cardiac function, the nitric oxide content of left ventricular tissue and serum were measured. Data showed that Berberine significantly depressed the left ventricular systolic pressure (LVSP), the maximum rate of contraction ($+dp/dt_{max}$) and heart rate(HR), raised the left ventricular end-diastolic pressure(LVEDP); elevated the nitric oxide content of left ventricular tissue and serum. It suggested that Berberine could prevent the heart hyperaction caused by L-thyroxine, and such effects were significantly correlated with the cardiac NO content.

Key words: Berberine, L-thyroxine, NO

The study was supported by the NSFC Grant of China.

P120062

Effect of epidermal growth factor receptor activation on Iks channel

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Objection: To determine whether epidermal growth factor receptor (EGFR) activation affects the Iks channel and to explore the receptor-channel interaction mechanism. Methods: The mRNA of human KCNQ1, KCNE1, EGFR were expressed heterologously in Xenopus laevis oocytes. Membrane currents were measured with the double electrode voltage-clamp technique. EGFR was activated by using EGF. Results: EGFR activation decreased the KCNQ1/KCNE1 current and increase KCNQ1 current, which was prevented by application of genistein, an inhibitor of tyrosine kinase. Conclusion: EGFR activation decreased KCNQ1/KCNE1 current via tyrosine phosphorylation of KCNE1.

Key words: voltage-clamp; phosphorylation; Iks channel; EGFR

P120063

Disruption of Ca^{2+} Homeostasis in Aconitine-induced Toxicity of Cultured Cardiomyocytes

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In present study, we discussed mechanism of aconitine (ACO) induced Ca^{2+}-dependent arrhythmia. We characterized the cytotoxicity, alterations of cytosolic Ca^{2+} signal, expressions of Ca^{2+} handling proteins in ACO-induced primary cardiomyocytes by MTT, LDH release, comet assay, Ca^{2+} imaging, RT-PCR and Western blot. It is shown that treatment with ACO results in not only distinct cytotoxicity of cell viability, cytomorphology, spontaneous beating and DNA damage, but disruption of cytosolic Ca^{2+} signal and upregulation of L-type Ca^{2+} channel, SR Ca^{2+} release channel (RyR2) and Na^+/Ca^{2+} exchanger proteins. While application of Na^+ channel and RyR_2 inhibitors tetrodotoxin and ruthenium red can partly reverse the ACO-induced abnormity. It is concluded that ACO induces the disruption of intracellular Ca^{2+} homeostasis and thus the unbalance of EC coupling, which might the potential reason of its arrhythmic cytotoxicity, and special inhibitors appear to play important roles in detoxification of ACO-induced Ca^{2+}-dependent arrhythmia.

Key words: aconitine, cytotoxicity, Ca^{2+} homeostasis, Ca^{2+}-dependent arrhythmia

Acknowledgment: The work was supported by the national Key Technologies R&D Programme. (2004BA721A11)

P120064

Investigation of the relaxant effect of C-type natriuretic peptide (CNP) in human penile small arteries.

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CNP is a known relaxant and hyperpolarizing agent in the cardiovascular system. The aim of the present study was to investigate the role of K^+-channels and hyperpolarization in the relaxant effect of CNP in human penile small arteries. Erectile tissue was obtained in connection with transsexual operations (n = 9), and the isometric tension and membrane potential was recorded. CNP (0.01-1 μM) evoked relaxation (70.7 ±6.3%) in phenylephrine-contracted blood vessels, which was inhibited in K^+ contracted preparations and in the presence of the combination of charybdotoxin + apamin and barium + ouabain, known inhibitors of different K^+-channels and Na^+/K^+ ATP-ase. Membrane potential recording showed that CNP (0.7 μM) induced smooth muscle cell hyperpolarization (1.6 ±0.2 mV). The present findings suggest that Ca^{2+}-activated and inward-rectifier potassium channels, sensitive to charybdotoxin, apamin and barium, respectively, and Na^+/K^+ ATP-ase, sensitive to ouabain, play an important role in the relaxant effect of CNP in human penile small arteries.

Key words: CNP, K^+ channels, Na^+/K^+ ATP-ase

P120065

Inhibition of Transient Outward and Ultra-Rapid Delayed Rectifier Potassium Currents and Sodium Current by Eicosapentaenoic Acid from Fish Oil in Human Atrial Myocytes

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Unsaturated fatty acids from fish oil was recently reported to exert a protective effect against atrial fibrillation in humans; however, ionic mechanisms are not fully understood. The present study was therefore designed to investigate effects of eicosapentaenoic acid (EPA, an important unsaturated fatty acids from fish oil) on transient outward and ultra-rapid delayed rectifier potassium currents (I_{to} and I_{Kur}), and voltage-gated sodium current (I_{Na}) in human atrial myocytes using whole-cell patch configuration. It was found that EPA inhibited Ito in a concentration-dependent manner (IC_{50} = 10.5 μM), without affecting time- and voltage-dependent kinetics of the current. In addition, the unique current I_{Kur} was suppressed by 1-50 μM EPA (IC_{50} = 12.2 μM) in human atrial cells. Moreover, EPA reduced I_{Na} in human atrial myocytes in a concentration-dependent manner (IC_{50} = 11.6 μM), negatively shifted the potential of I_{Na} availability, and slowed recovery of I_{Na} from inactivation. These results indicate that anti-atrial fibrillation of EPA in man is likely related to the inhibition of I_{to} and I_{Kur} (prolonging atrial action potential duration) and reduction of I_{Na} (stabilizing cardiac membrane potential).

P120066

The role of potassium channels in the relaxation of bovine coronary artery induced by hydrogen peroxide.

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Endothelium-derived hyperpolarizing factor (EDHF) hyperpolarizes vascular smooth muscle by opening K^+ channels and then elicits vasodilatation. Currently, hydrogen peroxide (H_2O_2) is a major candidate for EDHF. The aim of this study was to investigate the effect of H_2O_2 and the relation of this effect with K^+ channels in bovine coronary artery. H_2O_2 (10^{-7}-10^{-2} M) relaxed bovine coronary artery strips contracted with $PGF_{2\alpha}$ (10^{-5} M), in tissue baths (E_{max}: 94.2 ±3 %). Removal of endothelium did not change the effect of H_2O_2. The relaxation was not affected by tetraethylammonium (10^{-4} M, inhibitor of Ca^{+2}-activated K^+ channels), charybdotoxin (10^{-7} M, inhibitor of Ca^{+2}-activated K^+ and voltage sensitive K^+ channels) but inhibited by glibenclamide (10^{-6} M, inhibitor

of ATP-sensitive K^+ channels) significantly (E_{max}:54.5 ±2 %; P<0.05). On the other hand, H_2O_2 did not relax arteries contracted with 80 mM K^+ solution. It is concluded that H_2O_2 induces endothelium independent relaxation in bovine coronary artery and this relaxation is mediated, in part, by activation of ATP-sensitive K^+ channels. This conclusion supports the reports stating that H_2O_2 can be an EDHF.

Key words: hydrogen peroxide, coronary artery

P120067

Expression and Function of Na^+/Ca^{2+} Exchanger in Duodenal Epithelial Cells

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Na^+/Ca^{2+} exchanger (NCX) plays an important role in controlling cytosolic Ca^{2+} ($[Ca^{2+}]$cyt) in many mammalian cells. Since the expression and function of NCX in the duodenum are poorly understood, the purpose of the present study was to identify the localization and function of NCX in duodenal mucosa. NCX1 proteins were found to be mainly localized to the apical membranes of duodenal epithelial cells. 5-HT induced duodenal mucosal bicarbonate secretion (DMBS) in Ca^{2+}-containing solutions, but not in Ca^{2+}-free solutions. 5-HT stimulated DMBS was significantly attenuated by KB-R7943 (10 M), a selective inhibitor of the reversed mode of NCX. Acid significantly stimulated DMBS in control intact mice, whereas KB-R7943 (10 mg/kg, i.p.) attenuated this response by 93% (n=6, p<0.01). Acid-stimulated DMBS was intact in NCX+/+ mice, but was obviously impaired in NCX+/- mice (n=5, p<0.05). When NCX1 protein was knocked down with a specific siRNA in a duodenal epithelial cell line, the activity of NCX was also attenuated. Therefore, our data indicate that NCX1 is expressed in duodenal epithelial cells and plays an important role in the regulation of DMBS by controlling $[Ca^{2+}]$cyt.

Key words: NCX1; $[Ca^{2+}]$cyt; DMBS

P120068

Modulation of Transient Outward and Ultra-Rapid Delayed Rectifier Potassium Currents by Raloxifene in Human Atrial Myocytes

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Raloxifene (a selective estrogen receptor modulator) showed cardiovascular protective in humans. However, it is unclear whether raloxifene would affect human cardiac repolarization currents. The present study was therefore to investigate effects of raloxifene on transient outward and ultra-rapid delayed rectifier potassium currents (I_{to} and I_{Kur}) in human atrial myocytes using whole-cell patch technique. It was found that Ito was inhibited by raloxifene with IC_{50} of 1.8 μM. Time-dependent recovery from inactivation was slowed, and time to peak and time-dependent inactivation of I_{to} was significantly accelerated, while voltage-dependence of activation and inactivation of I_{to1} were not affected by raloxifene. Importantly, raloxifene substantially suppressed the unique current I_{Kur} (IC_{50} = 0.7 μM) in human atrial cells. These effects were not affected by the estrogen receptor antagonist ICI 182780. Our results indicate that the estrogen receptor modulator raloxifene directly inhibits the repolarization potassium currents I_{to} and I_{Kur} in human atrial myocytes, suggesting that raloxifene may have beneficial effects on supraventricular arrhythmias in man.

P120069

Discriminative Modulation of Zolpidem on the Sympathetic Nervous System at the Spinal Level

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Zolpidem modulates GABA$_A$ mediated currents at benzodiazepine receptors with subtype selectivity. This study reports on the influence of zolpidem on GABA$_A$-mediated responses in sympathetic preganglionic neurons (SPNs). Whole cell recordings were obtained from SPNs of thoracic spinal cord slices (300 μm) of rats (10-14days). Inhibitory postsynaptic potentials (IPSPs) were evoked by stimulating fibres descending in lateral funiculus (Lf) or interneurons in the central autonomic area (CAA) and were isolated in kynurenic acid (2 mM). At a low concentration (0.3-0.5 μM), zolpidem induced an initial increase in IPSP amplitude from both Lf (115.0 ±12.4%, n=10) and CAA (129.5 ±11.9%) stimulation. However, a secondary sustained increase was also observed on those IPSPs elicited by Lf stimulation (110.1 ±9.2% to 116.1 ±11.5%), an effect not induced in CAA IPSPs. At higher concentrations (1-10 μM, n=5), increase in IPSP amplitude was related to drug concentration. These results indicate that the effects of Zolpidem on SPNs might be via different GABA$_A$ receptors subunits or combinations.

Key words: GABA$_A$, Zolpidem, SPN

We acknowledge the support of the British Heart Foundation.

P120070

Calmodulin kinase II phosphorylation and calmodulin binding prevent run-down of L-type Ca^{2+} channels

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We have previously reported that the basal activity of L-type Ca^{2+} channels is suppressed by inhibitors of calmodulin (CaM) dependent protein kinase II (CaMKII), and that CaM represses the channels after run down. However, the CaM effect becomes smaller with the longer run-down time. This study is to investigate the relations of CaMKII and CaM in maintaining the Ca^{2+} channel basal activity. Single Ca^{2+} channel activities are recorded with patch clamp technique in guinea-pig ventricular myocytes. A GST-fusion peptide containing a.a. 1509-1791 of the C-terminal region of guinea-pig Cav1.2 (CT-1) is prepared. After run down, CaMKIIα-T286D, a constitutively active CaMKII produces Ca^{2+} channel activity to only 2-10% of the basal activity. However, in the presence of CaMKIIα-T286D, the time-dependent nature of the CaM effect is abolished. In pull-down assay, CT-1 treated with CaMKII shows a higher affinity for CaM than that treated with phosphatase. Conclusion: Both phosphorylation of the channel protein with CaMKII and binding of CaM to the channel may be required for maintaining basal activity of the Ca^{2+} channels.

Key words: calmodulin, CaMKII, Ca^{2+} channel, run-down.

P120071

The role of potassium channels in the vasodilating action of levosimendan on the human umbilical artery.

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Levosimendan is a calcium-sensitizing and inodilator agent which is working via potassium channels and under current investigation in the treatment of heart failure. We investigated the type of potassium channels, which play role on the dilatating effect of levosimendan on the contractile tone of the isolated human umbilical artery. The responses were recorded isometricaly by a force displacement transducer in isolated organ baths. Levosimendan (10 nM-3 μM) was added to organ baths after precontraction with serotonin (1 μM). Levosimendan-induced relaxations were tested in the presence of the large conductance Ca^{2+}-activated K^+ channel inhibitor tetraethylammonium (TEA, 1 mM), ATP-sensitive K^+ channel inhibitor glibenclamide (GLI, 10 μM) and the voltage-sensitive K^+ channel inhibitor 4-aminopyridine (4-AP, 1 mM). Levosimendan produced potent relaxation in the human umbilical artery. This relaxation was not affected by GLI. However, 4-AP and TEA inhibited levosimendan-induced relaxation significantly ($p<0.05$). In conclusion, the mechanism of this levosimendan-induced relaxation in the umbilical artery appears to be due to voltage-gated and large conductance Ca^{2+}-activated K^+ channel opening action.

Key words: Levosimendan, human umbilical artery, vasodlation, potassium channels

P120072

The Role of Intercellular Calcium Store in the Healing of Full Thickness Excisional Wounds in Rabbit

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Objective: The present study attempted to elucidate the role of intracellular calcium ions store in an in vivo setting, using dantrolene, an agent known to interfere with calcium release from sarcoplasmic reticulum. Materials and Methods: Full thickness excisional wounds (2 x 2 cm) were created down to the fascia layer at

the dorsal side of the rabbits. Daily tracing technique of the wound surface area, complemented with histological assessment was used to assess the healing effects of various concentrations of dantorlene (0.5, 1 and 2 % in eucerin base). The results were compared with non-treated and vehicle treated-control wounds. Results: The rate of reduction in wound surface area was not found to be significantly different among all treatment groups. Furthermore, no apparent changes in the histological parameters were observed. Conclusion: The intracellular calcium store does not contribute a significant role in the process of wound healing.

Key words: Endoplasmic reticulum, intracellular calcium store, dantrolene, rabbit, wound healing.

P120074

NARINGIN MODULATES GIRK1/GIRK4 POTASSIUM CHANNELS INDEPENDENT OF THE PRESENCE OF $GABA_B$ RECEPTOR SUBUNITS.

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Flavonoids are polyphenolic compounds present in large quantities in plants. $GABA_B$ receptors belong to G protein-coupled receptors. Compounds that modulate this receptor are potential therapeutics for the treatment for epilepsy and addiction. AIMS: To investigate the pharmacological properties of different flavonoids on $GABA_B$ receptors. METHODS: GB1b, GB2, GIRK1 and GIRK4 mRNA were microinjected into Xenopus oocytes. Two-electrode voltage clamp methods were performed 2-3 days after injection. RESULTS: The flavonoid glycoside naringin (100 μM) positively modulated $GABA_B$ receptors in the presence of GIRK1/GIRK4 ($I/I_{GABA(3\mu M)} = 0.77 \pm 0.15$; n = 8 oocytes). CGP36742, a $GABA_B$ receptor antagonist, did not block this modulation. It was found that naringin (100 μM) positively modulated GIRK1/GIRK4 in the absence of $GABA_B$ receptor subunits ($EC_{50} = 110 \pm 1.15$ μM; n = 3-18 oocytes). The effects of gossypin, flavone and resveratrol were also studied. CONCLUSION: Naringin and gossypin positively modulated GIRK1/GIRK4 potassium channels, while flavone and resveratrol negatively modulated these channels. The modulatory effects of these flavonoids are independent of the $GABA_B$ receptor subunits.

P120075

Left ventricular (LV) mechanical dysfunction and Ca^{2+} overload caused by oxidative stress: Role of Na^+-H^+ exchangers and voltage-gated Na^+ channels.

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Intracellular Ca^{2+} overload caused by oxidative stress may play a role in LV ischemia-reperfusion (IR) injury. This study examined the role of Na^+ loading by either Na^+-H^+ exchange (NHE) or late Na^+ current (I_{Na}) in H_2O_2-induced Ca^{2+} accumulation and LV dysfunction. Intracellular Ca^{2+} ($[Ca^{2+}]_i$, indo-1 fluorescence) and LV function were measured in isolated working rat hearts (n = 5/group) perfused at 37 °C with Krebs containing glucose (11 mM), insulin (100 $mU.L^{-1}$) and palmitate (1.2 mM). H_2O_2 (100 μM for 30 min) caused a transient (5 min) decrease in LV function to 31.9 ±6.3 % of baseline that recovered to 67.7 ±4.5 %, and a slow increase in diastolic and systolic $[Ca^{2+}]_i$ by 8.5 ±1.6 % and 16.0 ±1.5 %. Cariporide (5 μM), a selective inhibitor of NHE, did not affect responses to H_2O_2, but it reduced Ca^{2+} overload and LV dysfunction caused by IR. R56865 (1 μM), a selective inhibitor of late I_{Na}, reduced Ca^{2+} overload and LV dysfunction due to enhancement of late INa with Sea Anemone Toxin II (12 nM) or by IR. R56865 did not alter the adverse effects of H_2O_2. These results suggest that, in contrast to IR, NHE and late I_{Na} have no major roles in Ca^{2+} overload and LV dysfunction caused by oxidative stress.

P120076

Elevated ADMA level contributes to downregulation of small-conductance potassium channels (SK3) expression in endothelium of atherosclerotic mice

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Objective: To determine the role of endogenous inhibitor of nitric oxide synthase asymmetric dimethylarginine (ADMA) in the expression of small-conductance potassium channels (SK3) in endothelium of atherosclerotic mice. Methods: ApoE-/- mice were treated with and without ADMA (5 mg/kg/day, ip) for 4 weeks. Human umbilical vein endothelial cells (HUVECs) were incubated with lysophosphatidylcholine (LPC, 5 μg/ml) or ADMA (10 μM) for 48 h. Protein and mRNA levels of SK3 were determined by western blot and RT-PCR, respectively. Results: The levels of ADMA both in the plasma of apoE-/- mice and in the medium of LPC-treated HUVECs were markedly increased. ADMA-treatment greatly increased the downregulation of both protein and mRNA expressions of SK3 in the thoracic aortas of apoE-/- mice. Similarly, LPC or ADMA significantly downregulated both mRNA and protein expressions of SK3 in HUVECs. Conclusion: Elevated ADMA level may contribute to the downregulation of small-conductance potassium channels (SK3) expression in endothelium of atherosclerotic mice.

Key words: Small-conductance potassium channels; Asymmetric dimethylarginine; Endothelium; Atherosclerosis

P120077

Effects of N-n-butyl Haloperidol Iodide on L-type Calcium Channel in Rat Ventricular Myocytes

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Objective: The aim of this study was to investigate the effects of N-n-butyl haloperidol iodide (F_2) on L-type calcium channel (I_{Ca}) in rat ventricular myocytes. Methods: Cells were isolated enzymatically from rat ventricles. The whole-cell patch clamp technique was used to record I_{Ca}. Results: Our data showed that (1) F_2 reduced the voltage activated peak amplitude of I_{Ca} in a concentration dependent manner (0.1 to 10 $\mu mol \cdot L^{-1}$). F_2 up-shifted the current-voltage (I-V) curve of I_{Ca} without altering the maximal activation voltage, the reversal potential of I_{Ca}; (2) F_2 induced a marked leftward shift of the steady-state inactivation curves of I_{Ca}, but did not affect the activation curves of I_{Ca}; (3) F_2 markedly shifted the curve of time-dependent recovery of I_{Ca} from steady-state inactivation to the right, and prolonged the recovery time of I_{Ca} from inactivation (n = 10 cells, $p < 0.01$). Conclusions: F_2 inhibits I_{Ca} maybe due to acting on on the inactivated state of L-type calcium channels.

Key words: calcium channel; calcium; myocytes; patch clamp techniques

P120078

Effects of the hypoxia on the activity of Na^+, K^+-ATPase and αisoforms in rat brain slices

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Objective: Our previous studies have proved the changes of Na^+, K^+-ATPase αisoforms in different cerebral ischemic models. The present study is to identify the responsible changes of the activity of Na^+, K^+-ATPase and αisoforms in hippocampal and cortical slices induced by the hypoxia. Methods: The Na^+, K^+-ATPase activity, the pump current and the mRNA expressions of three αisoforms in normal and hypoxic slices were detected by spectrophotometry, patch-clamp and the RT-PCR techniques, respectively. Results: The changes of activity of Na^+, K^+-ATPase in hippocampal and cortical slices were different during hypoxia for 5, 10, 15, 30 and 60 minutes. The pump current was reduced after hypoxia for 10 min. In the hippocampal slices, the mRNA expression of α3 isoform was more than that of α2 or α1 isoform. But in cortical slice there was no significant difference in the mRNA expressions of three αisoforms. After hypoxia for 10 min, the mRNA expressions of three αisoforms were not changed both in hippocampal and cortical slices. Conclusion: These results suggest that the hypoxia could reduce the decrease of Na^+, K^+-ATPase activity, which might be not due to the changes in the expression of αisoforms mRNA.

P120079

MscL adaptation to sustained membrane stretch in liposomes under different amphipaths

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Our objective is to study the effects of two amphipaths, chlorpromazine (CPZ) and trinitrophenol (TNP), on the adaptation of mechanosensitive channel of large conductance (MscL). Purified MscL protein was reconstituted into liposomes, which were prepared using phosphotidylcholine. Single channel current was recorded in inside-out patches. To observe adaptation, negative pressure was applied to the pipette, the exponential decay of the currents was compared. Our results showed that (1) Increasing negative pressure caused adaptation decreased. When the channels maximally activated, MscL channels would not show any

adaptation. (2) MscL adaptation depends on the pressure changes exerted on the channels during an experiment and is not influenced by the membrane tension applied to pre-stress the membrane patch. (3) After adding TNP and CPZ inside the pipette, MscL responsiveness to membrane tension was altered, the adaptation was observed in all patches but was decreased in a concentration-dependent manner. Our results indicated amphipaths can alter the properties of MscL adaptation and may have a big role in the modulation of mechanosensitive channel function.

Key words: MscL, amphipaths, liposome, adaptation

This work was supported by the Australian Research Council.

P120080

INHIBITORY MECHANISM OF NICORANDIL ON CATECHOLAMINE SECRETION FROM THE RAT ADRENAL MEDULLA

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The present study was attempted to investigate the effect of nicorandil, which is an ATP-sensitive potassium channel opener, on secretion of catecholamines (CA) evoked by cholinergic stimulation and membrane depolarization from the isolated perfused rat adrenal gland. Collectively, these experimental results suggest that nicorandil causes the marked inhibition of CA secretion evoked by stimulation of cholinergic receptors as well as by membrane depolarization, indicating that this effect may be mediated by inhibiting both influx of extracellular calcium and the release of intracellular calcium in the rat adrenomedullary chromaffin cells. Furthermore, these findings suggest that these potassium channel openers-sensitive membrane potassium channels also play a modulatory role in regulating CA secretion.

P120081

1,4-diazabicyclo[2.2.2]octane derivatives: a novel class of voltage-gated potassium channel blockers

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Voltage-gated potassium (Kv) channels are targets for therapeutic drugs in the treatment of electrical disorders such as cardiac arrhythmia. Here, we synthesized three classes of novel polyammonium compounds incorporating the bicyclic unit 1,4-diazabicyclo[2.2.2]octane (DABCO) and tested their action on three representative mammalian Kv channels, Kv2.1, Kv3.4 and Kv4.2. Simple DABCO monostrings and diDABCO strings inhibited Kv2.1 and Kv3.4 channels, with potency increasing with string length. Kv2.1 and Kv3.4 were most sensitive to C_{16} monostrings, with IC_{50} values in the low micromolar range. For aromatic DiDABCO compounds, inhibition depended upon relative positioning of the two DABCO groups, with only the para forms showing activity. Kv4.2 channels were relatively insensitive to all compounds tested. MTSET protection studies suggested DABCO compounds bind in the outer pore. Thus, DABCO salts represent a new class of relatively potent Kv channel blockers. The potential for synthesis of an array of modular derivatives suggests that DABCO compounds hold promise as probes of Kv channel structure and identity, and as therapeutic agents.

P120082

A-TYPE POTASSIUM CURRENT IN MICROVASCULAR SMOOTH MUSCLE IS A $K_v1.5/K_v\beta1$ CO-ASSEMBLY

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Aim: to identify the A-type K^+ current in retinal microvascular smooth muscle (MVSM) cells using patch clamp techniques, RT-PCR, immunohistochemistry and neutralizing antibody studies. The A-type K^+ current was resistant to specific inhibitors of K_v3 and K_v4 channels, but blocked by the K_v1 antagonist correolide,. No effects were observed with pharmacological agents directed against $K_v1.1/2/3/6$ and 7 channels, but the current was blocked by riluzole, a $K_v1.4/K_v1.5$ inhibitor. It was unaffected by K^+-free solution but abolished by flecainide, suggesting involvement of $K_v1.5$- rather than $K_v1.4$ channels. Transcripts encoding $K_v1.5$ but not $K_v1.4$ were identified. Immunofluorescent labeling showed $K_v1.5$ localisation to the plasma membrane of MVSM but not $K_v1.4$. Anti-$K_v1.5$ antibody applied intracellularly inhibited the current: anti-$K_v1.4$ antibody had no effect. $K_v\beta1$ or $K_v\beta3$ subunits convert $K_v1.5$ currents from delayed rectifier to A-type currents. $K_v\beta1$ mRNA was detected in retinal arterioles, but not $K_v\beta3$. This data points to a likely co-assembly of $K_v1.5$ and $K_v\beta1$ subunits as the major component underlying the A-type K^+ current in retinal MVSM.

Key words: A-type K^+ current, $K_v1.5$, retina, arterioles

P13. Clinical Pharmacology - Clinical Trial for New Drugs

P130001

Study on the bioequivalence of cefdinir dispersible tablet in human being

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Objective: To study the bioequivalence of cefdinir dispersible tablet in human being. Method: Cefdinir dispersible tablet and cefdinir capsule were used as the investigational drug and the control drug respectively. Each drug (200 mg) was taken orally one time for each healthy volunteer. The interval of administration was 5 days. Blood drug levels at specified timepoints were determined by HPLC. Result: cefdinir consistents with the one open compartment model of oral administration. There were no significant differences between the main parameters, C_{max} (1.52 ±0.48 vs 1.42 ±0.39 μg/ml), T_{max} (3.08 ±0.73 vs 3.22 ±0.81 h), $t_{1/2}$ (2.04 ±0.53 vs 1.87 ±0.29 h), $AUC_{(0-t)}$ (7.12 ±1.85 vs 6.86 ±1.60 (g/ml) h), $AUC_{(0-inf)}$ (7.67 ±2.01 vs 7.38 ±1.85 (g/ml) h). The relative bioavailability of the investigational drug was 103.53 ±11.50%. RSD was 11.11%. The 90% confidence interval of $AUC_{(0-t)}$, $AUC_{(0-inf)}$ and C_{max} of the investigational drug were 80.05%-119.95%, 98.74%-108.52% and 70.12%-142.88% of that of the control respectively. Conclusion: the two agents were bioequivalence in vivo.

Key words: cefdinir dispersible tablet, bioequivalence

P130002

Pharmacokinetics, tolerability, and safety of pirfenidone (PFD), an antifibrotic agent, following single and multiple oral doses in healthy volunteers

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Objective: To assess the pharmacokinetics (PK), tolerability, and safety of single and multiple oral doses of PFD in healthy volunteers. The gender and food effects on the PK were also evaluated. Methods: PK studies of PFD were examined in an open-label, randomized, dose-escalating trial in forty-eight subjects (24 females, 24 males). PK were determined from serial blood and urine samples obtained up to 12 h after single 200, 400 or 600 mg doses of PFD, and 108 h after 400 mg three times daily. Results: Plasma levels and AUC of PFD were found to be proportional to the doses. PK parameters after multiple doses were similar to those obtained after single doses. Under fasted and fed conditions, T_{max} were 0.8 and 1.5 h; C_{max} were 13.0 and 9.2 mg/L, respectively. PFD was well tolerated. Conclusions: PFD displays linear PK in the dose range of 200 to 600 mg, and no accumulation occurs with repeated dosing. Concomitant food intake considerably reduces the rate of absorption of PFD, while no effects of gender on the PK were observed.

Key words: pirfenidone; antifibrotic agent; pharmacokinetics

P130003

A multicentric clinical study to evaluate the efficacy and tolerability of LL-2123 HP, a polyherbal formulation, in antitubercular drug induced hepatotoxicity

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A randomized, double blind, placebo controlled, multicentric clinical study was conducted to evaluate the possible protective effects of LL-2123 HP, a polyherbal preparation, against anti-TB chemotherapy induced hepatotoxicity in patients of pulmonary TB. After ethical clearance, preliminary screening, informed consent and baseline liver function tests, clinically diagnosed pulmonary TB pa-

tients (n = 103) were randomly divided into two groups and given placebo or LL-2123 HP (test drug), along with anti-TB chemotherapy for 8 weeks, and were followed up at regular intervals (at 1, 2, 4 and 8 weeks) for qualitative and quantitative measures of liver function. Analysis of data of 95 completed patients showed that there was a significant increase in body weight (49%) in the test drug group as compared to placebo (42%). Further, the anti-TB chemotherapy induced elevations in liver function markers in test and placebo groups were: SGOT(30% vs 85%), SGPT(28% vs 90%) and GGTP(25% vs50%) in the 8 week follow ups. Adverse effect profile of the test drug group was less severe as compared to that of placebo. The results indicate that LL-2123 HP was more efficacious and better tolerated than the placebo when used against anti-TB chemotherapy.

Key words: Anti-TB Chemotherapy, Hepatotoxicity, LL-2123 HP

The financial support from Lupin Limited (Mumbai) is gratefully acknowledged

P130004

Influence of Afobazol on the psychophysiological parameters of healthy vdunteers with different background personal traits

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The present study was undertaken and performed with the aim to evaluate the effect of the novel selective anxiolytic Afobazol and full benzodiazepine receptor agonist Phenazepam upon the operatory performance in healthy volunteers stratified into stress-resistant and stress-unresistant groups using psychological rating scales. Afobazol at a dose of 5 mg proved more effective when compared to 0.5 mg of Phenazepam in stress unresistant individuals as to psychophysiological functions assessment criterion and the absence of neither hypnosedative nor myorelaxant effects.

P130005

Disposition but not the cholesterol-lowering effect of ezetimibe in man is markedly influenced by co-medication of rifampicin, an inhibitor of hepatic OATP1B1

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Disposition of the sterol-lowering ezetimibe (EZ) is influenced by the intestinal efflux transporters Pglycoprotein and MRP2 and the glucuronosyltransferase UGT1A1. To evaluate their role in hepatic elimination of EZ and its glucuronide (GLUC), disposition of EZ was studied in presence of rifampicin, which inhibits MRP2 and hepatic drug uptake by OATP1B1. Disposition of EZ (20 mg, po) alone and in presence of rifampicin (600 mg, po) was measured cross-over in 8 healthy subjects (22-36 years, BMI 20.4-23.9, all SLCO1B1 *1a/*1a). EZ and GLUC in serum, urine and feces and the plant sterols campesterol and sitosterol in serum were quantified using LC-MS/MS. After rifampicin, AUC and fecal excretion of EZ were decreased (140 ±86.3 vs. 102 ±37.6 ng*h/ml, ns; 10.4 ±1.8 vs. 7.6 ±2.2 mg, $p < 0.05$) whereas AUC and renal excretion of GLUC were markedly increased (1030 ±370 vs. 2150 ±690 ng*h/ml; 2.0 ±1.2 vs. 4.9 ±1.9 mg, both $p < 0.05$). Rifampicin did not influence the effects of EZ on plant sterol absorption. Co-medication of rifampicin increases systemic exposure with GLUC most likely by inhibition of its intestinal secretion and hepatic uptake but does not influence the sterol lowering effect of EZ.

P130006

A multicentric clinical study to evaluate the efficacy and tolerability of LL-2123 HP, a polyherbal formulation, in antitubercular drug induced hepatotoxicity

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A randomized, double blind, placebo controlled, multicentric clinical study was conducted to evaluate the possible protective effects of LL-2123 HP, a polyherbal preparation, against anti-TB drug induced hepatotoxicity in patients of pulmonary TB. Clinically diagnosed pulmonary TB patients (n = 103) were randomly divided into two groups and given placebo or LL-2123 HP (test drug), along with anti-TB therapy for 8 weeks, and were followed up at regular intervals for qualitative and quantitative measures of liver function. Analysis of data of 95 completed patients showed that there was a significant increase in body weight (49%) in the test drug group as compared to placebo (42%). Further, the anti-TB chemotherapy induced elevations in liver function markers in test and placebo groups were: SGOT(30% vs 85%), SGPT(28% vs 90%) and GGTP(25% vs50%) in the 8 week follow ups. Adverse effect profile of the test drug group was less severe as compared to that of placebo. The results indicate that LL-2123 HP was more efficacious and better tolerated than the placebo when used against anti-TB chemotherapy.

P130007

Human Tolerance to ω-3 Unsaturated Fatty Acid Soft Capsules from Callorhinus Oil

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AIM: We observed the tolerance to ω-3 unsaturated fatty acid soft capsules from callorhinus oil in Chinese normal male and female volunteers. METHODS: Thirty normal adult volunteers are divided into five groups and they are administered 1g, 1.5g, 2.5g, 4g, 5g of soft capsules for 30 days respectively, PO, bid. Clinical symptoms and laboratory indexes before, midst and after administration are compared to evaluate the tolerance to ω-3 unsaturated fatty acid soft capsules. RESULTS: There is no significant difference among groups before, midst and after administration. Only in a few vdunteers occurs the side effect of mild diarrhea. CONCLUSION: Normal adult volunteers indicate good tolerance to ω-3 unsaturated fatty acid soft capsules from callorhinus oil. The recommended dosage of 5g/d is acceptable.

Key words: tolerance, unsaturated fatty acid, callorhinus oil

P130008

The double-blind controlled trial of bupropion and amitriptyline in the treatment of 229 patients with depressive disorders

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The outpatients or inpatients who met criteria for depressive episode in Chinese Classification and Diagnostic Criteria of Mental Disorders, 3rd ed. were randomly assigned to bupropion group (n = 115) and amitriptyline group (n = 114) for 6 weeks. The efficacy was evaluated with Hamilton Depression Scale (HAMD), Hamilton Anxiety Scale (HAMA) and Clinical global Impression Scale (CGI). The Safety and tolerability were assessed with Treatment Emergent Symptom Scale (TESS), hematology, clinical chemistry, urinalysis, electrocardiogram and vital sign. HAMD scores of bupropion reduced less than those of amitriptyline ((-16 ±8) vs (-20 ±7), $P < 0.01$). Bupropion was inferior to amitriptyline in the effect on anxiety/somatization and sleep disorders of HAMD and psychical anxiety of HAMA ($P < 0.05$). There were less drowsiness, dry mouth, tachycardia and weight gain of bupropion than those of amitriptyline ($P < 0.05$). The adverse effects of bupropion were fewer than those of amitriptyline, but its anxiolytic effect could be not as good as amitriptyline.

Key words: Bupropion; Amitriptyline; Depressive Disorders

P130010

Topical nimesulide gel treatment in knee osteoarthritis

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Aim: to investigate if topical nimesulide treatment has any beneficial effect in knee osteoarthritis patients. Seventy-four knee osteoarthritis outpatients were enrolled in this randomized, double-blind, placebo-controlled (5/2 treatment/placebo ratio; n = 51/23) study. WOMAC Osteoarthritis Index, NHS and patient & physician global satisfaction scores were used as efficacy measures at initial and final visit (1 month). Treatment group (TG) received topical nimesulide gel (Sulidin gel 1%) and placebo group (PG) an identical gel preparation (3x1) for 30 days. 70 patients completed the study. There was a significant improvement in the TG for all three main parameters and overall score of WOMAC between pre- and post-treatment values, whereas no significant change was observed in PG. There was a significant improvement at Energy level, Pain, Physical motion and NHPD scores in the TG whereas no improvement in any of the parameters for the PG. The average of patient and physician global satisfaction scores for TG and PG

were 3.3 to 1.8 and 3.7 to 1.5, respectively. The results indicate that the topical administration of nimesulide gel produces significant improvement in knee osteoarthritis patients.

P130011

PHARMACOKINETICS AND D2 RECEPTOR OCCUPANCY MODELING OF A NOVEL ANTIPSYCHOTIC, YKP1358

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Objective: YKP1358 is a novel 5-HT_{2A} and D_2 antagonist. We conducted a D_2 receptor occupancy study with YKP1358 in healthy volunteers using PET (Positron Emission Tomography), to measure the D_2 receptor occupancy and to characterize how it relates to plasma drug levels. Method: A single oral dose, dose escalation (100 mg, 200 mg, and 250 mg) study was performed in 10 healthy male volunteers using the PET radiotracer [^{11}C] raclopride. The D_2 occupancy of striatum was measured pre-dose, and at 2, 5, and 10 h after dosing of YKP1358. Serial blood samples were taken for determination of plasma levels of YKP1358. Results: D_2 occupancy of YKP1358 was 53%-83% at 2 h, 40%-64% at 5 h, and 20%-51% at 10 h. The dose-plasma level relationship showed large variability, but plasma level and D_2 occupancy of YKP1358 showed good relationships and were well predicted by a sigmoid E_{max} model using non-linear mixed effects modeling. Conclusions: D_2 occupancy of YKP1358 was related to plasma levels, and well predicted by a sigmoid E_{max} model. Using these results, the initial doses for achieving therapeutic ranges of D_2 occupancy of YKP1358 can be estimated for further patient studies.

Key words: Receptor occupancy, Schizophrenia

P130012

Dose-Escalating Study to Investigate Safety, Tolerability, and Pharmacokinetics of Lonicera japonica Extract in Healthy Volunteers

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Lonicera japonica (LJ) has been applied to of inflammatory diseases in Oriental medicine. We studied safety, tolerability, and pharmacokinetics of rising, single and multiple intravenous doses of extract from LJ, SKLJI in 80 healthy volunteers (56 for single, 24 for multiple; total 13 times for 4 days). A randomized, placebo-controlled, double blind, dose-escalation study after single and multiple dosing was conducted. Blood and urine samples were collected and subjects were monitored throughout the study. Seven and 14 cases of adverse events related with SKLJI were reported in single and multiple doses, respectively. They were mild, transient and relieved without an intervention. In single dose, T_{max} were 30 min for slow infusion, 5 min for bolus, respectively. $T_{1/2}$ was 1.4-1.6 h. Linear pharmacokinetic profiles were shown and interindividual variations were 15-30% in high dose. Pharmacokinetics of multiple doses was similar to that of single dose. The accumulation index was 0.93-1.08, and renal clearance was 5-12 L/h. SKLJI was safe and well tolerated as a single and multiple doses up to 100 mg. It showed linear pharmacokinetics, short $T_{1/2}$, little accumulation, and small interindividual variations.

P130013

Clinical observation and experimental research on the treatment of chronic pelvic inflammation by Baijiang Compound

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This study conducted clinical observation and experimental research of the treatment of chronic pelvic inflammation by Baijiang Compound. 220 subjects were divided into two groups at random. 111 patients in the trial group were treated with Baijiang Compound and the other 106 cases were given Qianjinpian as the control. The results showed that the total effective rate of trial and control group was 96% and 80% respectively, both groups were found effective in improving the clinical symptoms and hemorrheological and immunologic character. Animal experiments indicated lymphocyte transformation index, the level of serum IL-2 markedly decreased, IL-6 and indexes of hemorrheology such as all blood viscosity, plasma viscosity and HCT significantly increased in model control group. Different doses of Baijiang Compound improved these indexes of uterus in various degree; Morphological investigation also revealed the alleviation of inflammation in Baijiang Compound groups. The results above suggested that Baijiang Compound has significant therapeutic effects on chronic pelvic inflammation, which may be related to the improved blood circulation and regulated immune function.

P130014

An oral, rising, single-dose pharmacokinetic and safety study of pregabalin capsules in healthy volunteers

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Pregabalin has pain-relieving, anxiolytic, and anticonvulsant activity. Our objective was to determine the pharmacokinetic characteristics, safety, and tolerance of rising, single oral doses of pregabalin capsules (100, 200, and 300 mg) in healthy Korean volunteers. An oral, rising, single-dose, double-blind, randomized, placebo-controlled, parallel group, staggered-start study was conducted in 30 healthy male volunteers. Serial blood and urine samples were collected for pregabalin assay from Day 1 to 3. Safety evaluations were performed. Pregabalin was rapidly absorbed with individual T_{max} (0.5 ~2hr). Mean oral bioavailability was at least 94.5%. Mean values by dose group for renal clearance and oral clearance were similar. Values for $t_{1/2}$ were independent of dose (5 ~8hr). Pregabalin C_{max} and AUC(0-∞) appeared to increase less than proportionally with dose. All Adverse Events (AEs) were mild and transient. No clinically significant laboratory abnormalities, vital signs or ECG measurements were observed. Pregabalin C_{max} and AUC values increased with increasing dose; however, the increases were slightly less than dose proportional. Pregabalin was generally safe and well tolerated with only mild AEs.

P130015

Survey of adenosine effect on sperm motility.

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Objective: adenosine as a nucleoside naturally finds in all of human tissues. It combined with phosphorous groups and produce energy. These mechanisms active cells such as spermatozoids. We surveyed the effect of adenosine on sperm motility. Material and Methods: this study was carried out as case-control. We added adenosine in 2, 5 and 10 mmol concentrations of adenosine in Ham's F10 as case and Ham's F10 culture as control to 10 sample of normal semen and then compared sperm motility in samples after 15 minutes. Results: we found sperm motility increase in all of concentration of adenosine. There was significant correlation between sperm motility and adenosine in 5 mmol concentration. Conclusions: Our founding shows that adenosine in 5 mmol concentration increase sperm motility. We recommend using of adenosine for increasing of sperm motility.

P130016

A new acetaminophen (APAP) antipyretic and analgesic treatment strategy in children: using an initial loading dose

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A new antipyretic and analgesic APAP dosing schedule has been evaluated after revisiting APAP pharmacokinetics and pharmacokinetic-pharmacodynamic relationship. A lag-time to APAP maximal effect, ranging 7 to 20h, related to the time to obtain steady-state plasma concentrations and to a 1-2h lag-time in the time course to maximal antipyretic effect compared to time to maximal plasma concentration. To decrease this lag-time, the use of an initial APAP 30 mg/kg loading dose (twice a usual dose), followed by the usual 15 mg/kg/6h maintenance dose schedule has been suggested. Three controlled clinical trials in children were conducted: - In febrile children a single 30 mg/kg (loading dose) demonstrated superiority to a 15 mg/kg single dose in time to 38.5C (-30 min), time below this temperature (+1h). - Results of a repeated-dose trial confirmed these findings. - Post-operative analgesic efficacy, clinical and biological safety were evaluated for 24 hours. A preventive postoperative nalbuphine-sparing ef-

fect that improved postoperative analgesia was observed in 1/3 more of the patients in the loading dose group. Excellent clinical and biological (liver enzymes) safety was recorded in both groups.

P130017

SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF CKD-501, A NOVEL PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA/GAMMA DUAL AGONIST

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CKD-501 is a novel peroxisome proliferator-activated receptor α/γ dual agonist for the treatment of diabetes mellitus. This study aimed to investigate safety, tolerability, and pharmacokinetics of CKD-501 in healthy volunteers. A randomized, placebo-controlled, double-blind, parallel-group, dose-rising study was performed. Thirty-six healthy male subjects received single oral doses ranging from 0.5-8 mg CKD-501 or placebo. In the multiple-dose study, 24 subjects received 1-4 mg once daily for 7 days. Serial blood and urine samples were collected. No serious adverse events (AEs) were observed and AEs reported were all of mild severity. In the single-dose study, mean C_{max} and AUCinf increased linearly up to the 8 mg dose level. T_{max} and $t_{1/2}$ ranged from 0.5-4 h and 7.8-9.8 h, respectively. Less than 1% CKD-501 was excreted in urine. After multiple dosing, accumulation index was around 1.2. Mean apparent clearance, T_{max} and $t_{1/2}$ in steady state were independent of dose and time. Single oral doses up to 8 mg CKD-501 and multiple doses up to 4 mg were safe and well tolerated. Mean C_{max} and AUCinf were dose proportional and there was no remarkable accumulation after multiple dosing.

Key words: PPAR, clinical trial

P130018

Studies on correlation between dissolution in vitro and absorption in vivo of levofloxacin tablets

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Aim: To investigate relationship between dissolution in vitro and absorption in vivo of levofloxacin tablets from two pharmaceutical coorperations (A, B). Methods: The dissolution of levofloxacin was determined according to Chinese pharmacopoeia. Levofloxacin concentration in plasma was determined by RP-HPLC after levofloxacin tablets were given to 18 volunteers. Their pharmacokonetic parameters were obtained by 3P97 program, the absorption percentage was calculated according to Wàngner-Nelson formula. Results: Bioequivalence of two preparations calculated by two one-side test showed they were bioequivalent The dissolution parameters of levofloxacin tablets acquired in different rotation speed all meet the requirement of Chinese pharmacopoeia. The linear regressive equation was established between the absorption percentage in vivo fa and accumulate release percentage in vitro (50 r m-1) ft as faA = 2.0176ftA + 0.7279, rA = 0.957; faB = 1.8929ftB + 0.7749, rB = 0.955 ($P < 0.05$). Conclusion: There was a significant relationship between absorption in vivo and dissolution performed in condition of rotation speed 50 r m-1 in vitro.

Key words: levofloxacin; HPLC; bioavailability; in vivo and in vitro correlation

P130019

Study on bioequivalence of metformin hydrochloride sustained release tablets

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AIM: To study if metformin hydrochloride sustained release tablets were bioequivalent to Metformin hydrochloride tablets. Methods: 36 male healthy volunteers were divided into two groups randomly. Single dosage group: sustained release tablets and control tablets were administered orally to the same subject respectively only once; Multiple dosage group: sustained release tablets were administered orally once a day, or control tablets twice a day for seven consecutive days. The plasma concentrations at different times were measured by HPLC. Pharmacokinetic parameters were calculated. Results: Single dosage group: all parameters except $T_{1/2}$ had significant difference ($p < 0.01$). Multiple dosage group: all parameters except Css and AUCss had significant difference ($p < 0.001$). All parameters of the last dosage of two preparations had significant difference ($p < 0.01$). Relative bioavailability of the sustained release tablets for single dosage was similar to that for multiple dosage. Conclusion: Metformin hydrochloride sustained release tablets could be released slowly, and were bioequivalent to the control tablets.

Key words: bioequivalence, metformin hydrochloride

P14. Clinical Pharmacology - Pharmacovigilance

P140001

SELF MEDICATION WITHOUT PHYSICIAN PRESCRIPTION IN AMBERES NEIGHBORHOOD, CARTAGENA, COLOMBIA IN 2003

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BACKGROUND: Self medication represent a big public health problem in undeveloped countries, most of people living in Cartagena Colombia are classified in poverty 75%,. OBJECTIVE: Determinate the characteristic of phenomenon of self medication in the population under study. Methods: A descriptive transversal study realized that include a sample of 580 houses, obtained from the census (1993) and question survey instrument. RESULTS: Base on the total population the percentage of self medication was (61.2%), distributed; women (40.6%) man (20.6%). The most commonly drugs used was commercial acetaminophen (Dolex), metronidazole, atropine sulphate plus difenoxilate (Lomotil) Aluminum hidroxile, mg hidroxile plus simeticone (Mylanta). The most common pathologies were; fever, stomachache, acute infection respiratory disease. Conclusions: The frequency of self medcation in the population under study was higher than developed countries. The self medication was higher in housewife than others conditios We need more information using analytic studies to determinate what are the factors that can influence in this behavior.

P140002

Intensive pharmacovigilance of Grow Colony Stimulate Factor (ior G-CSF) in patients with cancer in Cienfuegos, Cuba

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Neutropenia and infections limit chemo and/or radiotherapy, the use of Grow Colony Stimulate Factor (Leukocim) as primary/secondary prophylaxis or as treatment help to recover myelosupressor effects with favorable profile of tolerance, it was a study of intensive drug vigilance to measure safety of the product by checking 39 clinical records with 73 neutropenic episodes included in the national, phase IV and open clinical trial; the adverse events were determined by clinical and laboratory parameters and were classified taking into account intensity and relation of causality; 38,36% episodes presented adverse events, from them 23,28% presented 1 event, with 2 12,33% and with 3 2,74%, the most frequent adverse effects were: hyperuricemia (14,63%), pain in bones (12,19%) and fever (12,19%), most of them had mild intensity (58,54%) and 60.97% were classified as possible, 7 patients died due to their clinical condtion not because of the treatment, the drug was safe since it reported known adverse effects.

Key words: ior (G-CSF), Leukocim, neutropenic, adverse events, clinical trial.

P140003

Intensive pharmacovigilance of IFN α2b in the treatment of multiple sclerosis, during clinical trial

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It was carried out a pharmacovigilance study to evaluate the adverse reactions of the IFN α2b, which is elaborated in the Center of Genetic Investigations and Biotechndology (CIGB), 70 clinical data of patients that are included in the national clinical trial, phase IV, randomized and blind double were reviewed. From these data adverse reactions, including quantity and type were picked up. They were classified into: light, moderate and serious. It was applied Karch and Lasagnas algorithm to evaluate the force of causality between drug administration ans adverse reaction and classify them in: definitive, probable, possible, conditional and not related. 53 presented 113 adverse reactions to IFN α2b. The most frequent adverse reactions were: fever 17.87%, migraine 14.97%, chills 10.

625%, arthralgia 10.62%, asthenia 9.66% and myalgia 7.72%. These adverse reactions were in its magority colateral effects and they were classified as definitive. 197 had a favorable result. No patient reported antibodies anti-IFN α2b by intramuscular via and It is safe and it could be used in the treatment of MS by intramuscular via.

Key words: Multiple sclerosis, pharmacovigilance, events effects, Interferon.

P140004

Reversed phase high performance liquid chromatography for detection of *Mitragyna speciosa* **- derived mitragynine**

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A simple high performance liquid chromatographic (HPLC) technique for detection of mitragynine in serum was developed. Mitragynine extracted from fresh leaves of *Mitragyna speciosa* was used as a reference compound. HPLC separation was a reversed-phase isocratic mode and consisted of a C_{18} SunFire™ column (250 x 4.6 mm i.d., 5 μm particle size) heated to 35 °C, a methanol-water (80:20, v/v) mobile phase, flow rate of 0.8 ml/min and ultraviolet detection at 225 nm. Acenapthene was used as an internal standard. Mitragynine spiked in normal human serum was extracted with diethyl ether after sample alkalinization. Chromatographic results revealed good separation of mitragynine and the internal standard with the retention times of approximately 10 and 15 min, respectively. Diethyl ether extraction of serums spiked with mitragynine (1-10 μg/ml) yielded an average of 90.25% recovery. Limit of detection and limit of quantification were 0.03 and 0.14 μg/ml, respectively. This analytical method is useful in analysis of mitragynine in blood.

Key words: Mitragynine; HPLC; *Mitragyna speciosa*

Acknowledgement: This work was granted by the Thai Government Budget (2005-2006).

P140006

DRUG-RELATED HOSPITAL ADMISSIONS AT THE GERMAN PHARMACOVIGILANCE CENTERS (PVCs)

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Methods: In 4 German PVCs all non-elective hospital admissions to medical wards were screened prospectively for adverse drug reactions (ADR) from Jan. 1999 to Sept. 2004. Prescription data were obtained from regional pharmacy computing centers. For estimation of incidence, the exposed population was defined as medication users living in postal code areas contributing to the first 75% of all cumulative hospital admissions. Results: In 5,468 patients admission was caused by an ADR (2.98% of all admitted patients). Antithrombotics, NSAIDs and antidiabetics and cardiovascular drugs are the leading drug classes responsible for the DRA. 58% of patients were > 70 yrs. Per 1,000 patients exposed to indometacin, diclofenac, ibuprofen and celecoxib the calculated incidences [95% CI] of DRA came to 1.3 [0.9, 1.9], 0.7 [0.6, 0.8], 0.4 [0.3, 0.5] and 1.1 [0.5, 2.0], respectively. The established system allows for rapid and high-quality ADR-reporting and valid calculation of ADR incidence and will be further developed in the frame of national Pharmacovigilance Centers.

Key words: pharmacovigilance centers–admissions - internal medicine - incidence.

Supported by BfArM, grant no. Fo 2.1-68502-201

P140007

Acute vanishing bile duct syndrome after celecoxib therapy

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Vanishing bile duct syndrome (VBDS) represents a group of biliary diseases characterized by a progressive loss of intrahepatic interlobular bile ducts. It has been associated with aetiology as primary biliary cirrhosis or sclerosing cholangitis, but acute VBDS is often drug related. Celecoxib, a COX-2 inhibitor, appears to have a low incidence of hepatic injury. We report a case with severe and rapidly cholestatic jaundice associated to an acute VBDS after celecoxib treatment. A 71-year-old Moroccan women, usually treated for arterial hypertension by altizide, spironolactone and ricardipine, developed a cholestatic jaundice with a severe pruritus, elevated liver function tests after a 5 days celecoxib treatment for arthritic scapulalgia. All the differential diagnosis were eliminated. A liver biopsy realised one month after the onset, revealed a cytolytic and cholestatic hepatitis, associated with a VBDS. She was treated by ursodeoxycholic acid, rifampicin, ondansetron and potassium. She died one year after the beginning of symptoms. According to our knowledge, this is the first case of VBDS associated with celecoxib.

The mechanism is not fully understood. Toxic and immune causes have been suggested.

P140008

Renal Insufficiency and Failure Associated with Lianbizhi Injection Intravenous Therapy

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Objective: to analyze the 23 reports received in 2003-2005 in Shanghai FDA on the renal adverse events as a result of the use of Lianbizhi Injection. Patients and Methods: the epidemiology of LBZI-associated RAEs in Shanghai were described. Results: Among 23 patients, 96% patients were male. The mean serum creatinine level was 433.1 ±295.1 μmol/L. The mean recovery time of renal function was 13 days after RAE onset. In 13 (57%) of the 23 patients, a kidney biopsy was performed. Acute renal tubular necrosis occurred on 4 (17%) patients, light pathological changes of glomerulus on 5 (22%) patients, Acute interstitial nephritis on 5(22%) patients. 88% patients use LBZI and other drugs together when RAEs happened. Conclusions: doctors and pharmacists should realized the importance of reviewing indications for LBZI use and implementing precautions during its administration.

Key words: LBZI, renal adverse events

P140009

Case reports of increased warfarin effect by concomitant use of glucosamine

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Glucosamine is an endogenous substance which is approved as a drug for relief of symptoms in osteoarthritis. No interaction studies had been performed at the time of approval and little is known about interaction potential between glucosamine and other drugs. Three cases of interactions between warfarin and glucosamine have been reported to the Swedish spontaneous reporting system: Two female and one male patients, aged 69, 76 and 81 years, respectively, were treated with warfarin since long with stable International Normalised Ratio (INR). Due to pain from osteoarthritis treatment with glucosamine was started. INR increased from ≤2.1 to 2.5, 4.2 and > 8, respectively, in the patients during glucosamine treatment (weeks or months). The patient with INR > 8 also experienced hematuria, but recovered after stopping glucosamine. INR returned to previous levels in the other two patients after stopping glucosamine. In conclusion glucosamine may potentiate the warfarin effect. The mechanism for the interaction is unclear. More frequent monitoring of warfarin effect may be necessary when glucosamine is used concomitantly.

P15. Clinical Pharmacology - Therapeutic Drug Monitoring

P150001

Study on warfarin plasma concentration and its correlation with international normalized ratio

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Objective: To develop a method for plasma warfarin determination, evaluate the association of plasma warfarin concentration and international normalized ratio (INR), and confirm the significance of warfarin concentration determination for warfarin therapeutic monitoring. Method: Fifty-eight patients undertook cardiac valve replacement and on anticoagulation with warfarin were randomly selected for this trial. We determined the warfarin plasma concentration by high performance liquid chromatography method we developed, INR by ACL200 automated coagu-

lometer and analyzed the association of warfarin dosage and concentration with INR, respectively. Result: The method developed displayed precise RSD of < 5.27% for interday and < 6.89% for intraday. The assay was linear at the range of 0.12 - 3 μg/ml (r = 0.9995) with mean recovery of 94.58%. The coefficients of correlation between warfarin dosage or concentration and INR were 0.21 (0.1 < p < 0.2) or 0.30 (0.02 < p < 0.1) respectively. Conclusion: The method described proved to be accurate, reproducible and specific for plasma warfarin measurement. Warfarin concentration monitoring is helpful and needed for the patient whose ideal INR is difficult to target.

Key words: Warfarin; Anticoagulation; plasma concentration, INR; therapeutic monitoring

P150002

POSTMARKETING SURVEILLANCE, ROLE OF THE REGULATORY AUTHORITY OF DRUGS.

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Most of the developed countries and an important number of countries in development, have implanted programs, systems or methods, with more or smaller level of complexity for the post marketing control of drugs that have been approved by the Regulatory Authority of Drugs. To carry out an analysis of the pos marketing surveillance system and the role of our National Regulatory Authority. A wide review of the information related with the activity of pos marketing surveillance was analysed which included the sanitary measures adopted and investigations carried out during the years 2001 at the 2005, as a consequence of problems of quality, effectiveness and safety. The final results have proven that there is a national surveillance system integrated by the Regulatory Authority of Drugs (CECMED) and other institutions. As a results of it, the Regulatory Authority of Drugs have issued some safety measures in order to avoid risks in health system. The Regulatory Authority has actively worked with the objective of implement an adecuate the national post marketing surveillance system being of vital importance for the development of the appropriate legal base according to the international tendencies.

P150003

Transdermal Absorption of Repellent DEET and Sunscreen Oxybenzone

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Purpose: To investigate systemic absorption of repellent DEET and sunscreen oxybenzone from topical skin application in vivo. Methods: Three commercial repellent and sunscreen products were applied to 18 piglets. Skin strips, blood and urine samples were collected at predetermined intervals for up to 48 hours. Concentrations of DEET and oxybenzone were analyzed with HPLC. Results: Overall recovery of DEET in skin strips at 2, 12 and 48 hours amounted 45%, 22% and 7% respectively, while those of oxybenzone were 22%, 14% and 18% respectively. Combined repellent/sunscreen preparation produced statistically higher ($p \leq 0.05$) recovery of DEET (69%, 35%, 75%) and oxybenzone (58%, 25%, 84%) than its single - component counterparts. DEET and oxybenzone reached peak plasma concentrations 2 hours after the application; concentration of DEET (314 ±15 μg/mL) and oxybenzone (29 ±3 μg/mL) from the combined preparation was statistically higher than its single - component counterparts (DEET: 215 ±12 μg/mL, oxybenzone: 21 ±3 μg/mL). Conclusions: Repellent DEET and sunscreen oxybenzone penetrated systemically across the skin after topical application; the percutaneous absorption was enhanced with a combined preparation.

P150004

The Correlation of Lamotrigine Concentrations Between Saliva and Serum in Children With Epilepsy

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Objective: To develop a simple and sensitive method for the determination of lamotrigine (LTG) in serum and saliva by high performance liquid chromatography, study the correlation between LTG saliva and serum concentration in children with epilepsy. Method: Collected 27 patients and 38 concentration data in our hospital, taking LTG for a minimum of 4 weeks. Blood samples were obtained by phlebotomy, patients spit a minimum of 0.5 ml into a cup to obtain saliva samples. Result: Linear regression analysis was made by LTG concentration (C) and the peak area ratio (Y) of LTG vs. internal standard (Flurazepam), the regression equation of serum and saliva respective were: Y = 0.1824C - 0.0080, 0.9998; Y = 0.1816C - 0.0119, 0.9997. The correlation between LTG serum and saliva concentration was: saliva (y) = 0.5443serum (x) - 0.5949 (n = 38, r = 0.9444, p < 0.01). Conclusion: A significant positive correlation was found between LTG serum and saliva concentration. Saliva may be a useful alternative to serum for therapeutic monitoring of LTG. As saliva collection is simpler and painless, children may particularly benefit from this method. Key words: Lamotrigine, saliva, therapeutic drug monitoring, HPLC

P150005

Retrospective analysis of dynamic theophylline blood levels in 90 cases with COPD

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Objective: To explore an ideal rang of the serum trough theophylline concentrations for patients with chronic obstructive pulmonary disease (COPD) in order to prevent the adverse effects by irrational medication. Method: It is a retrospective analysis of the dynamic blood trough concentration of theophylline in 90 cases with COPD selected from results of blood concentrations in previous 11 years in Beijing Hospital combined with the clinical effect and adverse effect at that time. Each case had at least five theophylline concentration results. Results: In the 90 cases 74.3% theophylline concentration results are fallen into 3 - 10 ug.ml^{-1}. In the rang of 3 - 10 ug.ml^{-1} 85.7% results corresponded to symptoms control 6.6% improvement of patient's condition. The dynamic theophylline concentrations of patients changed around 3 - 10 ug.ml^{-1}. Conclusion: It is suggested that for the COPD patients the serum theophylline concentrations should be controlled in the rang of 3 - 10 ug.ml^{-1}.

Key words: Theophylline, Blood trough concentration, Chronic Obstructive Pulmonary Disease

P150006

DIRECT COSTS OF DEPRESSION IN THE LOCAL HEALTH SERVICE OF TREVISO, ITALY IN THE YEAR 2004

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Objective: The present retrospective study was performed to quantify the direct costs of depression in an Italian Local Health Authority (LHA9) in 2004. Methods: Data were retrieved from local database of drug prescriptions and referred to 4,958 incidental patients (IPs), i.e. treated with tricyclic antidepressants (TCA) or selective serotonin reuptake inhibitors (SSRI) or other antidepressants and 8,678 prevalent patients (PP), i.e. those who had a prescription of antidepressants in the previous two years. Results: The total direct costs were 37,174,107.13, whose 42.7% was due to hospitalization. Cost/day for PP was 6.68, whereas, that for IP was 11.02 and 7.65, before and after antidepressant treatment, respectively. Women were more prescribed than men (4.8 vs 2.2%). The antidepressant Received Daily Doses (RDDs), except for SSRI, were lower than the respective Defined Daily Doses (DDDs). Conclusions: Cost/day for PP was lower than that for IP, because the latter exhibited a decrease in hospitalization. RDDs for TCA and other antidepressants were lower than their DDDs, probably because they were associated with a higher toxicity risk than SSRI.

Key words: Antidepressant direct costs

P150007

Simvastatin Reduces Specific Allergen-Induced Asthma Symptoms in mice

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Simvastatin as a cholesterol - lowering agent was reported to have an anti - inflammatory effect on allergic asthma in murine model, but its mechanism was not yet. Therefore, this study aimed to investigate anti - inflammatory mechanism of simvastatin in allergic asthma mouse model. BALB/c mice was sensitized and challenged with OVA. Simvastatin (40 mg/kg) was given i.p. injection three times before local nebulization. OVA - specific serum IgE was measured by ELISA, the recruitment of inflammatory cells into BAL fluid and lung tissues by Diff - Quik and H&E, mucus secretion by PAS staining, CD40L and VCAM - 1 expressions by immunohistochemistry, activity of MMPs in BAL fluids by gelatin zymography, mRNA and protein expression of cytokines and MMPs in lung tissues by RT - PCR and ELISA, the activity of NF - kappa B by EMSA. Simvastatin reduced serum IgE Ab level, number of total inflammatory cells,

eosinophiles and mast cells, activities of MMP-2 and -9 in BAL fluids, the CD40L and VCAM-1 expressions or the mRNA and protein level of IL-4, IL-13 and TNF-alpha, and NFkappa B activity in lung tissues in OVA-challenged allergic asthma in mice.

The data suggest that simvastatin may be used as a therapeutic agent of asthma

P150008

Liquid Chromatography and Flow Injection Analysis Assay Methods for Therapeutic Drug Monitoring of the Antibacterial Drug Cefuroxime Axetil

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Cefuroxime axetil is the pro-drug of cephalosporin cefuroxime that is used in treatment of common community-acquired infections. A liquid chromatographic method for therapeutic drug monitoring of cefuroxime axetil has been developed and validated, in this study. Cefuroxime axetil and indapamide (internal standard) were separated by a reverse phase column (Supelco Hypersil 5 micrometers, 150x4.6 mm ID, C18) using a mobile phase consisting of KH2PO4 (0.1 M) and acetonitrile (70:30, v/v, pH 4.0). The mobile phase was pumped at 1.0 mL.min^{-1} flow rate and cefuroxime axetil was detected by ultraviolet detection at 281 nm wavelength within an average analysis time of 11 min. Additionally a flow injection analysis was performed using a carrier stream of methanol: water (10:90, v/v) with a flow rate of 1.0 mL min-1. The LOD and LOQ concentrations of the methods were 1.35x10^{-7} M and 4.08x10-7 M for chromatography, 1.31x10^{-7} M and 4.00x10^{-7} M for FIA, respectively. The precision and the accuracy of the methods were found to be suitable for therapeutic drug monitoring of cefuroxime axetil.

Key words: Cefuroxime axetil, Therapeutic drug monitoring, Liquid chromatography, Flow injection analysis

P150009

Monitored Anaesthesia Care with remifentanil versus anaesthesia with propofol-alfentanil: Effects on in vitro fertilization outcome.

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Background and Goals: The aim of the study is to compare the effects of monitored anaesthesia care (MAC) with remifentanil versus general anaesthesia with propofol and alfentanil on in vitro fertilization (IVF) outcome. Material and Methods: Forty women, who underwent ultrasound transvaginal oocyte retrieval under either general anaesthesia with midazolam, alfentanil and propofol (group I, n=20) or under MAC with midazolam and remifentanil (group II, n=20) respectively, were compared for number of collected oocytes (CO), matured oocytes (MO), fertilization rate (FR), cleavage rate (CR), implantation rate (IR) and pregnancy rate (PR). These preliminary data were analyzed using the ANOVA in SPSS ($p<0.005$). Results: There were no significant differences in CO, MO, FR, CR, IR and PR between two groups (ANOVA). Data (Mean ± SD, p) are shown in the table:

	CO	MO	FR	CR	IR	PR
group I	6.6 ±5.1	6.25 ±5.0	70.2 ±23.9	93.5 ±14	25.8 ±37.2	40.0 ±50.2
group II	7.8 ±3.9	7.25 ±3.8	68.5 ±24.3	85.7 ±24.5	16.6 ±32.8	25.0 ±44.4
p	0.39	0.72	0.83	0.22	0.41	0.32

Conclusions: MAC with remifentanil compared with general anaesthesia with propofol and alfentanil did not affect differently the IVF outcome.

Key words: propofol, remifentanil, oocyte retrieval

P150010

The influence of Ciprofloxacin on the changing of females' catamenia quantity

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Aim: We investigate Ciprofloxacin making females menses change to use it better in clinic. Methods: Study 186 cases of fair sex sufferers who come from different community in Kunming with using Ciprofloxacin during march, 2003-march, 2005, exception whose cycle catamenia's is deviant First we study sufferers' quantity of menses before they used Ciprofloxacin three months and after they used Ciprofloxacin one month. Then we balanced with using Ciprofloxacin. Results: 38.7% of sufferers' menses mete are manifold. 25% in them increased 30%-40% at their basic menses, 60% in them added 40%-60% at their basic menses, 15% in them added 60%-80% or upward 80% at their basic menses, they had to use hemostasia and be cured at all. It is important that all sufferers used Ciprofloxacin at the prophase of menses or during menses will increase sufferers' menses, but in the end of menses or during a period of time in ovulate, without electrophoresis. It is not distinctness connection with ave of using drug. Conclusion: Female sufferers, especially losed more blood(>80 ml/month) during menses, they do not use Ciprofloxacin at the prophase menses or during menses.

P150011

EFFECT OF KIDNEY DISEASE ON THE HEPATIC CYP2B6 ACTIVITY AS MEASURED BY BUPROPION PHARMACOKINETICS

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Our aim was to investigate the effect of kidney disease on the bupropion pharmacokinetics and on CYP2B6 activity. 17 healthy subjects and 10 patients with kidney disease received a single 150-mg oral dose of bupropion. Subjects were genotyped for variant alleles *4, *5 and *6 of CYP2B6. The bupropion AUC was 126% higher ($P<0.0001$; 95% CI, +72% to +180%), C_{max} 86% higher ($P=0.001$; 95% CI, +40% to +131%) and $t_{1/2}$ 140% longer ($P=0.001$; 95% CI, +76% to +204%) in the renal-impaired patients. The clearance of bupropion was 64% lower ($P<0.0001$; 95% CI, -20% to -106%) in the patients with kidney disease. In renal-impaired subjects, the hydroxybupropion/bupropion AUC ratio was reduced by 66% ($P=<0.0001$; 95% CI, -19% to -114%) and hydrobupropion/bupropion AUC ratio by 69% ($P=.001$; 95% CI, +8% to -146%) compared to controls. Bupropion clearance was significantly reduced in subjects with renal impairment. The most plausible explanation is the suppressed CYP2B6 activity. Patients with renal impairment are likely to need dose adjustments when treated with bupropion.

P150012

Population pharmacokinetics of mycophendic acid in Chinese adult renal transplant recipients during the first month after transplantation

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This study was aimed to investigate the population pharmacokinetics (PK) of mycophenolic acid (MPA) in Chinese adult renal transplant recipients during the first month after transplantation. PK data for MPA and covariate information were collected from 45 patients who underwent renal transplantation at two transplantation centers. At least one whole PK profile was obtained in 40 patients and total of 871 concentration-time points were available. Population analysis was performed using NONMEM and the final model was evaluated by bootstrap method. The best base model was a two-compartment model with a typical population (SE%) apparent oral clearance (CL/F) of 31.6 l/h (5.8%) and apparent volume of the central compartment of 50.4 l (12.7%). CL/F increased significantly with increasing weight. The results were in close agreement with the bootstrapped estimates. For the first time, population PK parameters for MPA in Chinese patients were determined and the proposed model may be helpful in optimizing MPA therapy.

Key words: mycophenolic acid, population pharmacokinetics, renal transplantation

P150013

PK/PD Modeling of Antisecretory Effect of Omeprazole and Its Application in Dose Regimen Optimization

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Objective: to develop a PK/PD model of antisecretory effect of omeprazole (OME) and use it to optimize the dose regimen for the treatment of gastroesophageal reflux disease (GERD). Method: Thirteen healthy volunteers received

an oral administration of omeprazole 40 mg and 24 hour intragastric pH measurement was performed before and after drug dosing to calculate the acid secretion inhibition%. Blood samples were also drawn for pharmacokinetic analysis. Results: A mechanism-based PK/PD model of OME was successfully developed and the experimental data could be satisfactorily fit to the model. According to the simulation analysis, the costeffective initial dose regimen for Chinese GERD patients was suggested as 10 mg bid or 20 mg qd, only half of the presently recommended daily dose, which was further proved through a double-blind randomized clinical trial with 152 GERD patients. Conclusion: PK/PD modelling and simulation may be an efficient approach to optimize the medication dose regimen.

Key words: omeprazole; PK/PD model; cost-effectiveness; randomized clinical trial

P150014

Effects of intraoperative volume replacement on propofol blood levels and depth of anesthesia in patients undergoing major surgery.

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Background and Goals: The aim of the study is to investigate the effect of intraoperative volume replacement on propofol kinetic and dynamic parameters in patients undergoing major surgery. Material and Methods: In eight adults with a high volume replacement of up to 10% of total body fluids, propofol blood levels (using chromatographic assay HPLC with a fluorescent detector) and anesthesia depth (using Bispectral Index: BIS) were studied at preset intervals, during major surgery. The percentage of blood loss (PBL), propofol infusion rates (PIR), propofol concentrations (Cprop) in μg/ml and BIS values were recorded. Findings were analyzed by descriptive statistic analysis. Correlation between BIS values and Cprop was analyzed using correlation coefficient R^2. Results: Data (Mean ±SD) were: PBL: 10 ±6.0, PIR: 4.8 ±1.7 mg/kg/hour and R^2: 0.5238 ±0.0719, respectively. Mean ±SD of BIS values and Cprop are shown in the table:

min	30	90	150	210
Cprop	2.1 ±1.4	1.8 ±0.9	2.5 ±1.4	2.6 ±2.4
BIS	44.6 ±5.7	44.6 ±6.4	44 ±10	39.2 ±7.8

Conclusions: Propofol blood level and anaesthetic effect seems to remain unchanged in patients with volume replacement during major surgical procedures.

Key words: propofol, volume replacement, BIS

P150015

Effects of Na Channel (ENaC)- Na/Ca Exchange (NCX) Inhibitors Amiloride (AM) and Benzamil (BZ) on the Renal Afferent Arteriolar Myogenic Response

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ENaC is suggested to be required for myogenic signaling and renal autoregulation, as AM and BZ inhibit myogenic tone in the mouse renal interlobar artery (IA) (Am J Physiol 289: F891, 2005). Since the IA is not a resistance vessel, we examined the effects of AM and BZ on myogenic responses of the afferent arteriole (AA) using the in vitro perfused hydronephrotic rat kidney (Circ Res, 90:1316, 2002). In controls, increasing renal arterial pressure from 80 to 120 and 140 mmHg reduced AA diameters (SEM) from 17.8(0.7) to 15.1(0.7) and 12.3 (1.1) microns. Following treatment with 0.1 and 1.0 micromol/L BZ, the same manipulation reduced AA diameters from 17.8(0.7) to 13.1(1.2) and 10.1(1.4) microns, and from 16.5(0.8) to 10.5(1.6) and 8.1(1.2) microns, respectively ($P < 0.05$). Thus, BZ did not inhibit, but rather potentiated myogenic reactivity. Similar observations were obtained with AM (1-10 micromol/L). These findings do not support the premise that ENaC is required for myogenic signaling. The potentiating effects of BZ and AM may relate to the actions of these agents on NCX, and may indicate an important role of this transporter in AA Ca handling and reactivity. (supported by grants from CIHR)

P150016

Mechanism-based Pharmacokinetic-Pharmacodynamic modeling of bendazac lysine

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AIM: Bendazac lysine (BDL) is a aldose reductase (AR) inhibitor. To establish its mechanism-based modeling of pharmacokinetics and pharmacodynamic (PK-PD) for clinic use. METHODS: Ten Chinses healthy volunteers received a single dose 400 mg of BDL per oral. blood concentration of BDL was determined by HPLC-UV method, The inhibitory potency of BDL was measured by purifying AR from human erythrocytes through ionexchange chromatography (DE-52). PK and PD parameters were caculated by Computer Aids Pharmacokinetic and Pharmacodynamic Modeling (CAPP). RESULTS: The time concentration curve of BDL was fitted to onecompartment model. Its Ke(h-1), Ka(h-1) and Vd/F(L/kg) were 0.187, 4.377, 5.66 respectively, Time effectoncentration curves were fitted to E_{max} model. Its IC50(μmol/L), E_{max}(%), γ were 25.2, 0.97, 1.72 respectively, this dosing rate of BDL can be caculated by estimation of IC50 based on enzyme-binding studies in vitro. CONCLUSION: It was indicated that integrated PK-PD model by using plasma concentration and enzyme-inhibition data in vitro could be predicted the effect-time profiles.

Key word: PK-PD modeling; Bendazac lysine; Aldose reductase inhibition

P150017

Phase I clinical tolerability and pharmacokinetics studies on secnidazole vagina effervescent tablets

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The studies were carried on to evaluate the tolerability and pharmacokinetics of secnidazole vagina effervescent tablets. In tolerability trials, 20 volunteers were randomly divided into four groups with single doses: 125, 250, 500, 750 mg. The studies of multi-doses were carried on according to the results of single-dose groups. In pharmacokinetics trials, the volunteers were also divided into different single-dose and multi-dose groups. The concentrations of secnidazole in plasma were determined by HPLC and the parameters of pharmacokinetics were calculated by DAS software. After single-dose administrations, most clinical symptoms, vital signs and laboratory tests were normal. There were no significant clinical changes or ADRs. No severe ADRs were observed after multi-dose administrations, Only 3 cases of slight were observed in 500 mg group. The pharmacokinetic parameters (250, 500, 750 mg for single-dose groups and 500 mg for multi-dose group) were as follows: $T_{1/2\beta}$ were 18.84, 15.25, 21.86 and 22.74h; Ka were 0.173, 0.108, 0.090 and 0.208; T_{max} were 12.22, 14.00, 18.00 and 11.60h; C_{max} were 3.585, 5.415, 7.996 and 14.303 mg·L^{-1}; AUC_{0-tn} were 104.0, 181.7, 266.3 and 496.3 mg·h·L^{-1}; $MRT_{0\sim tn}$ were 28.89, 30.21, 31.97 and 26.98h, respectively.

P16. Clinical Pharmacology-Drug Utilization

P160001

Cardiovascular drugs utilization in Croatia during a four-year period

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The aim of this work was to identify and present changes in the utilization of CV drugs in Croatia during the period 2000-2004 and discuss the underlying reasons. Information data on CV drugs utilization for the period 2001-2004 were obtained from the Croatian National Health Insurance. Drug utilization data are presented in defined daily doses/1000 inhabitants/day (DDD/1000). Comparing 2004 vs. 2001, total CV drugs utilization increased 49.53% from 176.80 to 264.37 DDD/1000. The statins had the highest share rise from 5.88% to 10.90%. Drugs acting on the renin-angiotensin system had the largest share (32%). The utilization of angiotensin II antagonists and the combination of ACE inhibitors and diuretics increased from 1.12 to 6.96 and from 10.07 to 26.85 DDD/1000, respectively. The utilization of the new calcium channel blockers (CCB) increased 1.27 times and the old CCB decreased 18%. The utilization of most CV drugs in Croatia increased during this relatively short study period and we presume that the main reason is a legal change (the new Insurance Act) with the introduction of supplementary health insurance.

Key words: cardiovascular drugs, drug utilization, health insurance

P160002

Use of renal risk drugs in hospitalised patients with mild to moderate renal impairment

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To investigate use of renal risk drugs in hospitalised patients with mild to moderate renal impairment (RI). A sample of 821 patients was drawn from 5 general hospitals. We recorded demographic data, drugs used, drugs described to be risky (RR- drugs) in RI and laboratory data. Four grades of renal impairment were identified on basis of levels of GFR and serum creatinine. Drug related problems (DRPs) were regularly searched for. 156 patients (19 %) were found to have reduced renal function: 46 patients (29 %) had diminished renal reserve, 86 patients (55 %) had mild and 24 patients (15 %) had moderate RI. Mean number of drugs used in patients with and without RI: on admission 6 vs 4.2; started in hospitals 4.4 vs 3.9; total number of RR- drugs 6.1 vs 4.6. In patients with RI an average of 3.2 DRPs/patient was recorded as compared to 2.4 DRPs/patient in those without RI. On average 28 % of RR- drugs were associated with DRPs. A high proportion of DRPs were acknowledged by the multidisciplinary team and acted upon. Conclusion: Among patients admitted to general hospitals a considerable proportion had RI. RR- drugs were widely used in these patients and DRPs were frequently associated with the use of RR- drugs.

P160003

The validity of medication lists in hospital files and discharge letters

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Aim was to compare medication lists in hospital files and discharge letters with information on prescription only medication (POM) use collected during home visits among recently hospitalised patients. Patients were visited within one week after discharge from hospital and interviewed about POM use. Stored drugs were inspected. We compared drug lists in hospital files and discharge letters to the list obtained during the home visit. 83 surgical and 117 medical patients were included (median age 75 years). 6 patients stored no POM, 194 patients stored 1189 POM. Among the 954 POM reported used at discharge 768 POM (81 %) were registered in hospital files. Only 453 (47 %) of used POM were registered in discharge letters. 66 POM users had no medication list in their discharge letter. 63 POM were used in obvious disagreement with prescribed regimen. Patients knew little about side effects and drug interactions. Approximately 1/5 of used POM are unknown to the hospital and half of used POM are not registered in discharge letters. Lack of communication between health care sectors may cause inappropriate drug therapy.

P160004

Epidemiology of clopidogrel drug- drug interactions, and their clinical consequences

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Clopidogrel is a prodrug that needs to be activated by CYP3A4 in order to inhibit platelet aggregation. The objective of the present study was to evaluate the prevalence and clinical consequences of potential drug interactions of clopidogrel and atorvastatin, CYP3A4 inhibitors and inducers in hospital inpatients. The study population comprised 726 clopidogrel- treated patients in 3.5 years. There were 127 patients (17.5 %) using concomitantly atorvastatin, 33 (4.5 %) a CYP3A4 inhibitor and 12 (1.7 %) a CYP3A4 inducer. The demographic characteristics or prevalence of diabetes, hypertension or heart failure of the patients in interaction groups did not differ from the control group. Co- administration between clopidogrel and atorvastatin, CYP3A4 inhibitors or inducers did not have effect on haematological laboratory test values. During one- year follow- up the incidence of any cardiovascular event was 84 (66 %) in the atorvastatin group, 14 (42 %) in the CYP3A4 inhibitor group, 3 (25 %) in the CYP3A4 inducer group and 279 (50 %) in the control group according to patient records. In conclusion our preliminary data do not support loss of efficacy of clopidogrel used concomitantly with CYP3A4 inhibiting drugs.

P160005

PREDICTORS OF HOSPITALIZATION FOR CARDIOVASCULAR DISEASE IN A POPULATION TREATED WITH STATINS

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Objective: To evaluate the predictors of first hospital admission for cardiovascular disease in hypercholesterolemic patients treated with statins (S) in Local Health Authority n.9 (LHA9) Treviso, Italy, 1994- 2003. Methods: Data of S treated patients were retrieved from databases of LHA9. Cholesterol data were detected during the S treatment. Cardiovascular admissions were included only after S therapy. The clinical complexity of the patient was evaluated trough a proxy- variable consisting in co- prescriptions (antidiabetics, antihypertensive and aspirin). Results: The patients enrolled were 5,028. Each variable (age, gender, compliance, S, number of co- treatments, and goal achievement), associated with time to admission, were inserted in a Cox regression model. The risk of first admission increases with age and gender (male vs female). Patients with polytherapy were more prone to be hospitalized. The risk of hospitalization increases with compliance, patients more compliant are older and have more risk factors. Conclusions: The study seems to indicate that old male patients with polytherapy are more at risk of first admission in spite of good compliance with S therapy.

P160006

Chemoprophylaxis in General Surgery Departments in Croatia, Serbia and Greece

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Aim: The aim of the present study was to compare Chemoprophylaxis in General Surgery Clinics of three major University Hospitals in Croatia, Serbia and Greece, and to check if general guidelines for Surgical Chemoprophylaxis are met. Methods: All surgeons replied to the same questionnaire, which checked: 1) application of chemoprophylaxis, 2) duration and time of initiation of chemoprophylaxis and 3) the kind of antibiotics used. Results: In clean surgery, Croatian surgeons used chemoprophylaxis only exceptionally, Greek surgeons used chemoprophylaxis only in patients with a high risk for a post- surgical infection, while Serbian surgeons always used chemoprophylaxis. In contaminated surgery and in laparoscopy, Greek and Serbian surgeons always used chemoprophylaxis, while Croatian surgeons used chemoprophylaxis in some operations. Chemoprophylaxis was almost always initiated during the initiation of anesthesia and its duration varied. All surgeons used a beta lactam but Croatian surgeons used also gentamicin in some cases. Conclusions: Non- conformance to the guidelines is observed in surgical chemoprophylaxis in the three countries studied.

Key words: surgical chemoprophylaxis

P160007

Intraoperative propofol in the prevention of side effects from epidural morphine

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We examined the efficacy of intraoperative propofol to prevent postoperative nausea and vomiting (PONV) or pruritus induced by epidural morphine administration during hysterectomy. Seventy patients ASA I, II undergoing combined epidural and general anesthesia for hysterectomy were randomly assigned to two groups: a) group P: anesthesia was induced with propofol and fentanyl, and maintained with propofol- N2O, b) group S: anesthesia was induced with thiopental and fentanyl and maintained with sevoflurane- N2O. All patients received 3 mg epidural morphine. The incidence of pruritus and PONV were evaluated the first hour and every 4 hours for the first 12 hours postoperatively. The total incidence of pruritus was significantly higher ($p = 0.024$) at group S (65.6 %) compared to group P (29 %). Significantly less patients ($p < 0.05$) of group P needed treatment for PONV the 1st postoperative hour, however there was no difference in the overall incidence of PONV in the two groups. Intraoperative propofol seems to reduce the incidence of pruritus induced by epidural morphine. It also seems to protect patients against PONV only for the 1st hour post-

operatively, while no protection was detected the next eleven hours.

P160008

Clinical evolution of patients treated with transfer factor.

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In Cuba is marketing transfer factor (TF), an immunomodulator used in several diseases, but the effectiveness of this drug has not been assessed in aftermarket research studies. Then, we performed a descriptive cross-sectional study in 9 hospitals located in the City of Havana, from April 2001 to April 2002, to evaluate the clinical evolution of patients treated with this immunostimulant. The rate of relapses occurred one year before and after the treatment was measured, others dates collected was therapeutic scheme, prescription reasons and immunological tests before and after treatment. The evaluation was made in 280 patients, it was satisfactory in 43.6 %, partly satisfactory in 39.4 % and unsatisfactory in 16.3 % of cases. Only 41.8 % of cases were applied supplementary tests prior to the prescription, but none was performed afterwards. The clinical evolution of the patients treated with TF improved after treatment, although cellular immunodeficiency was not confirmed for all the cases.

Key words: transfer factor, drug utilization studies, pharmacoepidemiology
Acknowledgement: National Network of Pharmacoepidemiology of Cuba

P160009

Effect of ethinyl estradiol on unsatisfactory colposcopy

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Oral contraceptive causes cervical eversion, which makes unsatisfactory colposcopy rare. A double-blind clinical trial with 50ug/day oral ethinyl estradiol for 10 days was performed on all patients evaluated for cervical dyspalsia with unsatisfactory colposcopy. Premenopausal patients started using the drug on the 5th day of the menstrual cycle and returned on the 10th day of treatment. Postmenopausal patients started the treatment at any convenient time. Forty patients entered into the study (20: ethinyl estradiol, 20: placebo). On colposcopy, TZ was fully visible in a significantly greater proportion of patients in estrogen group than in placebo group. TZ was not completely visible in 2 patients in estrogen group and 15 patients in placebo group. Fewer patients in estrogen group required diagnostic conization. No clinically significant side effects were reported, except vaginal bleeding in one case. The result of our study suggests that the use of 50 ug of Ethinyl Estradiol can ensure a satisfactory examination. The estrogen is useful for avoiding conization after unsatisfactory colposcopy in pre- and postmenopausal women.

Key words: unsatisfactory colposcopy, ethinyl estradiol

P160010

Artemisinin Combination Therapy - not the magic bullet

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The research aims to rationalise the usage of artemisinin based combination (ACT) for antimalarial chemotherapy. The great success of ACT with mefloquine in South East Asia has suggested other combinations, with amodiaquine, lumefantrine, sulfadoxine-pyrimethamine, chloroquine and chlorproguanil-dapsone, in Africa. All these combinations lack what was initially the requirement, similar pharmacokinetics of both codrugs, allowing selection of resistance to the co-drug. In addition, for many of the co-drugs there is already high or patchy resistance, which will be amplified when used as first-line treatment in endemic countries. In this paper we present a mathematical basis of a pharmacokinetic-pharmacodynamic model of ACT and a model of selection of resistance. Combining these we predict the rate of selection of resistance to the co-drugs under varying levels of initial resistance prevalence, transmission and population coverage. We find that the limits specified by the WHO for ACT are somewhat lenient, and that a specific evaluation is required for each setting.

P160011

Effect of ulinastatin on cellular immunity during total hip arthroplasty

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Objective: To evaluate effect of ulinastatin on cellular immunity of patients during total hip arthroplasty. Methods 24 ASA physical status I and II patients scheduled for total hip arthroplasty were randomly divided into two groups. Group I (n = 12) received intravenous infusion of ulinastatin after inducing of general anesthesia. Group II (n = 12) received same amount of normal saline instead of ulinastatin. Natural killer (NK) cells and T lymphocyte subpopulations (CD3+, CD4+ and CD8+ cells) were investigated before anesthesia, at the end of anesthesia and 24h after anesthesia. Results CD3+, CD4+ T lymphocytes and CD4+/CD8+ ratio decreased after anesthesia ($P < 0.05$). Those in group II decreased more significantly than in group I ($P < 0.05$). Conclusion Ulinastatin impair cellular immunity during total hip arthroplasty.

Key words: ulinastatin, total hip arthroplasty, cellular immunity

P160012

Drug utilization study of 2 statins in outpatients from a perspective of metabolic drug-drug interactions

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OBJECTIVE: To know metabolic interaction potential in concurrent therapy of simvastatin or atorvastatin, and promote their rational use. METHODS: Using a pharmacy administration software, we examined recipes containing either statin for outpatients during June-December, 2005. RESULTS: There were 21 substrates and 17 inhibitors of CYP3A4 prescribed with either statin and 8 drugs have brought clinically significant drug interactions. In either statin concurrent therapy, there were 35.2%~43.3% recipes containing CYP3A4 substrates and 3.6%~7.7% recipes containing CYP3A4 inhibitors. Moreover, there were 0.2% recipes containing two CYP3A4 inhibitors, 2.3% recipes containing one CYP3A4 inhibitor and one more CYP3A4 substrates and 3.7% recipes containing two more CYP3A4 substrates. CONCLUSION: The utilization of the two statins in their concurrent therapy is unsatisfactory and hence the increased risk of myopathy. Concomitant use of known CYP3A4 inhibitors should be avoided. More attentions should be paid in coadministration of the two statins with CYP3A4 substrates.

Key words: statins, metabolism, drug interaction, drug utilization

P160013

KETAMINE AND MIDAZOLAM FOR CONSCIOUS SEDATION

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Objective: Dental phobia is a deterrent to good dental health. Traditional oral sedatives used to alleviate anxiety gives unreliable results consequently many of these patients need general anaesthesia. Ketamine and midazolam is used orally as conscious sedation. It was decided to investigate the parenteral form given orally in healthy volunteers. Methods: A study in 10 healthy volunteers was conducted in which parenteral ketamine ($2.5 mg.kg^{-1}$) and midazolam ($0.14 mg.kg^{-1}$) were administered orally. Blood samples were drawn periodically. Patho-physiological parameters and vital signs were monitored. The data gathered was used to demonstrate a range of pharmacokinetic parameters. Results: No untoward events were recorded. Liver, blood chemistry and blood gases remained stable. An increase in pain threshold, anterograde amnesia, sedation and axiolysis was demonstrated. Bioavailability was demonstrated. Pk/pd effects were demonstrated. No untoward effects were noted and vital signs stayed intact. Conclusion: It can be concluded that the parenteral form of each drug in combination is effective and may be safely used when given orally.

Key words: conscious, sedation, dental

P160014

Towards rational use of drugs in Egypt

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A collaborative project between Egypt, Denmark and Sweden has been initiated to improve drug utilization in Egyptian hospitals (Tempus grant JEP-31033). Drug and therapeutics committees (DTCs) were founded from clinicians supported by pharmacological and pharmaceutical expertise. Data collected retrospectively led

to identify the most commonly used drugs and their irrational use. Through consultations at the initial phase (1 year), drug utilization of some commonly prescibed drugs fell by about 40%. Emphasis has been put on the principles of drug evaluation in the training of DTC members. Preliminary results suggest that it is possible for DTCs to change drug utilization towards a more rational approach.

Key words: Drug rationalization, Egyptian trial, Tempus

P160015

Comparison of pramipexole and modafinil on arousal and autonomic functions in healthy volunteers

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Dopaminergic neurones stimulate the noradrenergic locus coeruleus (LC), which increases arousal. The D_2/D_3 receptor agonist pramipexole is sedative, due to the stimulation of inhibitory autoreceptors on dopaminergic neurones. Modafinil increases arousal by activating the LC. We compared the effects of the two drugs on arousal and autonomic activity. 16 males participated in four weekly sessions (placebo, pramipexole 0.5 mg, modafinil 200 mg, pramipexole 0.5 mg + modafinil 200 mg). Alertness (critical flicker fusion frequency, visual analogue scales, pupillary fatigue waves), pupillary functions (pupillometry), blood pressure, heart rate, temperature, salivation were measured. Pramipexole reduced alertness and increased pupil diameter. Modafinil had no effect on alertness but tended to increase pupil diameter, blood pressure and temperature. The sedative effect of pramipexole may reflect the withdrawal of the dopaminergic activation of the LC. As the deactivation of the LC is expected to cause miosis, the mydriasis induced by pramipexole suggests a dopaminergic contribution to pupillary control which is independent of the LC. Modafinil showed sympathomimetic effects, consistent with LC activation.

Key words: pramipexole, modafinil, arousal, pupil

P160016

Inpatients consumption habits of psychotherapeutic agents at "10 de Octubre" Hospital.

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Patients with anxiety, insomnia, depression and psychosomatic disorders, sometimes, require psychotherapeutic agents. Although these drugs need enforced medical prescription, they are frequently used as self medications or prescibed to please patients. Our purpose was to know about consumption habits of these drugs in admitted patients in medical wards at "10 de Octubre" hospital, during 2004. A total of 920 in-patients under psychotropic drugs were interviewed; 100 of them were randomly selected to conduct this study. The most employed psychotherapeutic agents were anxiolytics (83%) and Diazepam had the first place (46%). Anxiolytics were used as self-medication in 22.9%, and 82% consumed them for longer periods than literature recommends Our results show an inadequate use of psychotherapeutic drugs and emphasize the need of educational campaigns directed to health personnel and general population to help achieve rational use of these drugs and improve quality of life.

Key words: psychotherapeutic drugs, consumption habits, inpatients.
Source of research: Survey on psychotherapeutic agents in admitted patients.

P160017

Pharmacokinetics of piroxicam patches in Chinese healthy volunteers

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Objective: To investigate the pharmacokinetics of piroxicam patches in Chinese healthy volunteers. Methods: 44 selected volunteers were divided into four groups by parallel design including a single-dose study of three groups in the dosage of 48～144 mg and a multi-dose investigation with 48 mg. The drug concentrations of plasma sample were determined by HPLC. The pharmacokinetic parameters were calculated by DAS 1.0 software. Results: The main pharmacokinetic parameters of three groups: C_{max} (34.57 ±8.01), (57.89 ±13.84) and (90.99 ± 20.77) $\mu g \cdot L^{-1}$; AUC_{0-t} (3148.0 ±552.8), (5157.0 ±1460.27) and (7662.08 ±1737.98) $\mu g \cdot L^{-1}$; t_{max} (48.64 ±16.35), (46.91 ±15.37) and (50.27 ± 14.91) h; $t_{1/2}$ (57.74 ±23.27), (58.63 ±16.73) and (58.91 ±20.23) h. There was a linear increase in C_{max}, $AUC_{0-\tau}$ and $AUC_{0-\infty}$ with increasing doses of piroxicam patches, but no singnificant differences were observed in Ka、$t_{1/2Ka}$、T_{max}、Ke、MRT、$t_{1/2}$、V/F、CL/F of three groups and in main pharmacokinetic parameters between the single-dose and the multi-dose study ($P > 0.05$) Conclusion: The pharmacokinetics of piroxicam patches nearly fit linear dynamic feature and no accumulation was observed for 14 days administration.

Key words: piroxicam patches; HPLC; pharmacokinetic

P160018

Clinical effect of 18,435 cases rheumatoid arthritis treated by Chinese ant (Polyrhachis vicina Roger) extract Preparation

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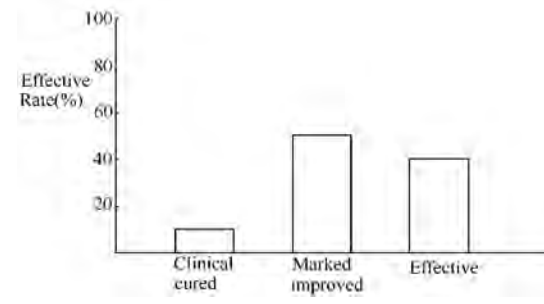
Aim: To summarize and analyse the results of clinical effect of 18,435 cases rheumatoid arthritis treated by Chinese ant extract preparations (CAEP). Methods: The CAEP maked in powder, capsules, pills, tablets or oral liquid, the dose range was from 2～10 grams per time, 2～3 times per a day, 30 days was a therapeutic duration. If the patient required, it will continued.



Results: 18,435 cases rheumatoid arthritis were treated by CAEP in 1981～2000 years, China. Among them, 1874 (10.2%) of the treated cases had complete resolution of their symptoms and signs with no recurrence during a 6 months follows up period; 8524 (46.2%) cases had marked improved; 7894 (42.8%) cases showed improvement; but 143 (0.78%) cases showed ineffective. Conclusion: Clinical effect of 18,435 cases rheumatoid arthritis treated by CAEP is effective, above all, in the early stage.

Clinical effect of 18,435 cases rheumatoid arthritis treated by Chinese ant (Polyrhachis vicina Roger) extract preparation (Control group treated with prednisone 30～40 mg/day and/or Indomethacin 75 mg/day, but only 22 cases, it was omitted for comparison)

Key words: Rheumatoid arthritis; Polyrhachis vicina Roger; Chinese ant extract preparations (CAEP)

P17. Pharmacokinetics and Drug Metabolism

P170001

INVOLVEMENT OF TRANSPORTERS IN NEUROTOXICITY

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The blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB), are the first lines for protecting the brain. A complex network of transporters expressed at BBB and BCSFB participates to solute exchanges between blood and brain. Influx transporters belonging to the Solute Carrier superfamily may facilitate the occurence of neurotoxic effects. Thus, the monocarboxylate transporter MCT1 transports across BBB the recreational drug of abuse g-hydroxybutyrate leading to seizures, respiratory depression and impaired consciousness. In contrast, efflux transporters like P-glycoprotein (Pgp), acts by pumping out endothelial cells towards the blood a wide variety of substrates including potential neurotoxic compounds. More recently, a second member, the Breast Cancer Resistance Protein (BCRP) was found co-localized with Pgp at BBB. The neuroprotective effect of these ABC transporters was demonstrated against xenobiotics like ivermectin, an antiparasite agent substrate of Pgp and dietary phototoxins which are substrates of BCRP. All these transporters play a critical role for protecting the brain from neurotoxic events.

Key words: neurotoxicity, ABC, SLC, transporter

P170002

Relationship between the metabolism of neferine in rat liver microsomes and cytochrome P450

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AIM: To identify which isoforms of cytochrome P450 were responsible for neferine (Nef) metabolism in rats. METHODS: Wistar rats were untreated or treated with various inducers including dexamethasone (DEX), phenobarbital (PB) and β-naphthoflavone (β-NF). Liver microsomes were obtained from these rats and incubated with Nef in the presence of NADP. After being variously treated, the rats received administration of Nef (9.4 mg·kg^{-1} or 18.8 mg·kg^{-1}, i.v.) and the plasma concentration of Nef were determined by HPLC. Results: The disppearing rate of Nef in the incubation solutions, which treated with DEX or PB, were quicker than that of control group ($p < 0.01$) and the rate of DEX group was quicker than that of PB group ($P < 0.01$), while no obvious difference between β-NF group and control group was observed ($P > 0.05$). The CL(s) of DEX and PB were larger than that of the control group($P < 0.01$), $t_{1/2}$ were smaller than the control group ($P < 0.01$), and the induction effect of DEX on the metabolism of Nef was stronger than that of PB($P < 0.05$). Conclusion: Our results suggest that both CYP3A and CYP2B are involved in Nef metabolism in rats, and CYP3A plays a major role.

Key words: neferine cytochrome P450 metabolism

P170003

Effects of Danshen and its tanshinone components on CYP3A-mediated metabdism of testosterone in rat and human liver in vitro

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The effects of Danshen (Salvia miltiorrhiza) and its components on CYP3A-mediated metabolism of testosterone in dexamethasone-treated rat liver and pooled human liver microsomes were studied in vitro. CYP3A activity was determined by measuring testosterone and 6β-hydroxytestosterone by HPLC. Danshen and its ethanolic extracts weakly inhibited CYP3A-mediated metabolism of testosterone in both rat and human liver. Inhibition of rat CYP3A (IC20) by isolated components of Danshen in potency order was dihydrotanshinone (14.5 μM) > cryptotanshinone (34.9 μM) > tanshinone IIA (45.0 μM) > tanshinone I (50.1 μM). Inhibition of human CYP3A4 (IC20) by isolated components of Danshen in potency order was dihydrotanshinone (0.6 μM) > tanshinone I (2.0 μM) > cryptotanshinone (10.7 μM) > tanshinone IIA (94.7 μM). Enzyme kinetic studies showed that the tanshinones were competitive inhibitors, except dihydrotanshinone. In conclusion, Danshen and its tanshinone components only weakly inhibited CYP3A activity and their potentials to cause significant drug-drug interactions with CYP3A substrates would be low.

Key words: Danshen (Salvia miltiorrhiza); Tanshinones; CYP3A inhibition

P170004

Pharmacokinetics of multidrug resistance modulator FG020326 in mice

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Objective: FG020326 is one of the homologues of FG020327 which were developed novel multidrug resistance modulators. This study was to establish a method to study its pharmacokinetics in mice. Methods The KM mice were used for the experiment. FG020326 was administered i.v. at a dose of 30 mg·kg^{-1}. Plasma concentration of FG020326 was detected by HPLC and the pharmacokinetic parameters were calculated by 3P97 software. Results The retention time of FG020326 was 7.9 min and the validated quantitation range was 162.5, 41600 ng·ml^{-1} ($r = 0.9998$); At the concentration of 650, 2600, 20800 ng·ml^{-1}, the recovery rates of extraction were 84.15 % ±7.09 %, 84.63 % ±6.06 %, 68.66 % ±4.14 %, and the recovery rates of method were 110.88 % ±8.91 %, 110.16 % ±7.88 %, 92.58 % ±5.58 %($n = 5$), respectively; The RSD of the precision within-day and between-day was less than 3.2 %. The concentration-time curve of FG020326 was well fitted to a two compartment model. $T_{1/2}$ and $T_{1/2}$ were 0.088 h and 5.33 h; k10, k21 and k12 were 1.60 h^{-1}, 0.64 h^{-1}, 5.81 h^{-1}, respectively, AUC0 was 14183 h·ng·ml^{-1} and Vd was 0.37 L. Conclusion The method is suitable and accurate for determination of FG020326 in mice plasma.

Key words: FG020326; pharmacokinetics; HPLC; MDR

P170005

Assessment of 3H-23-hydroxybetulinic acid uptake kinetics in human Caco-2 cell lines

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Objectives: 23-hydroxybetulinic (23-HBA) acid is a potential anti-tumor agent, whose uptake in Caco-2 cell line is not known. The aim of this study was to define the uptake mechanism and the kinetics of accumulation of 3H-23-HBA in caco-2 cells. Methods: The kinetics of 3H-23-HBA uptake in relation to time and dose dependency were examined. Cells were incubated in mixture of freshly radiolabelled 3H-23-HBA and cold 23-HBA in the presence or absence of specific transport inhibitors. The effect of low temperature was measured too. Apparent permeability coefficient (Papp) was measured through millicell system. Results: In Caco-2 cell lines, the mean Papp of 23-HBA was 3.84 ×10-5cm/s at concentration 0.11-10.11 μg/mL. 23-HBA uptake was time and concentration dependent. Its uptake rates were not markedly reduced by metabolic inhibitors (sodium azide and 2,4-dinitrophenol) and P-gp protein inhibitors (cyclosporine A, verapamil) and low temperature, these indicating the absorption process was not energy-dependent.

Conclusions: In vitro, the uptake of 23-HBA is good and the mechanism may be passive diffusion.

Key words: 23-hydroxybetulinic acid, caco-2 cell, uptake

P170006

Preliminary Biodistribution Studies in Animal of Peptide APRPGY Labeled with 131-Iodine as A Potential Tumor Angiogenesis Targeting Agent

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AIM: Angiogenesis is essential for tumor. In this work, we described the production of 131I-APRPGY and its preliminary distribution studies in mice. Methods: The preparation of 131I-APRPGY was carried out by Ch-T method, purified and characterized by HPLC. Biodistribution studies were carried out on ICR mice bearing hepatoma at different time after i.v. (5 uCi/200ul). Blood samples and interested tissues were collected, washed, weighted and counted ($n = 6$ for each time). The %ID/g and tumor/muscle ratio for each animal were calculated. Results: The yield of 131I-APRPGY is 55 % and its radiochemistry purity is above 95 %. The biodistribution of 131I-APRPGY showed a rapid elimination by kidneys. Tumor/muscle ratio of 131I-APRPGY was 2.1, 6.2, 3.3, 3.5, and 3.2 at 5, 10, 60, 120 and 240 mins, respectively. The accumulation of radioactivity in the tumor was 6.7 %ID/g at 10 mins and decreased within 240 mins to 3.2 %ID/g. In all other organs except kidney, the radioactivity was more rapidly eliminated. Conclusions: The high specific tumor uptake and predominantly renal excretion make APRPGY as a potential candidate targeting tumor angiogenesis. This peptide is worthy of further investigation.

P170007

Pharmacokinetics of paeoniflorin after intravenous administration of TGP in rats with adjuvant arthritis

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To investigate pharmacokinetics of paeoniflorin after intravenous administration of total glucosides of paeony (TGP) in rats with adjuvant arthritis (AA), the rats were induced by Freund complete adjuvant (FCA) with therapeutic adminstration via the caudal vein with TGP (6.25, 12.5, 25 mg/kg, d14-d21). whilst the same doses were injected to the normal rats. at the 22th day, plasma samples were collected at different time to construct pharmacokinetic profiles by plotting drug concentration versus time. Quantification of paeoniflorin in plasma was absorbance half-life ($t_{1/2}\alpha$), elimination half-life ($t_{1/2}\beta$), area under the plasma concentration time (AUC), and clearance (CL), estimated by an open two-compartmental model. The results showed that there were increased AUC values, decreased CL values and prolonged the terminal half-life of paeoniflorin in AA rats. These findings suggest that pharmacokinetic process of paeoniflorin expresses different change in rats with AA.

Key words: paeoniflorin; pharmacokinetic; adjuvant arthritis

P170008

Pharmacokinetic study on atomization inhalation furosemide in healthy mice

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Objective: To construct the determination method of furosemide in plasma and pulmonary tissue of mice. To study the pharmacokinetic parameters of atomization

inhalation furosemide in healthy mice and the relationship of the drug concentrations in plasma and pulmonary tissue homogenate liquid. Method: Divided the healthy mice into 2 groups in random. The high-dose group was given 0.155 mg/20g and the low-dose group was given 0.077 mg/20g atomization inhalation furosemide. Took out the plasma and pulmonary tissue at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180 min respectively (n = 8). The plasma and pulmonary tissue homogenate liquid were precipitated with acetonitrile, centrifuged and then got the supernatant to inject. Worked out the pharmacokinetic parameters of atomization inhalation furosemide with software DAS. Results: The minimum detection cncentrations in plasma and pulmonary tissue homogenate liquid were both 0.02 μg/ml. Conclusions: Constructed the determination method of furosemide in plasma and pulmonary tissue of mice. Determined the pharmacokinetic parameters of atomization inhalation furosemide in healthy mice.

Key words: furosemide pharmacokinetic mice

P170009

The fast metabolic feature of 5-Hydroxymethylfurfural in rats

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To detect the metabolic feature of 5-hydroxymethylfurfural in rats, We use HPLC to detect the prototype compound in the blood of rat after intravenous and oral administration of 5-hydroxymethylfurfural. There is no prototype drug that can be found even at two minutes after intravenous administration. But a metabolite of 5-hydroxymethylfurfural is detected. Also this metabolite exists in the blood of rat after oral administration of 5-hydroxymethylfurfural at 7 hours. The metabolite, with MH^+ ion at m/z 143, was detected by LC/MS. The mass of MH^+ ion (a sum of the molecular weight of 5-hydroxymethylfurfural plus 16 Dalton) was indicative of hydroxylation or carboxylation. 4-hydroxyl-5-hydroxymethyl-furfural, 3-hydroxyl-5-hydroxymethyl-furfural, 5-hydroxymethyl-furfural acid are the possible metabolite.

Key words: 5-Hydroxymethylfurfural, metabolite, hydroxylation, carboxylation,

P170010

Naltrexone microspheres: pharmacokinetics in rhesus monkeys and pharmacodynamics in rodent

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Subcutaneous or intramuscular injection of naltrexone (NTX) microspheres is a more effective method of producing chronic blokade of opiod receptors in treating alcohol and opiate dependencies to improve compliance. In the present experiments, pharmacodynamic studies of NTX microspheres (NTX loading: 20 %, LA/GA molar ratio in PLGA copolymer: 75:25, sterilized by γ-irradiation) after subcutaneous administration in mice and rats demonstrated that the preparation has a pronounced completely blocked effects to morphine analgesic response in the mice hot-plate test, rats tail flik test and to morphine physical dependence in mice compared to placebo microspheres. This antagonism began on day 1 following administration and lasted for about 40-45 days. Pharmacokinetics of NTX microspheres (NTX 200 mg/monkey and 8 mg/kg) were examined in rhesus monkeys by HPLC-MS method, the NTX plasma concentration exceeded a mean of 1 ng·ml^{-1} for 35 days after intramuscular injection. Clinical trials of the sustained-release preparation of naltrexone for treating alcohol and opiate dependency are currently ongoing.

Key words: naltrexone; microspheres; pharmacokinetics; pharmacodynamics;

P170011

Pharmacokinetic studies on single and multiple doses of oral igurati mod in healthy volunteers

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To determine the serum concentration and pharmacokinetic parameters of igurati-mod, 32 healthy volunteers were divided into four groups. The groups received three single oral doses (25, 50 or 75 mg) and one multiple oral doses (50 mg) of igurati mod, respectively. Serum concentration of igurattimod were measured by HPLC. The pharmacokinetic data were fit to a one-compartment model with first-order absorption. After single doses (25, 50 and 75 mg) of igurati mod, the following pharmacokinetic parameters were calculated, respectively: T_{max} 3.38 ± 0.92, 4.88 ± 1.96 and 4.33 ± 1.00 h; C_{max} 1.24 ± 0.22, 2.13 ± 0.54 and 3.59 ± 0.67 mg/L; AUC 20.93 ± 4.24, 34.89 ± 10.02 and 56.81 ± 8.02; $t_{1/2}$ 8.55 ± 3.01, 6.31 ± 3.15 and 7.30 ± 2.94 h. After multiple oral doses (50 mg) of igurati mod, the following pharmacokinetic parameters were calculated: T_{max} 3.63 ± 1.60 h; C_{max} 1.88 ± 0.31 mg/L; AUC 31.88 ± 4.52 mg h/L; $t_{1/2}$ 10.25 ± 7.17 h. Igurati mod exhibited linear kinetics across oral doses of 25, 50, and 75 mg. There were no serious adverse events, and igurati mod was well tolerated over the entire dose range.

Key words: igurati mod; pharmacokinetic; serum concentration; HPLC

P170012

Effect of Obesity on CYP2E1 Expression in Zucker Rats

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An elevation of CYP2E1 activity in-vivo during obesity has been reported by several researchers, but the increase of CYP2E1 activity in-vitro was well documented only in liver. This research aims to reveal the alteration of CYP2E1 expression in liver, kidney and fat from high fat dietary (HF) and genetically obese (GO) Zucker Rats compared with control. The elevated expression of CYP2E1 was determined by rt-PCR, Western blotting, microsomal activity and pharmacokinetics of Chlorzoxazone (CZX). It was found that enzyme CYP2E1 mRNA in abdominal fat and the protein content of CYP2E1 in liver and abdominal fat were increased in HF and GO rats. Accordingly, the microsomal CYP2E1 activities in liver and abdominal fat of HF and GO groups in-vitro exerted a higher rate of 6-hydroxychlorzoxazone (6OH) production. Furthermore, the AUC ratio of 6OH/CZX after an i.v. administration of CZX (20 mg/kg) in both HF and GO groups were significantly increased compared with that of control. In conclusion, the induction of CYP2E1 expression in abdominal fat and liver may lead to increasing in the metabolic degradation of CZX and decreasing in the pharmacological effect.

Key words: CYP2E1, Obesity, Zucker Rat

P170013

Pharmacokinetics of ZT-1 in experimental animals

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ZT-1 is a novel acetylcholinesterase (AChE) inhibitor, which was rapidly transformed to Hup A in vitro and in vivo. After ig administration of doses of PVP/ZT-1 (1, 2.5, 5.0 and 10.0 mg/kg) to rats, the Tpeak were all 15 min, the $T_{1/2}$ were about 6 ~ 7 h, the C_{max} were 0.59 ~ 3.37 nmol/mL, and the AUCs were 2.62 ~ 22.29 nmol h/mL for Hup A, respectively. Comparing the AUCs obtained from Hup A, the oral bioavailability of ZT-1 was 99.2%. After ig administration of ZT-1 2.5 mg/kg to dogs, the Tpeak, $T_{1/2}$, C_{max} and AUC were 1 ~ 3 h, 5.11 ~ 7.14 h, 2.58 ~ 3.44 nmol/mL and 19.40 ~ 25.15 nmol h/mL for Hup A, respectively. Tissue distribution results showed that Hup A was rapidly distributed in lung, liver, kidney and digestive tissues after ig 5 mg/kg of ZT-1 to rats. The drug levels in most tissues were much higher at 15 min than those at 2 or 6 h after dosing. The parent drug was not found in urine and feces during 0 ~ 48 h after oral dosing of 5 mg/kg ZT-1 to rats. The total excretion of the metabolite Hup A from feces, urine and bile amounted to 3.28%, 20.5% and 0.27% of the dose.

Key words: Pharmacokinetics, ZT-1

P170014

The quantification of the metabolites of dipfluzine in rats

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Objective: To determine the concentration of dipfluzine (Dip) and its metabolites in rat urine and feces. METHOD After a vein injection dose of Dip (0.2 mg.kg^{-1}) to rats, the urine and feces were collected within 24 h. Dip and its metabolites were separated and identified by HPLC methods. RESULTS In the rat urine, there were Dip, benzophenone, benzhydrol and 4-hydroxybenzophenone. The retention times were 32.7 min, 34.5 min, 30.9 min and 26.8 min, respectively. The higher contents in the metabolites of Dip were benzhydrol and 4-hydroxy-

benzophenone, with 38.08～49.44% and 29.72～34.20%, respectively; benzophenone was 0.68～1.44%, and the prototype drug was 1.76～2.81%. Benzhydrol and 4-hydroxy-benzophenone was transformed by benzophenone, the proportion of translation was 98.6%. There was only Dip in rat feces, which was 1.05～1.40%. CONCLUSION Dip was mostly excreted from the body by bile and kidney, the 3 metabolites were excreted by kidney.

Key word: dipfluzine, HPLC, Metabolites, Translation

P170015

Effect of bifendate on pharmacokinetics of cyclosporine A by intestinal administration in rat*

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The aim of this study was to investigate the pharmacokinetics of cyclosporin A (CsA) by intestinal administration with or without the presence of bifendate (BFD) in rat. After orally taking 20 mg·kg^{-1} BFD for 6 days, the BFD group was administered with 1 mg·kg^{-1} CsA plus 20 mg·kg^{-1} BFD through the proximal end of duodenum on the 7th day, while the CsA group was only administered with 1 mg·kg^{-1} CsA through the same site on the 7th day. The blood samples were collected from portal vein and the concentrations of CsA were determined by fluorescence polarization immunoassay (FPIA). The results showed that compared with CsA group, the average % decreases in C_{max} and AUC in BFD group were 55.7% and 49.7%, respectively ($P < 0.05$). $t_{1/2}$(ke), CL/F and V/F were significantly increased($P < 0.01$ or 0.05). No differences were observed between the other parameters in two groups. In conclusion, BFD can markedly decrease the bioavailability of CsA in rats. The interaction between BFD and CsA may occur in intestines.

Key words: bifendate; cyclosporin; drug interaction; pharmacokinetics

* This study was supported by Natural Science Foundation of Hubei Province (NO. 2004ABA209), China.

P170016

The theoretical investigation of the binding mode between human serum albumin and penicillins

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Aim To investigate theoretically the binding mode between human serum albumin and penicillins. Methods Molecular docking method was used to elucidate the binding modes between human serum albumin (HSA) and penicillins. Results The lowest binding energies (LBE) of apalcillin, oxacillin, carbenicillin, ampicillin, amoxicillin were 44.2, 46.5, 42.1, 40.9, 40.7 kcal/mol. Subdomain IIA and IB have better ability to binding penecillins than others. Conclusion Penicillins may be easier to bind to the cavity IIA and IB. The molecules with both polar and nonpolar parts may be easier to bind to the cavities of HSA.

Key words: human serum albumin, molecular docking, binding energy, binding cavity

Acknowlegment: This work was supported by the Tianjin Science Foundation (043185111-7) and the computation was supported by the Nankai star supercomputer. This work was also supported by the open fund of the Guangdong Key Lab of Computer Network (CN200409).

P170017

CODING REGION MUTATIONS IN UGT1A1 IMPAIR BILIRUBIN AND XENOBIOTIC GLUCURONIDATION

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UGT1A1 is solely responsible for bilirubin glucuronidation, and also contributes to the metabolism of drugs and xenobiotics. This study investigated the effects of the coding region mutations G71R, P229Q, F83L and Y486D on UGT1A1 activity and substrate selectivity. Variants were generated by site-directed mutagenesis using the wild-type cDNA as template. Wild-type and variant enzymes were stably expressed in HEK293 cells, and activity was measured using 4-methylumbeliferone (4MU), 1-naphthol (1NP), bilirubin (BIL), estradiol (EST) and naproxen (NAP) as the substrates. G71R and P229Q caused an approximately 40-70% reduction in the intrinsic or maximal clearances of BIL, 4MU, 1NP and EST. The F83L and Y486D mutations resulted in 90-99% loss of UGT1A1 activity. The Y486D mutation was also introduced into UGT1A3, UGT1A6 and UGT1A10, and resulted in almost complete loss of 4MU, 1NP and NAP glucuronidation activities. It is concluded that UGT1A1 coding region mutations associated with impaired bilirubin elimination also variably reduce xenobiotic glucuronidation, while Y486D greatly reduces all UGT1A activities.

Key word: UGT1A1 polymorphism

Acknowledgement: Thai Government and NH&MRC (Australia)

P170018

EFFECT OF TRAMADOLE ON SOME ANTIOXIDANT SYSTEMS IN ANIMALS WITH ULCER STRESS

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Influence of the synthetic opioid Tramadole on the ulcer stress caused by cold restraint stress was studied. Tramadole was applied to experimental animals before initiating stress, and animals were sacrifice after 3 hours stress condition. Antioxidative parameters - value of reduced glutathione - GSH and glutathione peroxidase - GSHPx, glutathione reductase - GSHR and peroxidase Px were determined in liver homogenate. The quantity of GSH was much lower in animals with ulcer, compared to control, while Tramadole showed protective effect (GSH content was higher than in animals with ulcer, but lower than control). Activity of GSHPx was reduced in animals with ulcer, comparing to the control, while in Tramadole-treated animals activity of enzyme was lower than in animals with ulcer, but not statistically significant. Activity of GSHR was higher in animals with ulcer, while treatment with Tramaddle produced activity higher than control, but lower than animals with ulcer. The activity of Px was reduced, but not statistically significant in the animals with ulcer, as well as in the Tramadole-treated animals.

P170019

Rifampin markedly reduces plasma concentrations of single and multiple oral doses of praziquantel in healthy volunteers

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In randomized, crossover design, a single or multiple oral doses of 40 and 25 mg/kg praziquantel alone or after pretreatment with 600 mg/kg rifampin orally for 5 days in 10 healthy volunteers were studied. Plasma concentrations of praziquantel were determined by HPLC. In the single-dose study, rifampin decreased praziquantel concentrations to undetectable levels in 7 of 10 subjects, whereas praziquantel concentrations were reduced by rifampin to undetectable levels in 5 of 10 subjects in the multiple-dose study. In 3 subjects with measurable concentrations in the single-dose study, rifampin significantly decreased the C_{max} and AUC_{0-24} of praziquantel by 81% and 85%, respectively whereas rifampin significantly decreased the C_{max} and $AUC_{(0-24)}$ of praziquantel by 74% and 80%, respectively in 5 subjects with measurable concentrations in the multiple-dose study. The C_{max} and $AUC_{(0-24)}$ of praziquantel in subjects whose praziquantel concentrations could not be detected in the single-dose study after rifampin pretreatment were reduced by approximately 99% and 94%, respectively and in the multiple-dose study, they were reduced by 98% and 89%, respectively.

P170020

HPLC method for determination of aildenafil in rat and dog serum

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Aim: A HPLC method was used to determine the concentration of aildenafil in rat and dog serum. Methods: An analytical C18 column and a variable wavelength detector at 292nm.. For rats, The mobile phase containing 45% methanol, 20% acetonitrile, 35% water, 1% triethylamine and 0.2% phosphate, was used at a flow rate of 1mL/min. For dogs, it containing 35% methanol, 20% acetonitrile, 45% water, 1% triethylamine and 0.2% phosphate. Results: For rats, the limit of quantitation was 20ng/mL. The recovery at 50, 200 and 1000ng/mL was 84.5, 98.9 and 91.2%, respectively. The relative standard derivative of inter-day and intra-day determination was less than 10%. For dog, the limit of quantitation was 5ng/mL. The recovery at 50, 200 and 1000ng/mL was 98.2, 93.2 and

93.6%, respectively. Conclusion: This bioanalytical method for determination of aildenafil in plasma possesses the characteristic with simple, sensitive and accurate. The validation for methodology is indicated that this bioanalytical method is suitable for pharmacokinetics study of aildenafil formulations.

Key words: aildenafil; bioanalytical method; HPLC

P170021

Pharmacokinetics of isoniazid in relation to NAT2 genotypes

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Objective: To evaluate the relationship between the pharmacokinetics of isoniazid (INH) and three different kinds of NAT2 genotypes in healthy Chinese subjects. Methods: Twenty-four subjects recruited from 120 volunteers whose genotypes were predetermined were classified into three groups according to their genotypes: w/w, w/m and m/m. Each subject received a single oral dose of 300 mg INH, plasma samples which were determined by the HPLC method were collected at different times. Results: Pharmacokintic parameters of INH for the three genotypes: $t_{1/2}$: 1.15±0.18, 1.76±0.17, 3.23±0.28 h; CL: 30.12±6.94, 19.20±5.19, 7.54±1.59 L×h×kg^{-1}; AUC(0-14): 9.81±2.40, 15.27±2.97, 36.57±7.31 mg×h×L^{-1}; respectively. The parameters of acetylisoniazid (AcINH): C_{max}: 5.59±1.38, 3.99±0.50, 1.38±0.24 mg×L^{-1}; T_{max}: 1.31±0.59, 2.50±0.93, 4.50±0.93 h; AUC(0-14): 36.88±7.41, 33.03±4.57, 13.87±2.33 mg×h×L^{-1}, respectively. There were significant differences in the pharmacokinetic parameters of INH and AcINH in three groups ($P<0.05$). Conclusion: The disposition of INH had marked differences in different NAT2 genotypes.

Key words: NAT2, INH, pharmacokinetics This project was supported by the Nation Natural Science Foundation of China (No.30472055).

P170022

Roles of pommelo fruit juices and cytochrome P450 3A5*3 in the metabolism and action of felodipine

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Three pommelo juices were tested for their CYP3A inhibition and clinical effects on oral felodipine in 12 Chinese with two CYP3A5*3 genotypes. Grapefruit (*Citrus paradisi* Macf., G), C. grandis Osbeck vs. Guanxi (P) and C. changshan-huyou Y.B. Chang (H) fruit juices were determined for their furanocoumarin (FC) contents, and their inhibition of CYP3A in human microsomes. In a four-way cross-over study, water (W), fruit juice of G, P or H (250 ml) was given alternatively with oral felodipine (10 mg), and heart rates, blood pressures and plasma felodipine were monitored for 12 hours. Comparing with G, P showed lower levels of FCs, and weaker CYP3A inhibition, whereas H showed almost no FCs, and no CYP3A inhibition. For all the clinical subjects, the orders of AUC and C_{max} were G>P>W and G>P>H&W, respectively. The order for heart rate increase was G>H&W. For the CYP3A5*3 subgroups, both orders for AUC and C_{max} were G>P&H&W for G/A, and G>P>W for G/G. The systolic blood pressures were lower in G/G than in G/A. In conclusion, FC is an index to predict citrus fruit juice-drug interaction; CYP3A5 may be involved in both the metabolism and action of felodipine.

Key words: furanocoumarin; pommelo; felodipine; Cytochrome P450 3A5*3 Project partly supported by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (0214-HD10)

P170023

Identification of the major metabolites of 3,4-dichlorophenyl-propenoyl-secbutylamine (DCPB), a novel antiepileptic drug, in rat plasma by HPLC-MS/MS

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Objective: To identify the major metabolites of 3,4-dichlorophenyl-propenoyl-secbutylamine (DCPB), a novel antiepileptic drug, in rat plasma by using high-performance liquid chromatography (HPLC) assay with electrospray ionization mass spectrometry (ESI-MS/MS). Methods: After an oral dose of DCPB (100 mg/kg) 6-8 hour, the metabolites in rat plasma were isolated and pretreated by HPLC (reversed-phase C18 column, 150×4.6 mm, 5 μm), in which mobile phase was composed of methanol and water (80:20, v/v). Subsequently, the metabolites were identified by LC-ESI-MS/MS. Results: The HPLC retention times of the three metabolites (M1, M2 and M3) were 1.76, 2.77, and 3.20 min, respectively, which appeared in front of DCPB spectrum peak (4.6 min) in rat plasma. The characteristics of LC-MS/MS were performed at m/z 216 (M1), m/z 215 (M2), m/z 287 (M3) and m/z 271 (unchanged drug of DCPB), respectively. Conclusion: The results suggested that the major metabolic pathways of DCPB might be hydrolysis of amide linkage (M1), N-dealkylation formed by the loss of secbutane (M2), and N-oxidation by hydroxy added to nitrogen (M3) in rats.

Key words: DCPB, metabolites, HPLC-MS/MS, antiepileptic drug.

P170024

DETECTION OF POLYCYCLIC AROMATIC HYDROCARBON EXPOSURE FROM AUTOMOBILE EXHAUST FUMES USING URINARY 1-HYDROXYPYRENE LEVEL AS AN INDEX

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Polycyclic aromatic hydrocarbons (PAHs) are bioactivated to reactive metabolites which can bind covalently to DNA and subsequently initiate mutation and carcinogenesis. The purpose of this study was to measure level of urinary 1-hydroxypyrene, a metabolite of PAHs, in subjects exposed to automobile exhaust fumes compared to non-exposed subjects. A urine sample was collected from individual subject after the end of working day and quantitated for 1-hydroxypyrene and creatinine by HPLC and spectrophotometric method, respectively. The results showed that average urinary 1-hydroxypyrene level in exposed subjects was significantly higher than non-exposed subjects, $P=0.000$. The ratio of urinary 1-hydroxypyrene / mol creatinine level, of the exposed subjects was significantly higher than that of the non-exposed subjects, $P=0.002$. Thus, automobile exhaust fume exposed subjects have a higher risk to be exposed to PAHs than the non-exposed subjects. Urinary 1-hydroxypyrene can be used as an index for an exposed of PAHs which are originated from automobile exhaust fume and other sources as well.

Key words: Polycyclic aromatic hydrocarbons, PAHs, 1-Hydroxypyrene Acknowledgement: Thanks to the Rachadapiseksompoj China Medical Board Reserch Fund and MUA-CU Thesis Grant.

P170025

Pharmacokinetics and disposition of bisbenzisoselenazolone-ketone (Se-2003), a novel antitumour drug, after oral administration in rats

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Objective: To investigate the pharmacokinetics and distribution of bisbenzisoselenazolone-ketone (Se-2003), a novel antitumour drug, after oral administration in rats. Methods: The concentration of selerium in the biosamples were determined by the method of fluorescence with wavelengths of excitation and emission at 376 and 520 nm, respectively, after a single oral administration of Se-2003 (20, 40, 80 and 120 mg/kg) in rats. Result: The plasma concentration of Se-2003 was increased as dose-dependent manner within the range of 20-120 mg/kg in rats. AUC0-t were 19.74, 29.86, 48.42 and 115.88 (μg/ml)h, and $t_{1/2}$ were 9.58, 10.34, and 35.40 h, respectively. Se-2003 was widely distributed into the various tissues, especially the higher concentrations of drug were observed in liver and kidney. The excretive major routes of Se-2003 were via the feces (52.71%) and urine (5.99%) within 48 h. The total excretion was approximately 58.70% of the total dose. Conclusion: The major pharmacokinetic parameters indicated that Se-2003 was rapidly absorbed, widely distributed into tissues, and slowly eliminated by urine and feces in rats.

Key words: Se-2003, pharmacokinetics, fluorescence.

P170026

Metabolite Profiling of Isovaltratin Family Oligosaccharides in Rats

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Electrospray ionization multi-stage tandem mass spectrometry and liquid chromatography coupling (LC/MS^n) were applied to identify trace-level in vivo metabolites after the gavage of two oligosaccharides to rats. Based on the relationship between the characteristic fragmentation reactions and the structural features of related compounds of known isovalertatins, the parent components and potential in vivo metabolites in urine, feces, and ileum incubation samples were analyzed in detail by two independent qualitative parameters, retention time and collision-induced dissociation fragmentation ions with a sensitive and specific solid-phase extraction plus LC/MS^n method. Nine and seven metabolites were successfully characterized from the above bio-samples after given isovalertatins M23 and D23 to rats, respectively. These biotransformation products resulted from the reducing terminus α-glucose hydrolysis, non-reducing terminus α-glucose hydrolysis, and isovaleryl de-esterification hydrolysis of parent isovalertatins, which mainly taken place in rat intestine tract.
Key words: metabolite profiling, oligosaccharides, isovalertatin, liquid chromatography/mass spectrometry
Acknowledgements: This work was supported in part by Grant # 2003AA2Z3347D of the National High Technology Program (863 program) of China.

P170027

Effect of berberine on cytochrome P450 total content and CYP1A2 metabolic activity in chemic-immune liver injury induced by DEN and BCG in rats

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Objective: To study the effect of berberine on chemic-immune liver injury induced by diethylinitrosamine (DEN) and Bacille Calmette Guerin (BCG) in rats. Methods: The liver injury was induced by a single dose of DEN (150 mg/kg, i.p.) and BCG (60 mg/kg, i.v., 2 weeks) in rats. The levels of alanine aminotransferase (ALT) and nitrite in serum and CYP450 total content in liver were determined by the method of spectrophotography. CYP1A2 activity was assessed by the concentration of probe drug caffeine (CAF) and phenacetin (PHE) in plasma and hepatic microsome using HPLC method. Result: After stimulation of DEN and BCG, the levels of ALT and nitrite, and CYP1A2 activity in plasma were increased, but CYP450 total content was decreased significantly ($p < 0.05$). Administration of berberine (50 mg/kg, i.g.) reversed the effects of DEN and BCG on ALT and nitrite level, CYP450 total content, and CYP1A2 catalytic activity in vivo. Conclusion: This result suggested that berberine improved the liver injury induced by DEN and BCG in rats, and the mechanism might be inhibition for CYP1A2 activity which contributes to toxic xenobiotic metabolism.
Key word: berberine, immune liver injury, CYP1A2

P170028

Stereoselectivity of epidermal carboxylesterase metabolism as observed in HaCaT keratinocytes

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Aim: To study stereoselectivity and molecular mechanism of epidermal carboxylesterase metabolism as observed in HaCaT keratinocytes. Methods: Ketoprofen ethyl ester was used as a model drug, and HaCaT cell homogenates was applied for studying the stereoselectivity of carboxylesterase metabolism. The concentrations of all samples were assayed by HPLC. Human liver L02 cell strain was used as control of carboxylesterase expression, and RT-PCR was used for studying the expression of carboxylesterase. Results: The main metabolite of ketoprofen ethyl ester in HaCaT cell homogenates was R-ketoprofen. Human craboxylesterase (hCE)-2 was highly expressed in HaCaT keratinocytes. However, the expression of hCE-1 was very weak or not detectable. Conclusion: hCE-2 is more abundant carboxylesterase in HaCaT keratinocytes that may be responsible for stereoselective hydrolysis of ketoprofen ethyl esters. This pilot study reinforces the methods of improving transdermal absorption by prodrugs.
Key words: carboxylesterase; HaCaT cell line; ketoprofen ethyl ester; stereoselectivity
Acknowledgements: We thank Dr. Q.S. Mi and Prof. J. Gu for kindly providing the HaCaT cell lines. This work was supported by the Natural Science Foundation of China (Grants No. 30271553 and No. 30572269).

P170029

Determination and pharmacokinetics of phencynonate and its optical isomers in rat

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Pharmacokinetics of phencynonate and its two isomers were investigated in rat by the method of liquid chromatographic assay with electrospray ionization mass spectrometry detection (LC-ESI-MS). The lower limit of quantification was at 1 ng/mL in blood. The precision was obtained from 2.92 to 9.76%. Extraction recoveries were in the range of 69.6-79.1%. The main pharmacokinetic parameters of phencynonate were as follows: $T_{1/2}\alpha$ 0.68 h, $T_{1/2}\beta$ 3.98 h, $T_{1/2}$Ka 0.013 h, T_{max} 0.076 h, C_{max} 54.08 ng/mL, AUC 77.70 ng h/L. There were some differences for the level of the blood drug concentration of phencynonate raceme and the two optical isomers after dosing the phencynonate and the R and the S-isomers, respectively. There was the relationship between the pharmacodynamics and the pharmacokinetics for the configuration to the chiral drug. It provided important information for developing a novel chiral drug and the clinical use of phencynonate.
Key words: phencynonate; isomer; LC-MS; pharmacokinetics.
Acknowledgement: This work was supported by the Major program of National Natural Science Foundation of China (No 203900508).

P170030

Liquid chromatography-tandem mass spectrometry method for determination of thiencynonate in rat plasma

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A sensitive and specific high-performance liquid chromatography-tandem mass spectrometry method (LC/ESI/MS) was developed and validated for the identification and quantification of the novel lead compound of anticholinergic drug thiencynonate in rat plasma. Simultaneous MS detection of thiencynonate and IS was performed at m/z 364.4 (thiencynonate), m/z 358 (phencynonate), and the SRM of the two compounds was both at 156. Thiencynonate eluted at approximately 2.8 min, phencynonate eluted at approximately 2.9 min and no endogenous materials interfered with their measurement. Linearity was obtained over the concentration range of 1~100 ng/mL in rat plasma. The lower limit of quantification was reproducible at 1 ng/mL in rat plasma. The precision measured was obtained from 2.47 to 9.28% in rat plasma. Extraction recoveries were in the range of 67.63-76.76% in plasma. This method was successfully applied to the identification and quantification of thiencynonate in pharmacokinetic studies.
Key words: Thiencynonate; lead compound; LC-MS; Quantification
Acknowledgement: This work was supported by the Major program of National Natural Science Foundation of China (No 203900508).

P170031

Pharmacokinetics profiles of penehyclidine, a novel anticholinergic agent in humans, rabbits and mice

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A sensitive and specific gas chromatographic-mass spectrometry with selected ion monitoring (GC-MS/SIM) method has been developed and validated for quantification of penehyclidine (PH) in human and animals. The lower limit of quantification was reproducible at 50 pg/ml in both human and animal blood. The within-day and between-day precisions were no more than 9%. The concentration-time profile of PH raceme and its four optical isomers were all best fitted to first order absorption two-compartment open model after i.m a single dose in human, rabbits and mice. The differences in absorption, distribution and elimination of PH and its isomers among the species were found. The main pharmacokinetic parameters of PH for the species were as follows: $t_{1/2}\alpha$ 0.41, 0.12 and 0.23 h, $t_{1/2}\beta$ 10.4, 8.4 and 3.3 h, t1/1Ka 0.16, 0.024 and 0.013 h, t_{max} 0.56, 0.024 and 0.067 h, C_{max} 13.2, 30.

2 and 18.7 ng/mL, AUC 133.2, 107.6 and 50.4 ng h/mL. The results provided the important information for developing a novel anti-cholinergic drug and for obtaining a more effectual remedy in clinical practice.

Key words: penehydidine; pharmacokinetics; species; GC-MS/SIM

P170032

Pharmacokinetic and pharmacodynamic profiles of penehydidine and its optical isomers, a novel anticholinergic agent

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The profiles of pharmacokinetics and pharmacodynamics of penehydidine (PH) raceme and its four optical isomers were investigated and compared. The blood and tissue concentration of PH raceme and its four optical isomers were determined by gas chromatography-mass spectrometry with selected ion monitoring. The affinity and relative efficacy were tested using radioligand-binding assay with central muscarinic acetylcholine receptors (mAChR) on the heart, intestinal muscle and submandibular gland of guinea pig. It existed the differences in the absorption, the distribution and the elimination between PH raceme and its four isomers. The distribution in mice was shown that the tissue concentration of R-2 isomer had a high level which R-2 isomer had a great affinity to mAChR. The order of affinity of PH and its isomers to mAChR in the tested tissues was the same, i.e., RR'> PH> SR'> RS'> SS'. Among four tested tissues, PH and its isomers had a relative higher selectivity to mAChR in submandibular glands. There was the relationship between the pharmacodynamics and the pharmacokinetics for R-configuration to the chiral drug.

Key words: penehydidine; isomer; pharmacokinetics; pharmacodynamics

P170033

Expression and transport activity of breast cancer resistance protein (Bcrp/Abcg2) in dually perfused rat placenta and HRP-1 cell line

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The purpose of this study was to describe the role of BCRP in transplacental pharmacokinetics using rat placental HRP-1 cell line and dually perfused rat placenta. Expression of Bcrp was revealed at mRNA and protein levels. Cell accumulation studies confirmed Bcrp-dependent uptake of BODIPY FL prazosin. In the placental perfusion studies, a pharmacokinetic model was applied to distinguish between passive and Bcrp-mediated transplacental passage of cimetidine as a model substrate. Bcrp was shown to hinder materno-fetal transport of the drug; feto maternal clearance of cimetidine was found to be 25 times higher than that in the opposite direction. This asymmetry was partly eliminated by BCRP inhibitors (fumitremorgin C or GF120918) and completely abolished at high cimetidine concentrations. In addition, Bcrp was found to actively remove cimetidine from the fetal compartment to the maternal one even against concentration gradient and establish a two-fold maternal-to-fetal concentration ratio. We propose a two-level defensive role of Bcrp in placenta: the transporter (i) reduces passage of its substrates from mother to fetus but also (ii) removes the drug already present in the fetal circulation.

P170034

The role of pharmacokinetic researches in optimisation of new anxiolytics drug formulations

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The aim of this study was development of anxiolytic drug formulations based on combined pharmacokinetic and pharmacodynamic researches. Derivatives of 1,4-benzodiazepine, Buspirone and benzimidazole were studied. For the determination of anxiolytics and their metabolites in blood plasma, high performance liquid chromatography was used. Anxiolytic, sedative and myorelaxant effects were evaluated. Extent and rate of desalkylation, also their pharmacological activity spectrum and adverse reactions were depend from excipient amounts. After preclinic pharmacokinetic and pharmacodynamic evaluations of Fenazepam, Gidazepam and Piricapirone different formulations, an advantage of transdermal delivery systems and solid dispersion systems was demonstrated in comparison with others administration ways. Intensity and duration of anxiolytic action largely depends on the rate of transdermal transfer and steady state drug concentrations in blood plasma.

Key words: pharmacokinetics, pharmacodynamics, anxiolytics, drug development

P170035

Bioequivalence study of two marketed brands of stavudine 40 mg capsules in healthy Thai male volunteers.

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This study evaluated the bioequivalence of two marketed brands of stvudine 40 mg capsules. A randomized, two way, crossover study was conducted in 26 fasting healthy volunteers. Blood samples were collected throughout a 10-h period after administration of reference product (R) and test product (T). The plasma stavudine concentration were determined via HPLC technique. Bioequivalence between the products was determined by calculating 90% confidence interval (90% CI) for the ratios of C_{max}, $AUC_{0-\infty}$ values for the test and reference products, using logarithmic transformed data. The 90% confidence intervals for the ratios of C_{max} (86.49-105.44%), $AUC_{0-\infty}$ (92.15-103.63%) values for the test and reference products were within the 80-125% interval, proposed by Thai FDA. Two formulations were considered bioequivalent, in the rate and extent of absorption.

Key words: Bioequivalece, stavudine (d4T), Pharmacokinetics

Acknowledgement: Government Pharmaceutical Organization (GPO), Thailand.

P170036

Cytochrome b5 Increases the Rate of Catalysis by Cytochrome P450 2B4

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In order to elucidate the mechanism by which cyt b5 enhances the efficiency of catalysis by cyt P450 2B4 in a reconstituted system, the kinetics of product formation by cyt P450 2B4 in the presence of cyt b5 and cyt P450 reductase (CPR) were compared. The kinetics of cyclohexanol formation from cyclohexane were determined with a chemical quench flow instrument. Product was quantified by gas chromatography-mass spectrometry (detection limit > 6.2 nmol/mL). Under single turnover conditions cyt P450 2B4 monophasically catalyzes the oxidation of cyclohexane which is ~10-fold faster in the presence of cyt b5 than with CPR. In contrast, when both cyt b5 and CPR were present, the kinetics of cyclohexanol formation were biphasic. The fast and slow phases correspond to the rate constants observed with cyt b5 and CPR respectively, while the phase amplitudes were proportional to the molar ratio of cyt P450 to cyt b5 and CPR. Conclusion: 1) Catalysis occurs more rapidly with cyt b5 than with CPR likely due to favorable conformation in cyt P450; 2) CPR and cyt b5 compete for a binding site on cyt P450.

P170037

Pharmacokinetics and Metabolism of a Novel Antifibrotic Drug Pirfenidone, in Rats and Beagle Dogs Following Oral and Intravenous Administration

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Pirfenidone (PF, RUIXING Genomics, Inc, Shanghai, China), a novel compound has therapeutic potential for IPF. The pharmacokinetics and metabolism of Pirfenidone (PF) had been investigated in rats and beagle dogs. After oral administration in rats and beagle dogs, plasma concentration curves of PF are best fitted to one-compartment model. After intravenous injection of PF, the C-T curve could be described by two compartment model and indicated the absolute bioavailability of 51.59% in rats and 80.59% in dogs. After a single oral dose of 100 mg/kg, the parent drug and its metabolites were detected in tissue rapidly and the relative concentrations of PF are highest in well-perfused tissues. About ten metabolites and few parent compound has been detected in urine and bile in rats. The ratio of plasma protein binding of the PF from rats and human were 64.09%-84.92% and 66.19%-77.78%. After oral administration of PF in rats for 6 days, this novel agent show an effect of induce on drug-metabolizing enzyme, especially on CYP3A. Totally, PF was rapidly absorbed, extensive metabolized and distributed throughout the body.

Key words: pirfenidone; metabolism; pharmacokinetics; artifibrotic drug

P170038

Pharmacokinetics and Metabolism of Neferine in rats

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Objective: To investigate the pharmacokinetics and metabolic pathway of Neferine, an alkaloid extracted from seeds embryo of Nelumbo nucifera, in Wistar rats. Methods NEF 10, 20 and 50 mg/kg was administrated per oral. The concentration of NEF was analyzed by high performance liquid chromatography. The metabolites were identified by liquid chromatography tandem mass spectrometry. The data were dealt with DAS program. Results The AUC0-24h of 10, 20, 50 mg/kg dose was 65.45, 92.094 and 126.107 mg/kg respectively and the MRT0-24h was 9, 10 and 11 h. NEF was rapidly distributed and the concentration was the highest in liver > lungs > kidney > heart > brain, in turn. Two major metabolites have been found. One is M1 (M/Z = 611, Rt = 5.6 min), which maybe LIEN. Another is M2 (M/Z = 611, Rt = 7.8 min), which maybe IL. Perhaps through desmethyl enzyme (CYP2D6), NEF was converted to the metabolites, because Quinidine, an inhibitor of CYP2D6, which was incubated with NEF, significantly inhibited this conversion. Conclusion These indicate that the pharmacokinetic characters of NEF are concentrated in tissues, quick transfered to M1 and M2, maybe involved in CYP2D6.

Key words: Neferine, Pharmacokinetics, Metabolism, CYP2D6

P170039

Pharmacokinetics of low-dose Bisoprolol/Hydrochlorothiazide Tablets in Healthy Chinese Volunteers

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To study the pharmacokinetics of low-dose of bisoprolol/hydrochlorothiazide tablets in healthy Chinese volunteers. This study had a randomized, open, three-dosage design. 48 volunteers, 24 males and 24 females, administered 2.5, 5 and 10 mg bisoprolol, combining with 6.25 mg hydrochlorothiazide, respectively. The plasma concentrations of bisoprolol and hydrochlorothiazide were measured until 48 h post-dose by HPLC. The urine concentrations of bisoprolol and hydrochlorothiazide were also measured. Noncompartmental pharmacokinetic parameters were derived. No statistically significant racial differences in the pharmacokinetic parameters were observed. Bisoprolol was well absorbed (t_{max} 2.4 h). C_{max} was 14.4, 30.0 and 65.8 ng/ml, and AUC0-48 was 200, 414 and 915 ng/ml*h, respectively. Bisoprolol's pharmacokinetics process was linear and dose proportional in both groups. On average, 36% of the bisoprolol dose and 41% of the hydrochlorothiazide dose were recovered in urine as parent compound. The pharmacokinetics of bisoprolol and hydrochlorothiazide are essentially identical between Chinese and Caucasian volunteers.

Key words: bisoprolol; hydrochlorothiazide; HPLC; pharmacokinetics

P170040

Glucuronidation of active components of a Gegen-Danshen herbal product: pure compound in comparison to mixture

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Objective: Gegen and Danshen are widely used herbs with multiple active components. The present study is to determine if glucuronidation reaction of these components, is different with pure compound when compared to mixture (combination). Method: A specific HPLC assay was developed for the active components. Glucuronidation reaction of these components was carried out by incubation either as single individual compound or mixture with pooled Human Liver Microsome (HLM). Results: Of the 10 components, only Daidzein was found to be metabolized to form glucuronidated conjugate when incubated alone with HLM. No glucuronidation of Daidzein was observed with the mixture of 10 components incubated together. Subsequently, inhibition of Daidzein glucuronidation by Saiviandic acid B was observed when they were coincubated together. Conclusion: Glucuronidation reaction of certain component present in the Gegen-Danshen product is different when incubated as individual pure compound vs that as a mixture and the observed difference was attributable to the presence of Salvianolic acid B.

Key words: Glucuronidation; Gegen-Danshen; Herbal medicine

Acknowledgement: AoE grant (AoE/B-10/01) supported by UGC, HKSAR

P170041

CHIMERIC UDP-GLUCURONOSYLTRANSFERASE (UGT) 2B7 AND 2B15 PROTEINS DEFINE DOMAINS ASSOCIATED WITH SUBSTRATE SELECTIVITY AND AUTOACTIVATION.

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Despite their role in the metabolism of drugs and endogenous compounds, the structural features of UGTs responsible for substrate binding and selectivities remain poorly understood. Since UGT2B7 and UGT2B15 exhibit distinct, but overlapping substrate selectivities, UGT2B7-UGT2B15 chimeras were constructed to identify the domains involved in substrate binding. A UGT2B7-15-7 chimera incorporating amino acids 61-194 of UGT2B15 glucuronidated the UGT2B15 substrates testosterone and phenolphthalein, but not the UGT2B7 substrates zidovudine and 11α-hydroxyprogesterone. Glucuronidation of 4-methylumbelliferone (4MU) by UGT2B7-15(61-194)-7 and UGT2B15 followed Michaelis-Menten and weak substrate inhibition kinetics, respectively. Sigmoidal kinetics, characteristic of autoactivation, were observed for the UGT2B7 catalysed reaction. Like UGT2B7, the UGT2B7-15(61-157)-7, UGT2B7-15(91-157)-7 and UGT2B7-15(61-91)-7 chimeric proteins exhibited sigmoidal 4MU glucuronidation kinetics. It is concluded that residues 60-194 are responsible for substrate binding and selectivity of UGT2B15, while residues 158-194 of UGT2B7 facilitate the binding of multiple 4MU molecules within the active site.

P170042

Bioequivalence of Cefditoren in human and pharmacokinetics of absorption in rat

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Objective: We investigated bioequivalence of cefditoren (CDTR) between cefditoren pivoxil (CDTR-PI) tablet (R) and CDTR-PI granule (T) in human and examined the change from CDTR-PI to CDTR in rat intestine. Methods: HPLC method was developed for plasma concentration of CDTR and CDTR-PI. A randomized crossover design was performed in 24 healthy male volunteers. The intestinal absorption of CDTR-PI and CDTR was examined in isolated rat intestine by HPLC. Results: C_{max} of T and R were 1.922 ±0.529 μg ml^{-1} and 1.950 ±0.582 μg ml^{-1}; AUC0→8h of T and R were 6.337 ±2.083 μg $ml^{-1}h^{-1}$, 6.012 ±1.957 μg $ml^{-1}h^{-1}$ for CDTR, respectively. The rapidly intestinal hydrolysis from CDTR-PI to CDTR obviously decreased by Orlistat, an inhibitor of esterase. Conclusion: The pharmacokinetic parameters showed bioequivalence between T and R in human. Orlistat inhibited the hydrolysis of CDTR-PI to CDTR in rat intestine. As CDTR is similar to Cefalexin in chemical structure, a challenge is doing to understand whether CDTR is absorbed by PepT1 in rat intestine.

Key words: Cefditoren; HPLC; PepT1

P170043

New mathematical methods in pharmacokinetic modeling of verapamil first-pass metabolism and bioavailability

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The methods based on linear dynamic system, artificial-neural-network, fuzzy-logic, fractal, liver blood flow rate-limited model and spline-convolution integrals were used. Sets of data were generated by introducing simulated random errors corresponding to a coefficient of variation of 1%, 5% and 10%, in the hypothetical sampled values of verapamil concentrations. Noise level of x% added to the function value Y is random number drown from a normal distribution with mean zero and SD = xY/100. Response errors of all tested methods were of the same order of magnitude as the noise level added to the data. The linear system, artificial-neural-network, fuzzy-logic and fractal approaches are based on numerical solutions of differential equations by iterative procedures that can not be completed without the use of computers. The liver blood flow rate-limited and spline-convolutional methods are based on exact mathematical solutions of algebraic equations. These methods are extremely useful in providing reasonable estimates of the first-pass metabolism of verapamil.

Key words: modeling, simulation, verapamil, bioavailability

Acknowledgement: Thanks to Mrs. Vesna Popovi for computer assistance.

P170044

The pharmacokinetics of levofloxacin and absolute bioavailability study of levofloxacin tablet in Chinese volunteers

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Objectives: To evaluate PK of levofloxacin via i.v and the absolute bioavailability of tablet in Chinese volunteers. Methods: ①20 subjects were administered single dose of 500 mg by i.v in 60 min and 90 min and 10 subjects accepted 500 mg once a day for 7 days; ②12 subjects were administered 500 mg tablet. Plasma and urine drug concentrations were determined by HPLC. Results: ① The mean C_{max} for 60 min and 90 min IV infusion were 7.44 mg/L and 6.75 mg/L; $T_{1/2}$ 6.4h and 6.62h; AUC 36.4h* mg/L and 37.4h* mg/L; ②Following the multiple IV dose, the mean $T_{1/2\beta}$ of day1 was similar to that of day 7. The mean UR_{24h} after first and last doses were 78.87% and 65.87% respectively. The differences of Log(C_{max})、Log($AUC_{0\sim\infty}$) and mean UR_{24h} between day1 and day7 were significant ($P<0.05$), while $T_{1/2\alpha}$ and $T_{1/2\beta}$ were not($P>0.05$). The accumulated factor(R) was 1.09; ③after oral dose, the mean C_{max} were 6.2 mg/L, T_{max} 0.9h,, $T_{1/2\beta}$6.6h and AUC 41.9h* mg/L. The absolute bioavailability was 108%. Conclusions: ①The PK parameters of two periods (60 min and 90 min) were similar; ②it is slightly accumulated following the 500 mg multiple dosing. ③the tablet was absorbed completely.

Key words: Levofloxacin, HPLC, pharmacokinetics

P170045

Experimental Study of Midazolam as a Probe for Evaluating Activity of Inhibited Hepatic CYP3A

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The present study was to establish a practical marker for evaluating midazolam (MDZ) as a probe for in vivo and in vitro metabolic activity of hepatic CYP3A in rats. For in vivo study, loading doses injection of ketoconazole (KTZ) followed by constant infusion were performed to achieve continuous inhibition on CYP3A with steadystate KTZ plasma concentrations. MDZ was injected 2 hours after starting the KTZ infusion. For in vitro study, MDZ was administrated to the hepatocyte suspensions with different doses of KTZ to attain a final MDZ concentration of 1.5 μg/ml. Blood, liver tissue and hepatocyte suspensions were sampled at the different time points for MDZ detection by an HPLC assay. The pharmacokinetic parameters of MDZ exhibited similar tendency for both in vivo and in vitro studies. CL(30,120), the clearance derived from MDZ plasma concentrations at 30 min and 120 min, proved perfectly correlated with CLs in vivo ($R=0.9126$, $p<0.01$) and in vitro ($R=0.9823$, $p<0.01$). Moreover, there were obviously negative correlation between CL(30,120) or CLs and KTZ concentrations. It indicated that CL(30,120) is a valid indicator for evaluating the drug-metabolizing function of hepatic CYP3A.

P170046

Determination of thiamin levels by HPLC in plasma of the patients undergoing hemodialysis

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A HPLC method for determination of thiamin (TA) level in human plasma based on pre-column oxidation of TA to thiochrome followed by fluorescence detection has been developed. The plasma of 9 Iranian patients on hemodialysis were analyzed and compared to healthy Iranian subjects. TA was extracted from Plasma by diethyl ether. Following oxidation with cyanogen bromide, were applied to a C8 column. The mobile phase was methand: 30 mM phosphate buffer (45:55) and 0.05% sodium lauryl sulfate. A precise and reproducible HPLC method was developed for determination of TA in plasma, the minimum detection limit was 0.2 ng/ml and the yield was 85%. The mean plasma TA level in Iranian healthy subjects was 3.07 ±0.95 ng/ml and in patients was 4.72 ±1.12 ng/ml and 4.29 ± 0.67 ng/ml before and after hemodialysis respectively. According to our findings the TA level in patients undergoing hemodialysis has no significant difference with healthy subjects and it seems that dietary TA is sufficient for the normal functions of the vitamin in the body, and taking TA supplementation is not necessary for these patients.

Key words: thiamin, hemodialysis, HPLC

We thank Razi Institute for drug research for financial support

P170047

Application of a Tumour Calculation Method to Biodistribution Studies with the Mouse B16F10 Lung Metastasis Model

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A new calculation method has been devised to estimate the mass of and drug deposit in the mouse B16F10 melanoma lung metastases and applied to biodistribution studies with 5 different kinds of radiolabelled compounds. The method was capable to dissociate drug contents in the tumour from the amount of tumour mass. Significantly high radioactivity accumulation in the tumour was observed with three tumour-specific radiotracers, ^{123}I-N-(2-diethylaminoethyl)-2-iodobenzamide (BZA2), ^{67}Ga (gallium citrate) and ^{18}F-fluorodeoxyglucose (FDG) (all Ps < 0.05, vs control lungs), whilst no significant difference was seen between the tumour and controls when a tumour non-specific tracer, ^{64}Cu (cupric chloride), was tested (all Ps > 0.05). Highly compatible data were achieved in repeated tests of an unknown compound, ^{67}Ga-silica nanoparticles (20 nm). These results substantiate the suitability of the calculation method used in biodistribution studies with the metastatic tumour model.

Key words: B16F10; Metastasis; Radioactive tracer; Biodistribution

Acknowledgement: The authors wish to thank Beverly Izard, Kerynne Belbin and Leigh Berwick for support in the experimental work.

P170048

The pharmacokinetics and plasma protein binding rate of osthol in normal rats

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Objective: To study the pharmacokinetics and plasma protein binding rate of osthol in the body of rats. Methods: Osthol of 30 mg·kg^{-1} was delivered to rats by intraperitoneal injection. At the appropriate time, animals were killed, plasma were collected and tissues were quickly removed. Plasma protein binding rate of osthol was determined with in vitro balance dialysis and HPLC-UV method. Results: After ip administration, the distribution of osthol in tissues and plasma balanced soonly with rapid distribution in livers, kidneys and spleens and with high drug contents and long mean residence time (MRT) in testicles and epididymises. At the dose of 2.0, 10.0, 20.0 μg·ml^{-1}, plasma protein binding rate of 48 hours and 72 hours were 68.23 ±1.25%, 69.31 ±1.53%, 53.03 ±1.93% and 81.53 ±4.31%, 70.50 ±4.68%, 77.21 ±1.37%. Conclusion: The pharmacokinetics of osthol consistented with one compartment open model. The distribution of osthol was general with a tendency to distribute in rich-blood-supplying and fattiness tissues; osthol could permeate blood-cerebral barrier. The plasma protein binding rate of osthol was about 76%.

Key words: osthol; pharmacokinetics; HPLC; plasma protein binding rate

P170049

Intestinal permeability of metformin using single-pass intestinal perfusion in rats

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Objective: To characterize the intestinal transport and mechanism of metformin in rats. Methods: The effective intestinal permeability(Peff) of metformin was investigated using single-pass intestinal perfusion (SPIP) technique in male, Waster rats. SPIP was performed in three isolated intestinal segments at same concentration and in a same isolated intestinal segment at three different concentrations. Besides, P-glycoprotein (P-gp) inhibitor verapamil was co-perfused with metformin in the duodenum segment. Results: Peff values of metformin in the jejunum and ileum were significantly lower than that in the duodenum at the same concentration. Besides, Peff values in the duodenum at high concentration were significantly lower than those at low and medium concentrations. Moreover the co-perfusion with verapamil did not increase the Peff value in the duodenum. Conclusion: Metformin could be absorbed from the whole intestine, with the main absorption site at duodenum, and was transported by both passive and ac-

tive, carrier-mediated, saturable mechanism. Metformin is neither a substrate nor inducer of P-gp.

P170050

An evaluation of the pharmacokinetics of single and multiple doses of gemifloxacin in Chinese healthy subjects

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Objectives: To investigate the pharmacokinetics of gemifloxacin in healthy Chinese subjects. Methods: ①12 subjects were given oral doses of 160 mg, 320 mg and 480 mg respectively; ②20 subjects were given 320 mg or matching placebo oral dose once daily for 7 consecutive days. The serum and urine concentrations of gemifloxacin were assayed by HPLC. Results: ① Following single doses of 160 mg, 320 mg and 480 mg, the means of C_{max} were 0.70 mg/L, 1.40 mg/L and 1.84 mg/L, respectively, T_{max} were 1.25h, 1.13h and 1.38h. The mean $t_{1/2}\beta$ were 7.00h, 6.72h and 6.91h, respectively. The mean AUC0-∞ were 4.14 mg*h/L, 7.54 mg*h/L and 11.66 mg h/L, respectively. The mean UR48hrs were 38.95%, 37.84% and 35.57%, respectively; ②After multiple doses, mean C_{max} were 1.55 mg/L on day 1 and 1.57 mg/L on day 7, T_{max} were 0.90h and 1.11h, respectively. The mean $t_{1/2}\beta$ were 6.14h and 7.78h, respectively. The mean UR48hrs were 37.19% and 41.65%, respectively. The mean accumulated factor was 1.13. Conclusions: ①The concentrations and AUCs had a linear relationship with dose. ②The multiple administrations caused a mild accumulation. ③About 40% of Gemifloxacin was excreted from kidney.

Key words: gemifloxacin, HPLC, pharmacokinetics

P170051

A New Sublingual Formulation of Propranolol for Rapid Absorption

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Objective: To demostrate that a specially formulated buffered propranolol (BP) could lead to rapid absorption and onset of action. Methods: Using special buffering technology, a 40 mg tablet of BP was formulated and administered sublingually to 8 healthy male subjects in a cross over manner with a conventionally formulated product, Inderal (I). Multiple propranolol plasma concentrations (PPC) were obtained post dose. Results: The mean PPC at 6 to 30 min after BP, but not at subsequent times, were significantly higher than that of I ($p < 0.05$). The mean time to reach a given therapeutic concentration was 8.5 min for BP as compared to 38.8 min for I ($p < 0.01$). Conclusion: The specially formulated sublingual BP yielded faster and higher initial PPC than the conventional tablet and may offer a new therapeutic modality for acute use in the future.

Key words: Sublingual; Propranolol; Pharmacokinetics

Acknowledgments: Supported by Direct Grant (No. CUHK 2041010) from The Chinese University of Hong Kong

P170052

Simultaneous determination of cefoperazone and tazobactam in human plasma and urine using liquid chromatography tandam mass spectrometry (LC-MS/MS)

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Objective: To establish a LC-MS/MS method to simultaneously determine cefoperazone (CPZ) and tazobactam (TZB) in plasma and urine. Methods: LC/MS conditions: Waters Atlantis dC18 (150 mmx2.1 mm, 5 μm) column was used with a mobile phase of 60:40 (v/v) ammonium formate-methonal solution. Negative ESI and SRM mode were employed. The characteristic fragments were m/z 528.0, 138.0 and 362.0 for CPZ (m/z 644.1), TZB (m/z 299.1) and I.S. cefuroxime (m/z 423.0). Plasma samples were pretreated with acetonitrile (1:3), dried with N2 and reconstructed with mobile phase. Urinary samples were diluted with buffer and analyzed directly following centrifugation. Results: The linearity for CPZ and TZB were in the range of 0.02-20 μg/ml and 0.01-10 μg/ml both in plasma and urine ($r^2 > 0.999$). The recovery of CPZ was 96.4% for plasma and 102.3% for urine and that of TZB was 91.7% for plasma and 99.6% for urine. The detection limit of CPZ and TZB at 10:1 (S/N) were 2.5 ng/ml and 0.2 ng/ml. Conclusions: The LC-MS/MS method established is a simple, accurate method and could be used for clinical pharmacokinetic study of cefoperazone-tazobactam.

Key words: cefoperazone, tazobactam, LC-MS/MS method

P170053

Establishment of a rapid assay of serum norvancomycin concentration

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Objective: To establish a rapid serum norvancomycin concentration assay for TDM. Methods: To assay norvancomycin serum concentrations of 239 samples from 10 young and elderly volunteers following an 800 mg I.V infusion of norvancomycin by FPIA and bioassay simultaneously. And the results were compared with that by HPLC method. Results: A linear regression equation of two assays was Y = 0.7534X - 0.5948, (X: the value from the FPIA method, Y: the value from the bioassay method, $R^2 = 0.9703$). Intra-day and inter-day precision (RSD) of the FPLA method for norvancomycin was ≤6.08% and ≤4.75, respectively. The range of the recovery was 87.74% to 114.34%. The serum drug concentration assayed by the FPIA method, which was modified by regression equation, was very similar to that by the HPLC method, Y = 1.016X + 0.0041 (X: the value from the FPIA method modified by regression equation, Y: the value from the HPLC, $R^2 = 0.9782$). Conclusions: FPIA method modified by regression equation was the rapid assay to determine norvancomycin serum concentration and could be used for TDM of norvancomycin in clinic.

Key word: norvancomycin; TDM; FPIA; HPLC

P170054

Effects of co-administering probenecid orally on pharmacokinetics of cefador

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Aim: To investigate the effects and quantitative relations of co-administering probenecid with different dosages on pharmacokinetics of cefaclor and approach the possible mechanisms involved as well. Methods: Monitoring plasma and urine cefaclor concentration. Cefaclor (50 mg·kg^{-1}) was co-administered with different dosages of probenecid (0, 100, 250, 625 mg·kg^{-1}). Blood and urine samples were collected according to the regular time schedule after intragastric administration. Results: Within the dosages of probenecid ranged from 0～250 mg·kg^{-1}, $T_{1/2ka}$, T_{max}, C_{max} and AUC of cefaclor increased in accordance with increasing dosage of co-administering probenecid while CL/F and V_d/F were decreased ($P < 0.01$); However, when the dosage of co-administering probenecid was 625 mg·kg^{-1}, C_{max} of cefaclor strikingly decreased ($P < 0.01$). Biological half life prolonged and urinary excretive accumulation percentage decreased obviously ($P < 0.01$). Conclusion: Co-administering probenecid can strikingly change pharmacokinetics of cefaclor and the influential degree of pharmacokinetics parameters is dependent on dosages of probenecid used in the experiment. Biological half life prolongs and urinary excretive accumulation percentage of cefaclor decreases obviously.

Key words: probenecid; cefaclor; pharmacokinetics; absorption

P170055

Suppression of CYP3A4 gene expression and function by RNAi interference in transgenic Chinese hamster cells lines expressing human liver CYP3A4

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Objective: To investigate the inhibitory effect of the CYP3A4 gene expression and function in transgenic Chinese hamster cells lines expressing human liver CYP3A4 (CHL3A4) by vector-expressed small hairpin interfering RNA (shRNA). Methods The shRNA expression vectors targeting CYP3A4 gene (CYP3A4 I、CYP3A4 II、CYP3A4 III) were designed and constructed. The cells were transfected with shRNA expression vector transiently, and the cells without shRNA transfection and with nonspecific shRNA transfection were used as controls. The inhibitory effect of shRNA expression vectors was detected by Western blot analysis. The activity of nifedipine oxydase in CHL3A4 S9 mix was measured by HPLC assay. Results CYP3A4III shRNA expressing vector significantly reduced the protein expression levels (75%) of the CYP3A4 gene by Western blot analysis. CYP3A4III shRNA expressing vector significantly inhibited the activity of nifedipine oxydase in S9 mix from CHL3A4 cells. Conclusions vector-based RNAi could suppress CYP3A4 expression and function in mammalian cells, and it

suggested that the use of RNAi was a promising new tool for the study of gene function.

Key words: RNA interference CYP3A4 Cyclophosphamide Nifedipine

P170056

Population pharmacokinetic analysis of norvancomyci

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Objectives: To investigate the population pharmacokinetics (PPK) of norvancomycin in different populations of patients and to provide a reliable approach to design a rational regimen in different groups of patients. Methods: NONMEM approach was chosen to establish a PPK model for patients given norvancomycin in this investigation. Results: ① Among 146 patients: the mean of the drug clearance (CL) and eliminate half-life ($T_{1/2}b$) were 0.23 L/h and 154.26 h in 14 patients with severe renal impairment, 2.17 L/h and 22.86 h in 16 patients with moderate renal impairment, and 4.01 L/h and 9.57 h in 45 patients with mild renal impairment, respectively. ② Comparison of 59 elderly patients with non-elderly patients showed 3.94 L/h versus 5.89 L/h for CL, 12.07h versus 6.79 h for $T_{1/2}b$, and 490.16 mg.h/L versus 283.92 mg.h/L for AUC24. ③ The increased volume of a peripheral distribution as co-administration of norvancomycin with diuretics. Conclusions: The PPK model of norvancomycin was effectively applied to design the regimen for patients with variable renal function..

Key word: norvancomycin; PPK; nonlinear mixed effect model; TDM

P170057

Study on transport of 5-aminosalicylate in Caco-2; L-MDR1 and MRP2 cell monolayers

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The purpose of this study was to investigate whether P-glycoprotein and MRP2 are involved in transport of 5-aminosalicylate(5-ASA). Permeability coefficients and transport rates of 5-ASA across Caco-2, L-MDR1 and MRP2 monolayers were measured. Transepithelial transport of digoxin across Caco-2 monolayers with addition of 5-ASA was also studied. The results showed that no differences of permeability coefficients and transport rates of 5-ASA at 5, 50 and 500uM between basal-to-apical and apical-to-basal direction were measurable across Caco-2, L-MDR1 and MRP2 monolayers ($P > 0.05$). Compared with control experiments, no significant differences were observed in basal-to-apical net transport and Papp of digoxin (5 uM) in the presence of 5-ASA (50uM-5mM) ($P > 0.05$). In conclusion, 5-ASA can not be regarded as a substrate of P-gp or MRP2. Inhibition or induction of P-glycoprotein by 5-ASA could be excluded. Further studies are needed to identify the nature of the involved active carrier system(s) in intestinal secretion of 5-ASA.

Key words: 5-aminosalicylate; intestinal transport; P-glycoprotein; cell lines

P170058

No Drug-drug Interaction Between Ketorolac and Ofloxacin Following Ocular Dosing of A Ketorolac/Ofloxacin Combination Solution to Healthy Subjects

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Study Objectives: To compare the pharmacokinetics (PK) of 1) ketorolac after ocular dosing of the ketorolac tromethamine 0.5% (Keto)/ofloxacin 0.3% (Oflox) combo with Keto alone; 2) ofloxacin after ocular dosing of Keto/Oflox combo with Oflox alone. Methods: 36 subjects (12 in each group) received either the Keto/Oflox combo, ACULAR, or OCUFLOX eye drops. Eye drops were applied to the right eye only every 30 minutes for 12 hours a day on the first two days and hourly for 12 hours a day on the next three days. Serial blood samples were collected on day 0 and day 4 after the last daily instillation. Serial tear samples were collected throughout the study period to evaluate the kinetic profiles of drugs in the tears. Plasma drug PK parameters included C_{max} and AUC on days 0 and 4, and $T_{1/2}$ on day 4. Results: Plasma and tear ketorolac PK profiles were similar between the Keto Alone and the Keto/Oflox Combo dosing groups. Plasma and tear ofloxacin PK profiles were also similar between the Oflox Alone and the Keto/Oflox Combo dosing groups. Conclusions: There is no drug-drug interaction between ketorolac and ofloxacin in the eye and in the systemic circulation after ocular dosing.

P170059

Xanthate (dithiocarbonate) metabolism by some monooxygenases

Yanev Stanislav*. Xanthates (salts of alkyl or aryl derivatives of dithiocarbonic acid, ROCS2K) upon pyrolytic reaction at 300 °C decompose to olefins. Our studies have shown that this pyrolytic cleavage of the xanthate molecule can be reproduced at 37 °C by biological enzymatic or nonenzymatic systems that generate active oxygen species such as hydroxyl radicals (Fe/EDTA/H_2O_2, xanthine-xanthine oxidase, hemoglobin, activated macrophages and possibly cytochrome P450 (CYP)). The primary change in the xanthate molecule after CYP attack is a one or two hydrogen abstraction from the first carbon atom of the alkyl chain. The resulting intermediate(s) is irreversibly bound to the enzyme protein. This metabolic transformation is supported only by CYP2B1/2B6 and CYP2E1. In this way the xanthates behave as potent and selective mechanism-based inactivators of some CYP enzymes. In comparison with CYP, xanthates are oxidized by some FMO's on the sulfur to the corresponding perxanthates. The same sulfur oxidation occurs in a purely chemical system containing hydrogen peroxide. The readiness of xanthates molecule to interact with different reactive oxygen species can explain their potent antioxidant and scavenger activity.

P170060

Disposition and sterol-lowering of ezetimibe in Mrp2-deficient rats with reference to intestinal and hepatic expression of Mdr1 and Ugt1a1

Oswald Stefan[1*], Westrup Sabine[2], Sieg mund Werner[3]. 1. SO. 2. SW. 3. WS. Disposition of ezetimibe (EZ) and its glucuronide (GLUC) is influenced by intestinal efflux because GLUC has high affinity for MRP2 and EZ binds to P-glycoprotein (Pgp) and MRP2.

To assess the overall meaning of Mrp2 for EZ, male wild-type and Mrp2-deficient (GY/TR-) Lewis rats (each N = 8) were administered EZ (5 mg/kg) and a sterol enriched diet for 14 days. EZ, GLUC and the plant sterols campesterol and sitosterol were quantified in serum, organs, feces and urine, respectively, relative to mRNA expression of Mdr1, Mrp2 and Ugt1a1 (TaqMan). In Mrp2-deficient rats, serum levels and fecal excretion of EZ were decreased (1.4 ±0.4 vs. 3.1 ±1.1 ng/ml; 115 ±48 vs. 361 ±102 ug/d, both $p < 0.01$). Serum levels and renal excretion of GLUC were increased (196 ±76 vs. 23 ±25 ng/ml; 7.8 ±3.1 vs. 0.4 ±0.4 ug/d, both $p < 0.01$) and intestinal clearance was decreased (0.3 ±0.3 vs. 15 ±17 ml/min; $p < 0.05$). The sterol lowering effect of EZ was reduced in correlation to GLUC levels (eg. campesterol r = -0.768). Hepatic Pgp and Ugt1a1 were significantly higher expressed. EZ in Mrp2-deficient rats is less active as caused by reduced intestinal secretion of GLUC and lower bioavailability of the parent EZ.

P170061

The New View on Mechanism of Enzymatic Hydrolysis of Dicarboxylic Acids Dicholine Esters by Human Butyrylcholinesterase

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Dicholine esters of dicarboxylic acids (DCh) are bioactive compounds and have a neuromuscular blocking action. Succinylcholine (ditiline) is the best known and widely used in anesthesiology. It has muscle relaxant action due to its hydrolysis by plasma butyrylcholinesterase (BuChE, EC 3.1.1.8). According to the classical view of kinetics the DChs' enzymatic hydrolysis could be divided in two stages. During the first stage the enzyme splits only one ester bond forming monocholine ester and choline. Monocholine is converted into dicarboxylic acid and choline in the second stage. The present research studies the mechanism of enzymatic hydrolysis of DChs with long hydrocarbon chain by human BuChE, which wasn't described before. The investigations were realized by using titration method by pH-stat. The enzymatic hydrolysis of DChs with long and short hydrocarbon chains were carried out and compared. The obtained results show that beside short chain DChs, dicarboxilyc acid was formed during the first stage of hydrolysis of long chain DChs. To explain the observed anomalous hydrolysis we suggest a new mechanism of kinetic.

Key words: dicholine esters, butyrylcholinesterase, anomalous hydrolysis

P170062

A novel liquid chromatography-tandem mass spectrometry based high throughput screening method to semi quantitatively determine reactive metabolite levels in-vitro

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A high sample throughput, semi-quantitative reactive metabolite (RM) screening approach is presented that combines a previously described high throughput RM detection method with a method that incorporates the use of novel quaternary ammonium glutathione analogs (QA-GSH) to semi-quantitatively determine RM levels. The first stage of the screening paradigm uses a liquid chromatography-multiple MRM tandem mass spectrometry technique to screen drug compounds for RM formation. The in-vitro biological assay consists of substrate, human liver microsome, an NADPH generating system and the analog of glutathione, glutathione ethyl ester (GSH-Oet). This first stage enables high throughput, low detection limit screening of drug compounds to detect RM that form stable conjugates with GSH-Oet. The second stage of the paradigm utilizes a novel QA-GSH internal standard method to semi-quantitatively determine the levels of RM formed during high throughput screening. The screening paradigm presented could be amenable for use during early discovery, does not rely on the use of radio-labeled material and could provide additional data necessary to guide RM go/no go decision-making.

P170063

Single dose pharmacokinetic study of thalidomide in patients with multiple myeloma.

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Thalidomide has recently been approved for use in patients of multiple myeloma (MM) in India, however the pharmacokinetic data in such patients is lacking. Therefore, a single dose pharmacokinetic study of thalidomide in MM patients was carried out. Nine MM patients satisfying the inclusion criteria were enrolled. After 10h fasting, thalidomide 200mg was administered and blood samples (0.5ml) were withdrawn at 0, 0.5, 1, 1.5, 3, 4, 6, 8, 12, 24 and 30h in tubes containing citrate phosphate dextrose adenine solution. Twenty-four hours urine samples were collected in a container with 5 ml HCl, and thoroughly mixed. The thalidomide concentrations in plasma and urine were determined by a reverse phase HPLC assay developed by us. Based on single compartmental model, the pharmacokinetic parameters are C_{max} 879.7 ±124 ng/ml, T_{max} 4.8 ±0.4h, kel 0.13 ±0.04/h and $t_{1/2}$ of 7.4 ±1.07h. The V_d and CL are 202.1 ±37 l and 25.4 l/h respectively, while 24h urinary excretion was 2.57 ±1.19 mg. The high values of V_d and CL in our study can be attributed to a significant tissue distribution of thalidomide in MM patients.

Key words: Thalidomide, Multiple myeloma, Pharmacokinetics.

Acknowledgement: Financial assistance by AIIMS is acknowledged.

P170064

CHARACTERISATION OF INTERACTIONS BETWEEN UDP-GLUCURONOSYLTRANSFERASE 2B7 (UGT2B7) SUBSTRATES USING MULTISITE KINETIC MODELLING

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Interactions between zidovudine (AZT), 4-methylumbelliferone (4MU) and 1-naphthol (1NP) glucuronidation by UGT2B7 were investigated using multisite kinetic models. AZT inhibited 4MU (Ki 176 uM) and 1NP (Ki 379 uM) glucuronidation by increasing S50 values with no significant change of V_{max} and sigmoidicity, suggesting that AZT inhibits at a distinct effector site. As demonstrated by increasing Km values, both 4MU and 1NP inhibited AZT glucuronidation with respective Ki values of 369 and 145 uM, and converted AZT glucuronidation from Michaelis-Menten to sigmoidal kinetics at high concentrations. 4MU activated 1NP glucuronidation (Ka 432 uM) by decreasing S50 values and sigmoidicity without changing V_{max}, suggesting that 4MU acts at a distinct effector site and mimics the cooperative effect of 1NP. In contrast, 1NP inhibited 4MU glucuronidation by decreasing V_{max} without significantly changed S50 and sigmoidicity, indicating 1NP may inhibit via a separator site (Ki 80 uM). Multisite kinetic modelling provides evidence of multiple substrate binding sites for UGT2B7 that may be regulated by distinct effector sites.

Key words: drug metabolism, UDP-glucuronosyltransferase, enzyme kinetics

P170065

Multiple dose pharmacokinetics of risperidone and 9-hydroxyrisperidone in Chinese female patients with schizophrenia

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Objective: To study the multiple dose clinical pharmacokinetics of risperidone and its main active metabolite, 9-hydroxyrisperidone, in Chinese female patients with schizophrenia. Methods: 23 Chinese female inpatients aged 18-65 years with schizophrenia completed the test. Plasma concentrations of risperidone and 9-hydroxy-risperidone were assayed by validated high performance liquid chromatography-mass spectrometry (HPLC-MS) methods. Results: Risperidone was rapidly absorbed (T_{max} was 1.6 hours) and the $T_{1/2}$ in plasma was short (3.2 h). 9-OH-RIS was quickly metabolized from parent drug with a mean T_{max} of 2.5 h and it had a long half-life of 24.7 h. The C^{ss}_{0-12} of risperidone and 9-hydroxy-risperidone were 36.9 ±33.1 and 110.6 ±30.5 μg·L^{-1}, respectively, and the AUC^{ss}_{0-12} were 443.2 ±397.4 and 1327.2 ±402.3 μg·h·L^{-1}, respectively. CL/F and V/F of risperidone were 8.7 ±6.2 L·h^{-1} and 34.1 ±24.3 L, respectively. Interindividual variations for pharmacokinetic parameters were quite large for risperidone. CONCLUSIONS: Systemic parameter exposure to risperidone and 9-hydroxy-risperidone in female Chinese schizophrenic patients is higher relative to published data in caucasion white patients. Larger studies of PK/PD relationship may be required to develop a reasonable clinical dosage regimen for Chinese female patients.

Key words: Risperidone, Metabolite, Pharmacokinetics.

P170066

Identification of an active metabolite of astilbin in rats

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Astilbin was a flavanone isolated from the rhizome of Smilax glabra, a Liliaceae plant. Our previous studies have revealed a unique immunosuppression of astilbin that is different from the immunosuppressive agents so far, selectively inhibiting the activated T lymphocytes. This character is quite of significance for the development of novel immunosuppressor. Herein, we describe the identification of 3'-O-methylastilbin in the blood and urine of rat after oral administration of astilbin. After in vitro incubation of astilbin with rat liver cytosol, a new metabolite of astilbin was isolated and characterized by MS and NMR techniques as 3'-O-methylated astilbin. Also this metabolite exists in the blood and urine of rat after oral administration of astilbin. To our knowledge this is the first time that 3'-O-methylastilbin has been identified as a metabolite of astilbin in rats. Furthermore, this new metabolite could inhibit the pro-inflammatory cytokines TNF-α and IFN-γ expression in vitro as astilbin did.

Key words: astilbin, active metabolite

Acknowledgement: Supported by National Natural Science Foundation of China (No. 30472174 and 20572043).

P170067

Rifampin significantly increased the clearance of risperidone

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Objective: to examine effect of rifampin on the pharmacokinetics of a single oral dose of risperidone in healthy volunteers. Material and Methods: The pharmacokinetic parameters of risperidone were determined in 10 healthy male volunteers using an open, randomised two-phases crossover design. In phase 1, each subject ingested a single dose of 4 mg of risperidone alone and in phase 2, each subject ingested the same dose of risperidone after pretreatment with 600 mg of rifampin given orally once daily for 5 days. Plasma concentrations of risperidone were determined by the HPLC method. Results: Rifampin significantly increased the clearance (Cl) of risperidone by 81% (i.e. 0.05 ±0.05 vs 0.27 ±0.60 l/kg/hr; $P < 0.05$) and the C_{max} and AUC_{0-last} were significantly decreased by 50% (32.44 ±19.16 vs 16.16 ±8.64 ng/ml; $P < 0.05$) and 72% (153.89 ±157.76 vs 42.66 ±24.72 ng/l/hr; $P < 0.01$), respectively. Conclusion: The alteration in the risperidone pharmacokinetic parameters should be the result of induction of CYP450, mainly CYP2D6 isozyme, by rifampin.

Key words: Risperidone; Rifampin; Pharmacokinetics

Acknowledgement: This study was supported by the Graduate School, Prince of

Songkla University, Thailand.

P170068

Stereospecific Disposition and Anti-Cancer/Anti-Oxidant Activity of the Chiral Flavonoids Eriocitrin and Eriodictyol

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The chiral flavanone glycoside eriocitrin is cleaved to the aglycone eriodictyol, mainly found in lemons. To develop a method to quantify eriodictyol, evaluate stereospecific disposition, anti-oxidant and anti-cancer activity. A high-performance liquid chromatographic method was developed to determine eriodictyol enantiomers on a Chiralpak OJ-RH column with UV detection. Eriodictyol (10 mg/kg) was intravenously administered to rats. Healthy volunteers drank lemonade (1,000 ml). Racemic eriodictyol was incubated with cancer cells and anti-oxidant activity examined. In both species, eriodctyol enantiomers were detected in urine primarily as R-glucuroconjugates. In lemons, R-eriocitrin predominates. Racemic eriodictyol in HCT-116 (colon) had an IC50 ~30 ug/ml. Anti-oxidant activity was greater for the aglycone. Eriodictyol has a rapid halflife in serum (7 hours) and excreted predominantly via non-renal routes. Racemic eriodictyol demonstrated a concentration-dependent anti-cancer and anti-oxidant activity. Eriodictyol is bioavailable, rapidly eliminated from the body with predominant non-renal excretion. Key Words: chiral, flavonoid, anti-oxidant, anti-cancer. Funded by the Organic Center for Education.

P170069

Anti-Cancer/Anti-Oxidant Activity and Pharmacokinetics of Pterostilbene

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To develop a high performance liquid chromatographic (HPLC) method to quantify trans-pterostilbene, evaluate anti-oxidant and anti-cancer activity, and examine pharmacokinetics in rats. HPLC separation was attained on a C18 column with fluorescence detection. The mobile phase used was acetonitrile/water 50/50 (v/v) with a flow rate of 1.0 ml/min and pinosylvin as an internal standard. Anti-oxidant and anti-cancer cell viability was examined in MDA-MB-231 (breast), and HCT-116 (colon). In rat liver microsomes, phase I and II metabolism was examined in vitro and a major glucuronidated metabolite evident. Male Sprague Dawley rats were dosed intravenously with pterostilbene (20 mg/kg) and a glucuronidated pterostilbene metabolite with halflife of ~8 hours was excreted in urine. Pterostilbene demonstrated a concentration-dependent anti-oxidant activity and anti cancer activity in all cell lines with an IC50 ~10 ug/mL in HCT-116 cells. Pterostilbene was detected in blueberries. The HPLC assay is sensitive, phase II metabolism predominates with a glucuronidated metabolite excreted in urine. Key Words: chiral, flavonoid, anti-oxidant, anti-cancer. Funded by the Organic Center for Education.

P170070

Pharmacokinetics of p53 fusion protein

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Aim: To investigate the pharmacokinetics of p53 fusion protein(PDT-p53), which is an Escherichia coli expressed p53 fused with HIV-tag. Methods: 125I-PDT-p53 was intravenously (iv) and intraperitoneally(ip) injected in rhesus monkeys or rats. Its concentration in serum samples was determined by trichloroacetic acid precipitation and SDS-polyacrylamide gel electrophoresis methods. The serum drug concentration-time data were analyzed by pharmacokinetic program DAS2.0. Results: The concentration-time curves of 125I-PDT-p53 were best fitted to a two-compartment open model. Following iv administration at a dose of 10, 20 and 40ug/kg in rhesus monkeys or rats, AUC0-24h linearly increased with dose, while Clearance rates, The terminal halflives ($T_{1/2}\beta$) and apparent volumes of distribution exhibited no significant difference among different dose groups. After ip administration at a dose of 40 mg/kg in rhesus monkeys or rats, Bioavailability were 96.47 ±9.54 and 95.83 ±8.91%, respectively. Conclusions: The pharmacokinetic behavior of PDT-p53 complies with linear kinetics within the examined dose range, $T_{1/2}\beta$ is approximately 10h in experimental subjects.

Key words: p53 fusion protein; pharmacokinetics.

P170071

Monitoring of Cyclosporine in Paediatric Renal Transplant Recipients

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Objective: There are few data on pharmacokinetics (PK) of cyclosporine (CsA) in children. The aim of the study was to determine mean exposure indexes (EI) for CsA in a paediatric renal transplant population. Methods: CsA PK monitoring, based on C0, C2 or AUC0-12h was performed in 98 renal transplant children, aged 9.7 ±4.5 year-old. CsA was associated with either azathioprine (AZA) or mycophenolate mofetil (MMF). 257 AUC0-12h were estimated using Kinetica. EI were compared between 4 post-graft periods and associated immunosuppressant groups. Results: All mean EI (C_0, C_2 and AUC_{0-12h}) significantly decreased along time ($p < 0.005$). AUC_{0-12h} significantly differed in patients on AZA from patients on MMF, during the first month (7.7 ±2.2 mg.h/L vs. 5.7 ±2.0 mg.h/L, $p < 0.001$), and between the third month and one year post-graft (4.9 ±1.5 mg.h/L vs. 4.3 ±1.2 mg.h/L, $p = 0.001$). Rejection occurred in 1 patient within one month, and 33 within one year. Conclusion: Mean EI in paediatric renal transplant population decreased along time and were lower when CsA was associated with MMF. Further studies are required to validate optimized EI.

Key words: CsA, PK, paediatric renal transplant population

P170072

A comparative pharmacokinetic study in healthy volunteers on the effect of carbamazepine and oxcarbazepine on CYP3A4

Andreasen Astrid-Helene, Brosen Kim*, Damkier Per. IST Clinical Pharmacology University of Southern Denmark Carbamazepine and oxcarbazepine are well-known inducers of drug metabolism via CYP3A4. Thus we performed a study in healthy volunteers to investigate the relative inductive effect of carbamazepine and oxcarbazepine, respectively, with the metabolism of quinidine as a marker for the CYP3A4 activity. Methods: Ten healthy, male volunteers participated in an open, cross-over, parallel-group study consisting of two periods separated by a 4-week wash-out period. They were randomised into group A and B; group A referring to 1200 mg oral oxcarbazepine daily for 17 days and group B to 800 mg oral carbamazepine for 17 days and vice versa in the 2nd period. A 200 mg oral quinidine full kinetics of plasma and urine was performed on day 17 in each period Results: Formation clearance of 3-hydroxyquinidine was increased by 89% (CI: 1.36-2.64; p = 0.0022) and 181% (CI: 2.20-3.60, p < 0.0001) after treatment with oxcarbazepine and carbamazepine, respectively, compared to baseline. Conclusion: We confirm a clinically significant inductive effect of both oxcarbazepine and carbamazepine. The inductive effect of carbamazepine was about 50% higher than that of oxcarbazepine.

P170073

Pharmacokinetics of Rosuvastatin in Chinese healthy volunteers

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Objective: To investigate the pharmacokinetics of rosuvastatin in chinese healthy volunteers. Methods: the single and multiple dose plasma concentrations after taking 5, 10 and 20 mg were determined LC-MS. The pharmacokinetic parameters were calculated by BAPP software. Results: The volunteers were taking a single-dose rosuvastatin 5, 10 and 20 mg, respectively. The parameters C_{max} were 6.54 ±2.06, 10.61 ±3.35 and 22.85 ±7.32 ng/ml, respectively; AUC_{0-72} were 77.83 ±25.43, 136.12 ±48.63 and 275.98 ±81.98 ng·h/ml, respectively; $t_{1/2}$ were 23.26 ±5.54, 25.64 ±14.02 and 20.54 ±5.80 h, respectively. The volunteers were taking multiple-dose rosuvastatin 5, 10 and 20 mg. The parameters C_{max} were 6.49 ±1.74, 12.72 ±5.60 and 22.17 ±9.09 ng/ml, respectively; AUC_{0-72} were 89.51 ±20.45, 185.34 ±61.75 and 303.41 ±83.81 ng·h/ml, respectively; $t_{1/2}$ were 21.65 ±7.63, 20.90 ±7.93 and 16.77 ±3.80 h, respectively; C_{SS} were 1.24 ±0.28, 2.57 ±0.86 and 4.21 ±1.16 ng/ml, respectively. The pharmacokinetics parameters are directly proportion to doses and no singnificant difference. Conclusion: The pharmacokinetics of rosuvastatin in the dosage of 5~20 mg fit linear dynamic feature and no accumulation was taking multiple-dose rosuvastatin in human body.

Key words: rosuvastatin; LC-MS; pharmacokinetics

P170074

D- Dopa Is Unidirectionally Converted to L- Dopa by D- Amino- Acid Oxidase Followed by Dopa Transaminase

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To study postulated chiral inversion of D- dopa to L- dopa and related mechanism, a method for enantiomeric separation of D- and L- dopa using high performance liquid chromatography (HPLC) was established. The results showed that in rat kidney homogenates D- dopa was indeed converted to L- dopa while L- dopa was not converted to D- dopa. Furthermore, sodium benzoate, a selective inhibitor of D- amino- acid oxidase (DAAO), blocked L- dopa generation in a concentration- dependant manner. Contrary to the kidney homogenates of wildtype ddY/DAAO+ mice, those of the mutant ddY/DAAO- mice lacking DAAO activity did not convert D- dopa to L- dopa unless exogenous DAAO protein was added. On the other hand, carbidopa, an inhibitor of dopa transaminase, significantly inhibited L- dopa production. All these results demonstrate that chiral inversion of Ddopa is unidirectional and further suggest that D- dopa is firstly oxidatively deaminated by DAAO to its alphaketo acid and then transaminated by dopa transaminase to L- dopa.

Key words: Chiral inversion, D- dopa, High performance liquid chromatograph

P170075

Effects of ursodeoxychdic acid on the CYP3A activity and pharmacokinetics of midazolam in rats

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Ursodeoxycholic acid (UDCA) is used for the treatment of a variety of chronic cholestatic liver diseases. Recently, the inductive effect of UDCA on CYP3A has been reported. The aim of this study was to clarify effects of UDCA on the CYP3A activity and pharmacokinetics of midazolam(MDZ) in rats. A single oral administration of UDCA (24.4 mg/kg) at 24 hr before the i.v. injection of MDZ in rats significantly reduced AUC of MDZ by 40.1%. After the treatment with UDCA (100 mg/kg/day, p.o.) for 7 days, the activity of MDZ hydroxylation in rat liver microsomes was significantly increased by 1.3- fold. The repeated treatment with UDCA for 7 days increased the mRNA level of CYP3A2 in the liver of rats. There were little significant differences of the activity for MDZ hydroxylation and levels of CYP3A mRNA in the rat intestine between treatments with vehicle and UDCA. The AUCs of MDZ following i.v. and p.o. administrations of MDZ were not significantly changed by 7- days treatment with UDCA. These results suggest that pharmacokinetics of MDZ may not be altered by the repeated treatment with UDCA in rats, although the activity and mRNA levels of CYP3A can be induced.

P170076

In Vitro Stability of Human Recombinant Cytochrome P450 Enzymes

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Human recombinant cytochrome P450 enzymes (rCYPs) are used extensively in pre- clinical drug development. In most in vitro drug metabolism studies an excess amount of parent compound is used and metabolite formation rate is monitored. Linearity of the latter with time is often assessed based on regression analysis on a few samples. Gentest and Cypex provide comprehensive data on the time- course of product formation in rCYP systems with multiple samples. Using these data the stability of different rCYPs with time was examined indirectly. The data were fitted using WinNonLin by a model incorporating the classical Michaelis- Menton equation with or without the assumption of enzyme stability. Assuming first- order enzyme degradation improved the fit for all data sets (based on the Akaike Information Criterion). The median value of t0.9 was 5.8 min, and estimates of half- lives (t0.5) for apparent decline in activity ranged from 11 to 231 min. The results suggest that typical time- linearity studies, with very few samples, may not allow enzyme instability to be identified leading, potentially, to inaccurate characterization of metabolite formation rates and apparent atypical kinetics.

P170077

Relationship between pKa, lipophilicity and solubility –a novel approach for measuring ionizable compounds

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The ionization constant (pKa), lipophilicity and solubility are important parameters for the physicochemical profiling of drug- like molecules. These parameters are inter- related and can be measured on one instrument by traditional and modified pH- metric methods. The relationship between pKa, lipophilicity and solubility is crucial in the investigation of new chemical entities and for creating new potent drugs. Chasing Equilibrium Solubility (CheqSol) is a new pH- metric method for the measurement of solubility of ionizable drug molecules. It requires accurate pKa values measured in the same experimental conditions as solubility. Equilibrium and kinetic values are obtained in the same measurement. The equilibrium solubility is obtained by adjusting the pH to precipitate or re- dissolve compounds and measuring the rates of precipitation and dissolution. Experimental results and graphs are presented for a range of well- known pharmaceutical compounds. The new approach allows the introduction of a new concept, classifying the compounds into "chasers" and "non- chasers" and providing useful information about the behavior of these molecules in the gastro- intestinal tract.

P170079

Tissue Specific, Inducible, and Hormonal Control of the Human UDP- Glucuronosyltransferase- 1 (UGT1) Locus

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The human UDP- glucuronosyltransferase 1 (UGT1) locus spans nearly 200 kb on chromosome 2 and encodes 9 UGT1A proteins which play a prominent role in drug and xenobiotic metabolism. Transgenic- UGT1 (Tg- UGT1) mice have been created and it demonstrated that tissue specific and xenobiotic receptor control of the UGT1A genes is influenced through circulating humoral factors. Human UGT1A1, UGT1A4 and UGT1A6 proteins in Tg- UGT1 mice are differentially expressed in the liver and gastrointestinal tract. Gene expression profiles confirm that all of the UGT1A genes can be regulated by the pregnane X receptor (PXR) activator pregnenolone- 16α- carbonitrile (PCN) and the Ah receptor ligand TCDD. Induction of UGT1A1 by PCN and TCDD may be highly dependent upon glucocorticoids, since sub- μmolar concentrations of dexamethasone actively promote PCN and TCDD induction of UGT1A1 in Tg- UGT1 primary hepatocytes. Hormonal control of the UGT1 locus is further verified in pregnant and nursing Tg- UGT1 mice. These results suggest that the Tg- UGT1 mice will be a useful model to examine the regulatory and functional properties of human glucuronidation. (Supported by United States Public Health Service Grants GM49135, and ES10337)

P170080

Measuring solubility of ionisable compounds by a novel pH- metric approach: CheqSol (Chasing Equilibrium Solubility)

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The primary objective of this study was to distinguish between two different behaviors of ionisable molecules when precipitating. Chasing Equilibrium Solubility (CheqSol) is a new pH- metric approach for the measurement of solubility of ionizable drug molecules. Equilibrium and kinetic values are obtained in the same measurement. The sample is dissolved, then titrated to a pH where the neutral species begin to precipitate. The concentration of sample at the point of initial precipitation, the kinetic solubility is recorded. The rate of change of pH is monitored, whilst strong acid and strong base are added alternately to force the sample to fluctuate between a supersaturated and "subsaturated" state. The process of chasing equilibrium is described. While many samples chase equilibrium, some samples don't, and the result is calculated differently. Most samples can be analyzed in less than 1 hour. The results are in good correlation with published values. Besides its speed and accuracy, this method confirms the result several times within the same experiment, and measures solubility in the presence of solid material without separation. The kinetic and equilibrium solubility values were measured for compounds with well- known pharmaceutical activity and compared with the values reported in the literature. The results are supported by recently

published papers.

P170081

THE RELATIVE BIOAVAILABILITY OF LORATADINE ADMINISTERED AS A CHEWING GUM FORMULATION IN HEALTHY VOLUNTEERS.

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The objective of this study was to investigate pharmacokinetics of loratadine and the metabolite desloratadine following administration of loratadine as: 20 mg conventional tablet, 20 mg smelt tablet, 30 mg medicated chewing gum with or without collection of saliva. Twelve healthy male volunteers participated in the open, four phases, cross - over trial. Plasma concentrations of loratadine and desloratadine, for 24 hours, were obtained by a HPLC method. Eleven of 12 subjects had an increase in relative bioavailability in the chewing gum formulation compared to conventional tablet (Median $AUC_{(0-\infty)}$: 23.77 h* ng/ ml and 8.48 h* ng/ ml, respectively). The median increase in $AUC_{(0-\infty)}$ was 2.68 (Geometric mean ratio: 2.68; 95% CI: 1.75 - 4.09). Pharmacokinetics of desloratadine were similar for conventional tablet, smelt tablet and chewing gum. Formulation of loratadine as a medicated chewing gum resulted in an almost three - fold increase in relative bioavailability compared to conventional tablet formulation. This is most likely due to a bypass of first - pass metabolism, as approximately 40 % of loratadine was absorbed via the oral mucosa this study.

Key words: Loratadine; pharmacokinetics; dosage forms; chewing gum

P170082

Determination of clomipramine and desmethylclomipromine in plasma by HPLC CoulArray electrolchemical detection

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To study the pharmacokinetics of clomipramine (CMI) and desmethylclomipromine (DCMI) in Chinese healthy volunteers, and the bioavailability of CMI hydrochloride tablet, we developed a method for the simultaneous determination of plasma CMI and DCMI levels by HPLC CoulArray electrolchemical detection. 1 mL plasma sample was extracted with 5 mL distilled diethyl ether, and re - extracted with 0.2 mL 0.1 M HCl. The HCl phase was evaporated to dryness with N2 steam and the residue was dissolved with mobile phase. The separation was done on a C18 Inertsil ODS - 3 HPLC column (5 μm, 150 ×4.6 mm). The mobile phase was composed of acetonitrile and sodium phosphate buffer (43: 57). Four channel CoulArray electrolchemical detector was used with the detection voltage of 360, 480, 620 and 760 mV. The extraction recovery of CMI and DCMI was 75% ~85%. The lowest detection concentration was 0.78ng/ ml. The intra - assay variance was 1.27% ~5.12%. The inter - assay variance was 4.45% ~9.39%. This method had been used for the pharmacokinetics and bioavailability study of CMI, and confirmed its sensitivity, specificity, precise and reproducibility.

Key words: clomipramine; desmethylclomipromine; HPLC

P170083

Mycophendlic acid metabolism in Wistar and Mrp2 transporter deficient TR - rat microsomes.

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Mycophenolic acid (MPA) is metabolised in the liver to mycophendate ether glucuronide (MPAGe) which undergoes enterohepatic recirculation via Mrp2. In the rat isolated perfused liver model, we have shown that the clearance of MPA was lower in TR- livers compared to controls suggesting that TR- rats have a lower capacity to metabolise MPA in situ. The aim of this study was to compare the in vitro formation of MPAGe in rat liver microsomes prepared from TR- and Wistar rats. Rat liver microsomes were prepared by differential centrifugation and incubated for 2 min at 37 °C in 5 mg/ L $MgCl_2$, 0.5 mg/ L alamethicin, 5 mM UDPGA, 25 - 1000 μM MPA and 1.0 mg or 0.5 mg protein for control and TR - rats respectively. MPAGe concentrations were determined by HPLC. Mean (SD) kinetic parameters for MPAGe formation were: K_m 0.47 (0.10) versus 0.50 (0.11) mM, V_{max} of 0.48 (0.10) versus 0.65 (0.13) nmol/ min/ mg and Cl_{int} 1.17 (0.24) versus 1.40 (0.21) μL/ min/ mg for control and TR- rats respectively. There was no significant difference in between controls and TR- rats. This suggests that in situ MPA metabolism was impaired, perhaps due to an accumulation of endogenous or exogenous compounds that may inhibit UGT's. Key words: Immunosuppressant, Mrp2, drug metabolism.

P170084

The development of a fluorescence technique for measuring the non - specific binding of drugs to human liver microsomes

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8 - Anilinonaphthalene - 1 - sulfonate (ANS) fluoresces when bound to the hydrophophic component of microsomes. Addition of drugs that bind to microsomes causes a change to baseline ANS fluorescence whereas non - binding compounds do not effect fluorescence. In this study sixteen drugs were characterised for non-specific binding to human liver microsomes using equilibrium dialysis and ANS fluorescence. Relationships between fu(mic), the concentration of bound drug, and percent ANS fluorescence increment/ decrement were determined. Statistically significant logarithmic relationships between fu(mic) and percent ANS fluorescence increment/ decrement for drug concentrations of 100 micromolar ($y = -43.40 \ln(x) + 4.49$; $r^2 = 0.92$) and 200 micromolar ($y = -73.51 \ln(x) + 11.44$; $r^2 = 0.90$) were obtained. There was a highly significant linear relationship between the concentration of drug bound to microsomes and percent ANS fluorescence increment/ decrement ($y = 1.13x$; $r^2 = 0.85$). Thus, drug induced changes in ANS fluorescence are considered an accurate measure of non - specific microsomal binding.

Key words: ANS, hepatic microsomes, drugs

P170085

Determination of Total plasma homocystein level with HPLC in patients with coronary artery disease.

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In this study we also determined plasma levels of homocysteine and other cardiovascular risk factors in - groups with coronary artery disease and a group of healthy controls. Materials And Methods: Total plasma homosysteine was measured in department of pharmacolgy with a modified HPLC method developed in our department, invdving previous derivitisation of plasma thiols with ammonium 7 - fluorobenzo - 2 - oxo - 1,3 - diazole - 4 sulfonate(SBD - F). Column: ODS 100 * 6 mm Mobile phase: 30% methand in acetate buffer (PH = 5.5). Detector: fluorescence, Excitation at 385nm and Emission at 515nm. Results: For homosysteine was 0.2 umol/l. The within day imprecision as 2.67% to 4.56% and the between day imprecision was 5.43% to 8.17%. The mean recovery of hemocysteine was 93% to 103%. The mean of total plasma homosysteine values in - patients with coronary artery disease (20.59umol/l) was significantly higher than control group (12.78 umol/l) (p - value = 0.001). Conclusion: This Hplc method is suitable for determination of total total homosysteine in research and clinical applications. The limit of detection (0.02 umol/l) and imprecision (CV between 2.67% and 8.17%)

P170087

EFFECT OF MORPHINE ON SOME ANTIOXIDANT SYSTEMS IN ANIMALS WITH ULCER STRESS

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In this paper we studied the influence of the natural opioid Morphine on the ulcer stress caused by cold restraint stress. Morphine was applied to the experimental animals before initiating stress. Animals were under the stress condtions during 3 hours. Aantioxidative parameters the value of reduced glutathione - GSH and glutathione peroxidase - GSHPx, glutathione reductase - GSHR and peroxidase were determined in liver homogenate. Quantity of GSH was lower in animals with ulcer, compared to control, while Morphine showed protective effect. Activity of GSHPx was reduced in animals with ulcer, comparing to the control, while in Morphinetreated animals activity oh this enzyme was higher than in animals with ulcer, and it is statistically significant. The activity of GSHR was much higher in animals with ulcer, while treatment with Morphine produced higher activity of this

enzyme , comparing to the contrd , and the same activity like animals with ulcer . There was no statistically significant change in the activity of Px in the animals with ulcer , either in the Morphine - treated animals .

P170088

Evaluation of the influence of potential transmembrane enhancer L - carnitine on the absorption of cholinesterase inhibitors using the rat intestine perfusion model .

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The methodological principle of bilateral in situ intestine perfusion : standardized washing of the intestine lumen from duodenum to caecum and the vascular bed from a . mesenterica superior to v . portae . Methoxytacrine (MEOTA) and galantamine (GAL) were the agents studied , and their concentrations during the perfusion process were monitored (MEOTA : scintillation spectrometry , GAL : HPLC) in both mesenterial and luminal perfusate . Basic absorption kinetics was determined in the first experimental group , in which either MEOTA or GAL were added to the luminal medium. Significant decrease of transintestinal transport of both MEOTA and GAL (presumably due to mutual competition on the carrier systems in the intestinal wall) was observed in the second experimental group , in which luminal perfusion was saturated by the combination of MEOTA + L - carnitine (CAR) or GAL + CAR. In the third group , which was being perorally premedicated by CAR for three days prior to perfusion , a significant increase of the transport of both drugs occurred (presumably due to the accelerating effect of CAR on the intestinal active transport) .

Key words : galantamine , methoxytacrine , perfusion, absorption .
Supported by grant IGA MZ CR NR7935 - 3/2004 .

P170089

Effect of cyp3A4 protein and mRNA expression by GBE in primary human hepatocytes

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Background : Human derived primary hepatocytes reserve the metabolism function and enzyme activity of liver , therefore , the technique has been used extensively in assessing the induction potential of drugs and other xenobiotics . In this study , CYP3A4 induction potential of GBE was evaluated and compared to rifampin in primary human hepatocytes . Methods : Freshly isolated hepatocytes were prepared according to the two - step collagenase perfusion procedure . The hepatocytes were cultured for 72h , followed by treatment for 72h with the GBE at 0.5 , 2 .5 ug/ ml and rifampin at 50 uM. Results : GBE(0.5ug/ ml , 2.5ug/ ml) can increase CYP3A4 protein and mRNA expression in primary human hepatocytes . GBE at 0.5ug/ ml inducted CYP3A4 protein to 789 % of control , to 80 % of rifampin at 50 uM. GBE at 2 .5ug/ ml inducted CYP3A4 protein to 906 % of control , to 98 % of rifampin at 50 uM. The expression of CYP3A4 mRNA were increased by 207 % by GBE at 0 .5ug/ ml . GBE at 2 .5ug/ ml inducted CYP3A4 mRNA to 201 % of control , to 120 % of rifampin at 50 uM. Conclusions : Our studies with the primary human hepatocytes suggest that GBE can significantly induct the expression of CYP3A4 protein and mRNA.

Key words : GBE ; CYP3A4 ; induction ;

P170090

The Constitutive Androstane Receptor Mediates Induction of Murine Cyp2c37 by Phenytoin

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This study utilizes knockout mice to determine which receptors mediate the induction of the CYP2C subfamily by drugs such as the anticonvulsant phenytoin (DPH) . Here , we report that two murine Cyp2c genes , Cyp2c37 and Cyp2c29 (like Cyp2b10) , are inducible by DPH and phenobarbital but not by the pregnane X receptor (PXR) agonist 5 - pregnen - 3β- ol - 20 - one - 16α- carbonitrile prenenolone (PCN) . Quantitative RT - PCR and immunoblots show that DPH and phenobarbital increase hepatic CYP2C37 mRNA and protein . We identified a putative constitutive androstane receptor response element (CAR - RE) - 2.8kb from the start of translation of the Cyp2c37 gene . Mutation of the CAR - RE in Cyp2c37 luciferase promoter constructs demonstrated that it is necessary for mCAR transactivation . The induction of CYP2C37 and CYP2C29 mRNA by DPH is abolished in CAR - null mice , suggesting that this induction is mediated by mCAR rather than PXR. However , induction of CYP3A11 mRNA by DPH was not abolished suggesting that the contribution of the nuclear receptors CAR and PXR to induction of P450 enzymes by DPH may be gene promoter dependent .

This research was supported by the intramural program of NIH/ NIEHS .

P170091

Use of a Regulated Secretion/ Aggregation Technology to Determine the Rate of Muscarinic M_1 Acetylcholine Receptor Plasma Membrane Delivery

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We sought to establish whether a regulated secretion/ aggregation technology (RPD™) could be used to determine the rate of human muscarinic M_1 acetylcholine (hM_1) receptor plasma membrane delivery . hM_1 receptors were expressed in CHO cells as C - terminal fusion proteins to a conditional aggregation domain (CAD) . The human growth hormone signal sequence fused to the N - terminus of the CAD targeted the fusion proteins to the endoplasmic reticulum, where they formed aggregates and were retained. Aggregates of CAD - hM_1 receptor fusion proteins could be disrupted in a concentration - dependent manner by the CAD - selective ligand AP21998 , allowing hM_1 receptors to traffic to the plasma membrane . hM_1 receptor plasma membrane expression was observed to peak after an 18 h incubation with AP21998 , then gradually decline to basal levels as the incubation continued out to 72 h . These expression data were fit using two different mathematical models to obtain estimates for the rate constants for hM_1 receptor plasma membrane delivery . Collectively , our data indicates that the RPD is a useful tool for characterizing the kinetics of receptor plasma membrane delivery .

Key words : trafficking , muscarinic , kinetics , receptors
Acknowledgment : The activities described were supported by an OHRS award for project number HR03 - 107S , from OCAST .

P170092

A pharmacokinetic study of paracetamol in Thai Beta - thalassemia / HbE patients

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Thalassemia may alter the pharmacokinetics of several drugs . Paracetamol , a common analgesic - antipyretics is extensively metabolized in the liver via glucuronidation . This study compares the pharmacokinetics of paracetamol (PCM) and its metabolites ; glucuronide (PCM - G) , sulfate (PCM - S) , cysteine (PCM - C) in sixteen patients and controls . After an overnight fast , a single dose of 1000 mg paracetamol (Tylenol) was given and blood samples were obtained at predose , 0.5 , 1 , 1.5 , 2 , 3 , 4 , 5 , 7 , and 9 hours after dosing for determination of the plasma levels of PCM and its metabolites by HPLC. There was no difference in maximum concentration of PCM between groups . However the elimination half - life of PCM was shorter in thalassemias . The body clearance of PCM was faster in thalassemias while the Vd of PCM did not change . The AUC of PCM - G and PCM - S increased in thalassemias whereas this parameter of PCM - C was slightly lower in the patients . Half - life of PCM metabolites was shorter in thalassemias . Thus the elimination of PCM and its metabolites in the patients was faster . Our data indicate that there is high PCM - G in the thalassemias with hyperbilirubinemia could be a strong factor to induce UGT expression .

Key words : acetaminophen , drug metabolism, thalassemia , UDP - glucuronosyltransferase
This work was supported by the National Center for Genetic Engineering and Biotechnology (BIOTEC) , and the Thailand Research Fund

P170093

effects of low doses of bile acids on blood glucose levels and some liver parameters in rats

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key words : bile salts , glycose , toxicity

The aim of our study was to compare hypoglycemic effects of cholic henodeoxycholic and chemicaly synthetized monoketocholic (MKH) acid and in combination with insulin (INS) after intranasal (IN) and intravenous (IV) administration. Their toxicity was tested by glutathione and protein content in liver. Anaesthetized rats received bile acids as 2% sodium salt solutions (2 mg/kg) as in combination with INS (10 IU/kg). Glucose levels were measured in 0, 15, 30, 60, 90, 120 and 180th min. Liver samples were taken in 0 min. and in every hour after IV injection (4 mg/kg). According to the initial time blood glucose levels were changing significantly after IN application of all bile salts. Areas under the curve had shown great hypoglycaemic effect ($p < 0,001$) in relation to controls and INS. There were no significant changes in glutathione and protein content compared to control groups. Our results confirmed that bile salts enhance the INS effects, but also indicate that they per se can affect blood glucose levels. Based on our results we might presume that IV or IN applied bile salts could be safely used as insulin promoters.

P170094

Absorptivity enhancement researches of curcumin in solid dispersions with the polymers PVP-K30[1]

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This research was focus enhancement of solubility and oral absorptivity of curcumin by Polyvinylpyrrolidone K30 (PVP). SDs in different ratios were prepared by co-evaporation in ethanol solution. The solubility of curcumin in PVP SDs (1:8) enhanced 857 times, compared to that of curcumin only. A sensitive HPLC method was developed to determine pharmacokinetics of curcumin in rat plasma after oral administration of curcumin in PVP SDs, Physical mixture (PM) and curcumin-CMC-Na (CMC), each sample was administered three level of dosages which contained curcumin 100, 200 and 400 mg/kg, respectively. The results showed that curcumin concentration of rat plasma administration of curcumin in PM and CMC were under the limited of detection even 4 h after oral administration. The bioavailability of curcumin was enhanced by PVP SDs, the concentration-time data was best fit for two-compartment model. The peak levels in blood for three level dosages were 74.558, 110.174 and 193.665 ng.mL^{-1} at about 45 min, respectively. PVP SDs could improve curcumin solubility and bioavailability.

Key words: Curcumin; Polyvinylpyrrolidone; solid dispersions; absorptivity;
[1] Project supported by the National Natural Science Foundation of China, No. 30170105; Supported Program of New Century Excellent Talent (No. NCET-04-0808); Supported Program of Fok Ying Tung Education Foundation (No. 91036)

P170095

Study of the Bioequivalence of Trimetazidine Hydrochloride Tablets in Chinese Healthy Volunteers

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Objective: To study the bioequivalence of Trimetazidine Hydrochloride Tablets in healthy volunteers. Methods: A single dose of 40 mg of tested (France Servier Pharmaceutical Factory) and reference formulation (Hubei Si-huan Pharmaceutical Company Limited) were given to 20 healthy volunteers in a randomised crossover study. The concentrations of Trimetazidine in plasma were determined by HPLC. The pharmacokinetics parameters were calculated and bioequivalence of two formulations were evaluated by DAS program. results: After a single dose, the pharmacokinetics parameters for Trimetazidine were as follows: C_{max} were (122.78 ±11.60) and (115.12 ±10.98) μg/L; T_{max} were (2.08 ±0.34) and (2.13 ±0.39) h; AUC(0-24) were (962.56 ±122.03) and (914.53 ± 86.16) μg·h/L; AUC(0-inf) were (1004.71 ±125.94) and (966.40 ±99.53) μg·h/L for tested and reference formulation respectively. The 90% confidential interval of AUC(0-24)、AUC(0-inf) and C_{max} of tested formulation were 100.4 ~109.5%、99.1 ~108.4% and 102.6 ~110.8% respectively. Conclution: the relative bioavailability was (105.41 ±11.22)%; The two formulations were bioequivalence.

Key words: Trimetazidine; pharmacokinetics; bioequivalence; HPLC

P170096

In vitro Metabolism of a Xanthone from a Tibetan Herbal Medicine, Halenia elliptica

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Halenia elliptica is a Tibetan herb used for the treatment of hepatitis and gastritis. An HPLC/DAD/APCI/MS method was developed for quantitative analysis of major xanthones present in the herb. We have also studied the metabolism of a major xanthone in rat liver microsomes. Thus, 1-hydroxy-2,3,5-trimethoxyxanthone (HM-1), the most abundant active constituent (around 8.8 mg per gram dried plant), was incubated for 1 hour with rat liver microsomes containing a NADP-generating system. The metabolites were isolated by chromatographic methods and their structures elucidated by using Nano-probe 1H-NMR, EI-MS and APCI MS. Five phase I metabolites were identified as demethylated and other derivatives.

Key words: xanthones, metabolism, Tibetan herbal medicine, Halenia elliptica
Acknowledgement: The project is partially supported by Natural Science Foundation of China (No.20372084). Miss Penolepe M.Y. Or is gratefully acknowledged for technical support.

P170097

Study on the conversion of novel drug Potassium 2-(1-Hydroxypentyl)-benzoate (dl-PHPB), a pro-drug of 3-n-butylphthalide (NBP)

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NBP is a novel agent for treatment of brain ischemia. PHPB, a potential prodrug of NBP, was designed to increase water solubility of NBP, which can be administrated intravenously and orally. The conversion of PHPB to NBP was investigated in vitro and in vivo. To determine the level of PHPB and active drug NBP, the HPLC method was used. In vitro, at concentration of 6, 30 and 60 μg/ml, 70% of PHPB was converted to NBP in 10 min, when PHPB was added into the plasma. However, when it was given 10 mg/kg intravenously to rats, PHPB was not detectable in blood. It was converted to NBP very fast. The half life ($t_{1/2}$) and AUC of NBP in blood were 6.9 min and 189 μg×min/ml, respectively. When given PHPB orally 100 mg/kg to rats, it converted to NBP also very fast. The T_{max}, C_{max} and AUC of NBP were 9.0 min, 15.8 μg/ml and 460 μg×min/ml, respectively. The pharmacokinetic studies showed that PHPB was metabolized quickly into NBP. The anticerebral ischemia effects of PHPB are mainly due to NBP release.

Key words: Pro-drug; conversion; pharmacokinetic; HPLC
Acknowledgements: The work was supported by the National Science Foundation of China, No.30371644 and National 973 Fundamental Project of China, No. 2004CB518906.

P170098

Pharmacokinetics of rosuvastatin in Chinese healthy volunteers

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Aim: To study the pharmacokinetics of rosuvastatin in Chinese healthy volunteers. METHODS: A single (5, 10, and 20 mg) and 7-d-repeated (10 mg/d) oral doses of rosuvastatin were perfored on 12 (6 males and 6 females) Chinese healthy volunteers. The concentration in plasma was determined by LC/MS/MS. Data were analyzed by a 3p97 program. Results: Geometric mean maximum plasma concentration (C_{max}) values of 9.7, 19.6 and 33.4 ng/ml were achieved at a median time to C_{max} of 3.5 hours after doses of 5, 10, and 20 mg, respectively. The corresponding geometric mean area under the plasma concentration-time curve from zero to time of the last measureable concentration (AUC0-t) were 66, 146, and 257 ng×h/ml. The apparent elimination half-life ($T_{1/2}$) were 12.6, 15.8, and 16.3. The main pharmacokinetic parameters of rosuvastatin after 7-d-repeated (10 mg/d) oral doses were as follows: t_{max}, $t_{1/2}$, C_{max} and AUC0-t were 3.6 h, 13.8 h, 17.3 ng/ml and 158 ng×h/ml, respectively. Conclusion: The C_{max} and AUC0-t were both linearly related to dose. The clinical dosage regime caused no drug accumulation.

P170099

Study on the pharmacokinetic characteristic of the effective components group of Xiao-xu-ming decoction in rats

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In this paper we studied on the pharmacokinetic characteristic of the effective components group of Xiao-xu-ming decoction in rats by HPLC-ESI-MS. Among those nearly ninety compounds which could be detected in blood after a single (gavage) dose orally, 36 components were found be absorbed as original drug, such as prim-O-glucosylcimifugin, cimifugin, 4'-O-β-glucopyranosyl-5-O-methylvisamminol, 5-O-methylvisamminol, Sec-glucosylhamaudol, liquiritin, cyclanoline, et al. Others were likely metabolites. The T_{max} of the most of the original drugs were at 0.5-1.5 hours, and the T_{max} of the most of the metabolites were at 1-1.5 hours. The total amount of the materials that could be detected in blood appeared two peaks at 0.5 hour and 1.5 hour. That means there is the intestines-liver cycle in the absorption procedure. At the time of 12 hours, most of the compounds, include metabolites, couldn't be detected in the blood, and the amounts of materials were about 10% of the maximum point. Our research work showed that it's reasonable to take this traditional Chinese medicine compound prescriptions twice per day.

Key words: pharmacokinetic, effective components group, Xiao-xu-ming decoction

P170100

Pharmacokinetics and bioequivalence of fluvastatin tablet in Chinese healthy volunteers

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Objective: To compare the pharmacokinetics and bioavailability of two tablets of fluvastatin in 20 Chinese healthy volunteers. Methods: According to the crossover design, each volunteer was orally given 40 mg fluvastatin. The concentrations in plasma were determined by RP-HPLC. Pharmacokinetic parameters were obtained using BAPP 2.0 program. Results: The pharmacokinetic parameters of fluvastatin were as follows: AUC were 524.63 ±308.92 and 540.65 ±228.82 μg × h ×L^{-1}; C_{max} were 517.45 ±252.06 and 491.38 ±211.44 μg ×L^{-1}; t_{max} were 0.57 ±0.13 and 0.62 ±0.18 h; $t_{1/2}$ were 1.71 ±0.68 and 1.52 ±0.63 h for test and reference tablets, respectively. The relative bioavailability was 98.75 ± 37.58%. The analysis of variance on pharmacokinetic parameters such as C_{max} and AUC indicated that there was no significant difference between the two tablets. All the 90% confidence intervals of the test/reference mean ratio of parameters were within the bioequivalence limits. Conclusion: Pharmacokinetic profiles showed no significant difference between the Caucasians and Chinese. The results of statistical analysis indicated the two tablets bioequivalent.

Key words: fluvastatin; RP-HPLC; pharmacokinetics; bioequivalence

P170101

Bioequivalent evaluation of two immediate release tablets of losartan/hydrochlorothiazide in healthy Chinese male volunteers

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The study was designed to evaluate the bioavailability of two losartan/hydrochlorothiazide tablet formulations. Twenty healthy male volunteers were administrated a 50/12.5 mg tablet of the test formulation (T) containing losartan/hydrochlorothiazide or a commercially original preparation as the reference formulation (R). The study was conducted according to an open, randomized, single-dose, two-period cross-over design with a wash-out period of 7 days. Blood samples were collected over 48 hours. Bioavailability was evaluated on the basis of plasma concentrations of losartan and hydrochlorothiazide, which were determined by a validated HPLC-ESI-MS method. In this study, the 90% confidence interval for AUC_{0-t} and C_{max} of losartan were between 0.86 and 1.12 (AUC_{0-t}) as well as between 0.89 and 1.34 (C_{max}); the 90% confidence interval for AUC_{0-t} and C_{max} of hydrochlorothiazide were between 0.85 and 1.00 (AUC_{0-t}) as well as between 0.75 and 1.02 (C_{max}) and thus within the acceptance ranges. Based on these statistical inferences, the test formulation was considered bioequivalent to the reference formulation.

Key words: Losartan; Hydrochlorothiazide; Bioequivalence; HPLC-MS

P170102

Preparation of ^{125}I-HSA-IFNα-2b and in vivo Evaluation in Rats

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Objectives: To evaluation behavior of albumin interferon α-2b fusion protein (HSA-IFNα-2b) in rats. Methods: 125I-HSA-IFNα-2b was prepared and assayed. 125I-HSA-IFNα-2b was injected subcutaneously (200 μg/kg), about 0.74 MBq per rat. Biodistribution and excretion of 125I-HSA-IFNα-2b in rats were evaluated. Results: The radiochemical purity of 125I-HSA-IFNα-2b was over 95%, the specific activity was 0.26 MBq/μg, the antiviral activity of HSA-IFNα-2b had almost no change. Biodistribution and excretion of 125I-HSA-IFNα-2b showed that radioactivity of 125I-HSA-IFNα-2b in blood reached highest, and eliminated slowly. Specific accumulation wasn't seen in any tissue. 125I-HSA-IFNα-2b was excreted mainly by kidney, The average accumulation excretory rates in urine were 80.10%; excreted partly by diachoresma; also can be excreted by bile after being metabolized by liver. Conclusions: HSA-IFNα-2b is a novel long-acting form of interferon α with long half life.

Key words: HSA-IFNα-2b, Iodine-125, Biodistribution, Excretion

* Founded by Jiangsu Province Health Department Graveness Project Foundation (H200208) and Jiangsu Province Nature Foundation (BK2003023)

P170103

BINDING OF SULPHADIMIDINE (SDM) TO CHICKEN PLASMA (CP)

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Binding of SDM to chicken albumin may not represent the actual in vivo binding to plasma proteins. A more realistic picture can be obtained by examining the interaction of SDM with CP. Several chickens were bled and their blood was collected and centrifuged. Plasma was pooled and divided into plastic vials. Ultrafiltration was used to examine binding of [^{14}C]-SDM to CP at 42°C. Solutions containing various concentrations of SDM were made with 0.1 M Na-phosphate buffer, pH 7.4, and were spiked with 0.04 μCi [^{14}C]-SDM. Each solution was mixed with CP in a ratio of 1:19 and aliquots (1 ml) were ultrafiltered at 1000 g for 5 min. The level of 14C of filtrate and initial solution were measured. The concentration of bound SDM was calculated from the difference between total and unbound concentration in CP and ultrafiltrate, respectively. Apparent affinity, K, and binding capacity, nP_t, were estimated by fitting binding data to the one class of saturable binding sites model. Percentage SDM bound varied relatively little as at concentrations of 2 μM and 100 μM, 30.2 ±0.5% and 26.4 ±0.7% of SDM were bound, respectively. Analysis of binding data gave values of 0.56 ± 0.01x10 M^{-1} and 95.0 ±3.0 μM for K and nP_t, respectively.

Key words: Chicken, Sulphadimidine, Plasma protein binding

P170104

Metabolism disposition of lansoprazole in relation to the CYP2C19 phenotype status

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Aim: To assess the possible involvement of CYP2C19 in the metabolism of lansoprazole in vivo. Methods: 31 Chinese subjects, extensive metabolizers (EMs, n=24) and poor metabolizers (PMs, n=7) of CYP2C19 phenotyped with use of index of omeprazole Copz/CopzOH, took an oral dose of 30 mg lansoprazole, and blood samples were collected up to 36 hours after dosing. Lansoprazole and its metabilites were measured by HPLC-UV. Results: AUC, CL were significantly greater, and lower, respectively, in PMs than in EMs group. The mean values for the AUC of hydroxylansoprazole and AUC ratio of hydroxylansoprazole to lansoprazole were significantly less in the PMs than in EMs group, whereas those for the AUC ratio of lansoprazole sulfone and AUC ratio of lansoprazole-sulfone to lansoprazole were greater in the former than in the latter group. In addition, the Copz/CopzOH correlated significantly with CL of lansoprazole. Conclusions: These results suggest that the hydroxylation of lansoprazole cosegrates with the genetically determined CYP2C19 polymorphism in the Chinese subjects.

Key words: CYP2C19, Chinese, polymorphism

Acknowledgements: lansoprazole and its two metabolites are gifts by Japanese TAKEDA.

P170105

Predictive possibilities of in vitro dissolution testing for clinical bioequivalence studies of oral tablet sulpiride formulations

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Antipsychotic drug sulpiride belongs to the Class 4 in the Biopharmaceutics Classification System- BCS (Amidon et al., Pharm. Res. 12:413, 1995) having low aqueous solubility and low intestinal permeability (absorption). Generally, the predictability of in vitro dissolution testing for in vivo bioequivalence studies (BeS) is poor in the case of the Class 4 drugs. Our aim was to evaluate this predictability in sulpiride formulations. Two in vitro - in vivo comparisons were performed. In each of them, two sulpiride formulations were compared in an in vitro dissolution test (artificial gastric juice; similarity factor f2 calculated - its value >50 suggests that two dissolution profiles are similar) and in a BeS in healthy volunteers (statistics of bioavailability parameters: two one - sided test procedure with null hypothesis of bioinequivalence). In both comparisons, there was a good in vitro - in vivo correlation (the first comparison: f2 = 22, 90% confidence intervals were 105 - 141 % for AUCinf; the second comparison: f2 = 56, 90% confidence intervals were 93 - 110 % for AUCinf). In spite of sulpiride belonging to the Class 4 in the BCS, in vitro dissolution testing predicted well the results of in vivo BeS.

P170106

Effects of CPU86017 on isolated rat left atrial contraction against the pharmacokinetic behavior in vitro

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Aim: To investigate the duration of effect of CPU86017 against the pharmacokinetic behavior in vitro. It was intend to explore why duration of pharmacological effect of CPU86017 is longer than that of plasma concentrations. Methods: The left atrium was suspended in an organ bath and driven electrically. K - H solution containing CPU86017 $5 * 10^{-5}$ M was infused into the bath at 1 ml/ min from 0 to 40 min and then free K - H solution was infused at 1 ml/ min from 40 to 100 min. The contractile force of atrium and levels of CPU86017 in bath were measured at different time. Results: The concentration of CPU86017 in bath increase from 0 to 40 minutes and decrease quickly from 40 min. The negative inotropism of CPU86017 emerges at 20 minutes and enhanced continuously until 70 min. A counter - clockwise hysteresis loop is involved in the effect - concentration curve. An apparent $T_{1/2}$ of pharmacological effect was about 1000 - fold as long as the pharmacokinetic $T_{1/2}$. Conclusions: The long - lasting effect of CPU86017 was due to the slow elimination rate from the effective compartment.

Key words: CPU86017; atrium; in vitro, PK- PD.

Supported by projects (no 30230170 & 30572193) from National Natural Science Foundation of China

P170108

Pharmacokinetics and bioequivalence of bismuth derived from two combined formulations of ranitidine and bismuth potassium citrate

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Aim: To evaluate the bioequivalence of bismuth derived from two combined formulations of ranitidine and bismuth. Methods: The bioavailability was carried out on 20 healthy male Chinese volunteers following a single oral dose (200 mg) of the test and reference products in the fasting state, in a randomized crossover design. After dosing, serial blood samples were collected within 24 h. Bismuth concentrations were analyzed by an ICPMS method. The non - compartmental method was used for pharmacokinetic analysis. Log transformed, C_{max} and AUC (0 - t) were tested for bioequivalence using ANOVA and Schuirmann two - one sided t - test. T_{max} was analyzed by Wilcoxon test. Results: The pharmacokinetic parameters of test and reference drug were as follows: C_{max}(11.80 ±7.36 vs 11.40 ±6.55 ng ×mL^{-1}), AUC(0 - t) (46.65 ±16.97 vs 47.03 ±21.49 ng ×h ×mL^{-1}), T_{max}(0.50 ±0.20 vs 0.50 ±0.20 h) and $t_{1/2}$(10.2 ±2.3 vs 13.0 ± 6.9 h). 90% confidence intervals for the test/reference ratio of C_{max} and AUC fell within the bioequivalence acceptable range 80～125%. No significant difference was obtained for T_{max}. CONCLUSION: Bismuth in two formulations were bioequivalent.

Key words: bismuth; ICP - MS; pharmacokinetics; bioequivalence

P170109

Simultaneous analysis of tramadol and metoprolol and their metabolites in biological samples by HPLC and application to a pharmacogenomic study

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Aim: To establish a HPLC method to simultaneously determine the concentrations of tramadol and its primary metabolite O- desmethyltramadol (M1), metoprolol and its metabolite α- hydroxymetoprolol (OHmet) in human plasma and urine in order to offer the methodology for a pharmacogenomic study. Methods Chromatography was performed with a zorbax C18 column and the mobile phase was a 0.05 M KH_2PO_4 - acetonitrile (90:10). The flow rate was 1 ml/ min. Fluorescence detection (λex 216nm/ λem 312nm) was used. The method was validated by selectivity, linearity, precision, accuracy, LOQ, recovery and stability. The preliminary test of pharmacogenomic study was conducted. Results In plasma, the linear range was 12.5 - 800 ng/ ml (tramadol), 5 - 320 ng/ ml (M1), 10 - 400 ng/ ml (metoprolol), 5 - 360 ng/ ml (OH- met). In urine, the linear range was 62.5 - 4000 ng/ ml (tramadol), 50 - 3200 ng/ ml (M1), 50 - 4000 ng/ ml (metoprold), 25 - 3600 ng/ ml (OH- met). The relative recovery was between 92% and 108% and the variations of within - day and between - day were no more than 10%. Conclusion This method is simple, reliable, sensitive and accurate, which is fitted to the pharmacogenomic study.

P170110

Ropivacaine plasma levels after thoracic epidural anaesthesia

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Study objective: The aim of this study was to determine ropivacaine plasma levels after thoracic epidural administration, in patients undergoing abdominal surgery under combined epidural-general anaesthesia. Methods: 15 patients were studied, aged 47 - 77 years. Epidural anaesthesia was performed at T10 - 11, T11 - T12 or T12 - L1 interspaces with 37,5 mg of ropivacaine. Blood samples were collected at 10, 40, 70, 100 and 130 minutes after ropivacaine administration. Determination of plasma levels was achieved with high performance liquid chromatography. Results: The highest plasma concentrations of ropivacaine were observed at 42 ±46 minutes. 60 percent of patients showed a peak of ropivacaine plasma levels 10 minutes after administration (C_{max}:0,65 ±0,47 μg/ ml). 26,6 percent of patients, showed a different peak at 70 and 130 minutes, and 13,4 percent a peak at 40 and 100 minutes equally. Mean elimination half life ($t_{1/2}$) was calculated to be 265,2 minutes. Conclusion: Ropivacaine plasma levels after thoracic epidural anaesthesia, peaked in a predictable manner in 60 percent of patients studied and $t_{1/2}$ was calculated to be 265,2 minutes.

Key words: Ropivacaine, plasma, epidural

P170111

RISPERIDONE DOES NOT INCREASE PLASMA CLOZAPINE AND NORCLOZAPINE CONCENTRATIONS IN PATIENTS

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Case reports that risperidone increases plasma clozapine concentrations are conflicting with a study based on therapeutic drug monitoring data that was analysed retrospectively. This prospective study determined whether risperidone influenced plasma clozapine and norclozapine in patients with chronic schizophrenia. Subjects received either clozapine alone (n = 14) or the clozapine/risperidone combination (n = 7). All patients drank tea or coffee and 75% were smokers; those who received medication that influenced CYP1A2 or CYP2D6 were excluded. After at least 4 weeks of treatment, blood was taken for HPLC analysis (12 hours after last clozapine dose). The Mann - Whitney U test showed no significant differences between the two groups with respect to clozapine concentrations (P = 0.941); norclozapine concentrations (P = 0.628); clozapine concentrations corrected for dose (P = 1.00) and norclozapine:clozapine ratio (P = 0.881), suggesting that risperidone does not affect clozapine or its active metabolite.

Key words: risperidone, clozapine, norclozapine

Acknowledgement: Study support from a University of Sydney Sesqui Grant.

P170112

Tolerability and Pharmacokinetics of a Single dose of Co-naphthoquine Tablets in Healthy Volunteers

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Objective Co-naphthoquine tablets is a new antimalarial drug which contains artemisinin (AS) and naphthoquine (NAP). The aim of this study was to assess the pharmacokinetics and safety of the two drugs in healthy male volunteers. Methods Safety and pharmacokinetics study of a single dose of Co-naphthoquine tablets (each tablet contains 125 mg AS and 50 mg NAP) in healthy male volunteers. 30 volunteers were enrolled, the oral dosages of these groups were 4, 8 and 16 tablets respectively. A method to detect the blood concentrations of AS and NAP were developed by HPLC-tandem mass spectrometry. Results The C_{max}, T_{max}, and the half-life ($t_{1/2}$,z) of AS in three groups were (427.30 ±143.01, 697.70 ±246.51, 892.60 ±219.78 ng/ml), (2.2 ±1.1, 2.4 ±1.1, 2.1 ±1.6 hr), and (4.0 ±0.6, 3.7 ±0.6, 4.9 ±1.9 hr). Those of NAP in each groups were (11.40 ±4.45, 27.44 ±16.21, 59.83 ±20.03 ng/ml), (3.5 ±5.2, 3.0 ±1.9, 2.5 ±1.1 hr), and (256.4 ±179.4, 276.4 ±107.5, 233.3 ±190.7 hr) respectively. Conclusion The Co-naphthoquine tablets were well tolerated by the subjects. The results suggest that there is a drug interaction between AS and NAP.

Key words: artemisinin, naphthoquine, Plasmodium falciparum, pharmacokinetics, tolerability

P170113

Influence of Age, Gender, Testosterone, Oral Contraceptives, and Ketoconazole on CYP3A Activity

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A placebo-controlled, double-blind, three-way crossover study evaluated the influence of age, gender, testosterone (TST), oral contraceptives (OCPs), and ketoconazole (KCZ) coadministration on CYP3A activity. Thirty nine human subjects were orally administered one dose of 0.0625 mg triazolam (TRZ) and/or three doses of 200 mg KCZ. Plasma concentrations of TRZ, KCZ, and TST were measured during 8h after dosage. Pharmacokinetic parameters for TRZ were consistent with established values. KCZ significantly increased C_{max}, T_{max}, $T_{1/2}$, AUC and decreased clearance (CL) of TRZ ($P < 0.001$). Individual 8h AUCs of TRZ were significantly correlated with the exposure of KCZ ($r_s = 0.653$, $P < 0.001$). Plasma TST levels in males reduced with age or after the treatment of KCZ. TST, OCPs, and gender did not affect the pharmacokinetic parameters of TRZ. In males, AUC ($r_s = 0.63$) significantly increased with age ($P < 0.05$). CL ($r_s = -0.63$) and CL/weight ($r_s = -0.61$) decreased with age ($P < 0.05$). However, these changes were not detected in females. In summary, 0.0625 mg TRZ could be used to monitor CYP3A activity. Gender, TST and OCPs did not influence CYP3A activity. CYP3A activity decreased in male elders, but not in females.

P170114

Indusion Compound: a Preferable Dosage Form to Enhance Bioavailability of Astragaloside IV in Intact Rat

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Purpose: To investigate the pharmacokinetics of inclusion compound of AGS-IV and its bioavailability. Methods: Twenty-four rats, were given inclusion compound of AGS-IV (5.0, 10.0 and 20.0 mg/kg, respectively) and aqueous solution of AGS-IV (2.0 mg/kg). Blood samples were drawn intermittently in each intact rat at 0.25, 0.50, 0.75, 1, 1.5, 2, 4, 8, 12, 24, 36, 48 and 60 h for oral dose, and 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 6, 10, 14 and 24 h for intravenous dose, respectively. The samples were prepared by SPE and analyzed by a LC-ESI-MS. Results: The inclusion compound of AGS-IV after oral doses (5.0, 10.0 and 20.0 mg/kg) was eliminated with $t_{1/2}\beta$ as 10.73 ±3.34, 11.47 ±3.28 and 12.88 ±2.03 hr, with CL as 0.88 ±0.09, 0.90 ±0.63 and 0.85 ±0.04 L/hr, with Vc as 6.73 ±1.78, 5.66 ±2.23 and 5.72 ±2.41 L, with AUC0-t as 1099.09 ±84.32, 2174.68 ±232.98 and 4800.24 ±214.86 ng.hr/ml, respectively. The bioavailability of AGS-IV was 10.3% for 5.0 mg/kg, 10.2% for 10.0 mg/kg and 11.2% for 20.0 mg/kg, respectively. Conclusion: Inclusion compound was a preferable dosage form to enhance bioavailability of AGS-IV.

Key words: Astragaloside IV; Inclusion compound; Absolute bioavailability; LC-ESI-MS

P170115

The Increased Emphasis of ADME Properties in Hit-to-Lead Drug Discovery

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Parallel chemistry, a new approach to identify and optimize drug leads, has been successful in synthesizing large libraries of compounds for novel therapeutic targets. As part of the lead generation process, it becomes crucial for the hit to lead (HTL) molecules to have good ADME (absorption, distribution, metabolism and excretion) and PK (pharmacokinetics) properties as well as good physicochemical properties for their clinical success. Even before the optimization process begins, potential issues in ADME area need to be identified so that they can be addressed in parallel with the more traditional aspect of potency. Consequently, in silico (computational) predction of ADME properties is required in drug design due to its ability of handling multiple chemical series, saving time and cost compared to routine laboratory work. In this presentation, several examples will be discussed to demonstrate how ADME strategies can be applied to early drug discovery to enable rapid progression of high quality hits into leads. These strategies include classical ADME tools, physicochemical properties, computational approaches and data visualization tools.

Key words: ADME, in silico, HTL

P170116

Determination and pharmacokinetics of phencynonate and its optical isomers in rat

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Phencynonate{N-methyl-9α-(3-azabicyclo[3,3,1] nonanyl)-2'-cyclopentyl-2'-hydroxyl-2'-phenylacetate} is a novel anticholinergic drug developed by the Beijing Institute of Pharmacology and Toxicology in China. Quantification and pharmacokinetics of phencynonate were investigated in rat by the method of high-performance liquid chromatographic assay with electro spray ionization mass spectrometry detection (LC-ESI-MS). The chromatography was on BetaBasic-18 column (150 mm × 2.1 mm i.d., 3 μm). The mobile phase composed of methanol and water (85:15, v/v), containing 0.05% formic acid, which was pumped at a flow-rate of 0.2 ml/min. Simultaneous MS detection of phencynonate and the internal standard of was performed at m/z 358.4 (phencynonate) and m/z 364.0 (thiencynonate). And the selected reaction ion monitoring (SRM) of the two compounds were both 156. The linearity was obtained over the concentration range of 1 ~ 100 ng/mL in rat blood. The lower limit of quantification was at 1 ng/mL in blood. The precision was obtained from 2.92 to 9.76%. Extraction recoveries were in the range of 69.6-79.1%. The concentration-time curves in rats were all best fitted to first order absorption two-compartments open model after i.m a single dose phencynonate (0.35 mg/kg). The main pharmacokinetic parameters of phencynonate were as follows: $T_{1/2}\alpha$ 0.68 h, $T_{1/2}\beta$ 3.98 h, $T_{1/2Ka}$ 0.013 h, T_{max} 0.076 h, C_{max} 54.08 ng/mL, AUC 77.70 ng·h/L. There were some differences for the level of the blood drug concentration of phencynonate raceme and the two optical isomers after dosing the phencynonate and the R and the S isomers, respectively. There was the relationship between the pharmacodynamics and the pharmacokinetics for the configuration to the chiral drug. It provided important information for developing a novel chiral drug and the clinical use of phencynonate.

Key words: phencynonate; isomer; liquid chromatography-mass spectrometry; quantification; pharmacokinetics.

This project was supported by the Major program of National Natural Science Foundation of China (No 203900508).

P170117

Determination of Dioxopromethazine Hydrochloride in human plasma and its pharmacokinetics in healthy Chinese volunteers

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Aim: To determine the concentration of dioxopromethazine hydrochloride in human plasma and investigate its pharmacokinetics in healthy volunteers following oral administration of a single dose of the medicine 9 mg. Methods: Plasm samples were processed by liquid-liquid extraction and the plasm concentrations of dioxopromethazine hydrochloride were assayed by High-performance liquid chromatography with fluorescence detection (HPLC-FLD). Results: Assay linearity was obtained in the range of (0.5 - 75.0) ug·L^{-1} ($r = 0.9999$). The recovery of dioxopromethazine hydrochloride from human plasma was more than 80 %. The intraday and interday relative standard deviations (RSD) for the lowest concentration examined (0.5 ug·L^{-1}) were 2.5 % and 6.7 %, respectively. The method was utilized to determine the concentration of dioxopromethazine hydrochloride in healthy volunteers. The concentration-time curve was fitted to a two-compartment model. Its main pharmacokinetic parameters were as follows: T_{max} were(2.17 ±1.70) h; c_{max} were (31.07 ±5.83) ug·L^{-1}; $T_{1/2}$ were (12.97 ±5.52) h. Conclution: the method described in this report was of high sensitivity, good selectivity and reproducibility for accurate determination of the plasma concentration of dioxopromethazine hydrochloride in human.

Key words: dioxopromethazine hydrochloride; high performance liquid chromatography; fluorescence detection; pharmacokinetics

P170118

Toxicokinetics of fipronil and fipronil sulfone in rabbits

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Aim: To study the toxicokinetics of fipronil and fipronil sulfone in rabbits and offer evidence for fipronil toxic clinical diagnosis and treatment. Methods: With diazepam as the internal standard, fipronil and fipronil sulfone were detectded by UV detector at 276 nm with the Hypersil-ODS C18 column and acetonitrile-methanol-water(26:24:50, V/V/V) as the mobile phase at a flow rate of 1.0 mL·min^{-1}. Six male rabbits were involved in the study and injected with fipronil 3 mg·kg^{-1}. The plasma fipronil and fipronil sulfone concentration were determined by HPLC. The plasma fipronil and fipronil sulfone concentration were calculated by 3p87 pharmacokinetical program. Results: After a single dose of 3 mg·kg^{-1} of fipronil intravenous injection to rabbits, the toxicokinetical parameters of fipronil was as follows: K_{10} was (2.08 ±0.83) h^{-1}, K_{12} was (0.34 ±0.07) h^{-1}, K_{21} was (0.27 ±0.05) h^{-1}, C_{max} was (3.48 ±0.52) mg·L^{-1}, $t_{1/2\alpha}$ was (0.31 ±0.11) h, $t_{1/2\beta}$ was (3.25 ±0.59) h, AUC was (4.96 ±1.22) mg·h·L^{-1}, Cl was (1.49 ±0.44) L·h^{-1}, V_1 was (0.67 ±0.15) L·kg^{-1}, V was (2.62 ±0.65) L·kg^{-1} respectively. The toxicokinetical parameters of fipronil sulfone was as follows: C_{max} was (1.10 ±0.12) mg·L^{-1}; $t_{1/2}$ was (81.28 ±4.82) h; AUC was (135.50 ±15.68) mg·h·L^{-1}; Cl was (0.05 ±0.005) L·h^{-1}, Vd was (2.32 ±0.11) L·kg^{-1} respectively. Conclusion: Intravenous injection administration, the kinetics of fipronil was fitted to two-compartment model and fipronil sulfone was fitted to one-compartment model. The half life of fipronil sulfone was longer than that of fipronil.

Key words: fipronil sulfone; fipronil; high performance liquid chromatography; toxicokinetics

P170119

Study on Bioequivalence of Voriconazole Dispersible Tablets in Healthy Volunteers

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Objective: To study bioequivalence of Voriconazole Dispersible Tablets in healthy volunteers. Methods: A single oral dose (200 mg of tested and reference formulation) were given to 20 healthy volunteers in a randomised crossover study. The concentrations of Voriconazole in plasma were determined by HPLC. The pharmacokinetics parameters were calculated and the bioequivalence of two formulations were evaluated by DAS program. Results: After a single dose, the pharmacokinetics parameters for Voriconazole were as follows: c_{max} were (1098.25 ±120.14) ng·ml^{-1} and (1037.01 ±81.18) ng·ml^{-1}; t_{max} were (1.35 ±0.29) h and (1.70 ±0.41) h; $AUC_{(0-24)}$ were (6720.05 ±717.19) ng·h·ml^{-1} and (6643.92 ±696.70) ng·h·ml^{-1}; $AUC_{(0-inf)}$ were (7080.97 ±747.33) ng·h·ml^{-1} and (7004.10 ±794.82) ng·h·ml^{-1} for T and R respectively. The 90 % confidential interval of C_{max}、$AUC_{(0-24)}$ and $AUC_{(0-inf)}$ of tested formulation to reference formulation were 102.1 %～109.2 %、95.0 %～107.6 % and 95.1 %～107.7 % respectively. Conclution: the relative bioavailability was (102.46 ±17.08) %, the results of the statistic analysis showed that the two formulations were bioequivalence.

Key words: voriconazole; bioequivalence; high performance liquid chromatography

P170120

Study on the distribution of ginsenoside Rg1 and its metabolites in brain

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Objective: It is well-known that ginsenoside Rg1 (Rg1) has a lot of biological activities in central neural system and it is metabolized into different products in vive, but there has few report about the pharmacokinetics and distribution of Rg1 and its metabolites. So in this paper we investigated if Rg1 and its metabolites could pass the blood brain barrier. Methods: HPLC-MS was applied to determine the concentration of Rg1 and its metabolites in rats' brain tissue at different times after orally administration. Results: Rg1 could be detected in cortex, hippocampus and striatum at one hour after orally administration in rats. The concentration of Rg1 reached its maximum at about eight hour, and it could be detected even twenty-four hour. However none of the metabolites were detected in rats' brain, which indicated that Rg1 exert its nootrophic effect and memory-enhancing and so on in central neural system but not its metabolites. Conclusion: Rg1 was metabolized out of the brain and could pass the blood brain barrier.

Key words: ginsenoside Rg1, metabolites, distribution, HPLC-MS

P170121

Determination of Captopril Concentration in Human Plasma by Reversed-phase HPLC

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Objective: A convenient, rapid and sensitive high-performance liquid chromatographic method was developed for the determination of captopril concentration in human plasma. Method: After oral administration of a single dose of captopril to each 20 volunteers at 50 mg, the plasma was collected and then captopril was immediately stabilized by froming an adduct with 4-Bromophenacyl Bromide (BPB). This adduct and the was treated by liquid-liquid extraction, and measured by high-performance liquid chromatography with UV detection. Results: The method was validated by linearity, precision and accuracy. The samples was steady in 24h after extraction. The standard curve was linear over a range of 10-500 ng ml^{-1}. The limit of quantitation was 10ng ml^{-1}. The average yield of captopril-adduct reached 99.1 %. The RSD was < 10 % in intra-batch and batch-by-batch tests. On the basis of elaborated method, a single-dose pharmacokinetics in 20 men has been investigated. The result of C_{max} was 332.88 ±141.39 ng ml^{-1}, Tpeak was 0.99 ±0.36 h, $AUC_{(0-t)}$ was 463.86 ±165.19 ng.h ml^{-1}, CL was 133.85 ±96.27 L hr^{-1}, $T_{1/2}$ was 1.80 ±0.64 h, Ke was 0.43 ±0.15 1 hr^{-1} and Vd was 412.91 ±536.05 L. Conclusion: The HPLC method possesser the feature with specify, convenient, sensitive and accurate to determine captopril concentration in plasma.

Key words: captopril, HPLC, pharmacokinetics, drug concentration in plasma

P170122

Determination of m-nisoldipine in Beagle dog plasma and the pharmacokinetics by RP-HPLC method

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Aim: To develop a sensitive and rapid HPLC method for the determination of m-nisoldipine (M-Nis) in Beagle dog plasma and to study its pharmacokinetics in Beagle dogs. Methods: M-Nis and nimodipine (Nimo, internal standard)

were extracted from plasma with diethyl ether. After liquid-liquid extraction, the sample was analyzed by HPLC with Diamond C18 analytical column (250 mm ×4.6 mm, 5 μm). The mobile phase consisted of Acetonitrile-20 mmol·L^{-1}-KH_2PO_4 (60:40) at the flow rate of 1.0 ml·min^{-1}. The UV detection wavelength was 237 nm. Results: The mean plasma concentration-time curves in Beagle dog plasma showed double peak concentrations after oral doses of 1.0, 2.5, and 12.5 mg·kg^{-1}. The time reaching to the first peak was 1h, and the time reaching to the second peak was 2~3h. The C_{max} was lower. Both the C_{max} and AUC increased proportionally with the dosages. Conclusion: M-Nis was absorbed quickly after oral administration. The lower C_{max} was possibly related to the first-pass effect, while the double peaks relevant to the hepatoenteral circulation.

Key words: m-nisoldipine; HPLC; plasma drug concentration; pharmacokinetics

P170123

Pharmacokinetic studies of Cinobufagin in male rats

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Object: To develop a RP-HPLC method for determination of cinobufagin in rat serum and to investigate the pharmacokinetics of cinobufagin. Methods: The separation was carried out by a reversed phase VP-ODS column (4.6 mm×150 mm, 5 mm) with a mobile phase consisting of methand-water (70:30, v/v), then detected at 290 nm. A total of 105 Wistar rats were included in this study. The pharmacokinetics of cinobufagin had been investigated in rats after intravenous administration 0.251, 0.503 and 1.006 mg·kg^{-1}. Results: The lowest limit of detection was 0.05 μg·mL^{-1}. The intraday and interday precisions were 8.33%-9.63% and 2.96%-3.25%, respectively. The mean recovery was 77.6%-81.3%. The calibration curve had the fine linearity in the concentration range 0.25 μg-4 μg·mL^{-1}. Conclusion: RP-HPLC method is simple, rapid, sensitive and accurate for determination of cinobufagin in rat serum. It was showed that the concentration-time curves of cinobufagin was fitted to a two compartment model with first elimination. The main pharmacokinetic parameters of cinobufagin (1, 0.5, 0.25 mg/kg) were $T_{1/2\alpha}$: 0.4830, 0.3777, 0.2723 h; $T_{1/2\beta}$: 4.4189, 5.8972, 2.4682 h; V(c): 2.5120, 8.6606, 27.9378 L·kg^{-1}; AUC: 12.1970, 8.4123, 2.9056 μg·h·mL^{-1}; CL: 2.0497, 5.9437, 34.4166 L·kg^{-1}·h^{-1}.

Key words: Cinobufagin;; Serum concentration; Pharmacokinetic parameters

P18. Pharmacogenetics and Pharmacogenomics

P180001

The effects of C3435T MDR-1 gene polymorphism of methotrexate (MTX) treatment outcome in patients with rheumatoid arthritis

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Rheumatoid arthritis (RA) is a disease of complex pathogenesis, and its treatment is mainly based on drugs modulating the course, e.g. methotrexate (MTX), sulfasalazine (SL), colchicine, gold salts or arechine (AR). Methotrexate is a substrate of efflux pump, i.e. P-glycoprotein (gp-170) encoded by MDR-1 gene, which can limit intracellular drug concentrations thus reducing its efficacy. The study was carried out on 235 rheumatoid arthritis patients treated with MTX (n=139), SL (n=70) or AR (n=26) as primary agents. MDR1 gene polymorphism was analyzed using PCR-RFLP method. It was found that patients with 3435 CC genotype significantly more often failed MTX medication as compared to 3435 TT subjects. However, the 3435 TT patients responded markedly better to corticosteroids. Any differences were observed among patients administered SL or a AR. It can be concluded that evaluation of MDR-1 C3435T polymorphism in rheumatoid arthritis patients enables individualization of RA treatment.

Key words: rheumatoid arthritis, methotrexate, MDR-1

The study was supported by grant 2P05B11029 for years 2005-2008 from the Ministry of Education and Science (Warsaw, Poland)

P180002

Tacrolimus dose requirement in relation to donor and recipient ABCB1 and CYP3A5 gene polymorphisms in Chinese liver transplant patients

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To investigate whether the heterogeneity in tacrolimus dose requirement is associated with ABCB1 and CYP3A5 gene polymorphisms in Chinese liver transplant patients during the 1st month after transplantation, ABCB1 and CYP3A5 genotyping were performed in recipients (n=50) and their corresponding donors (n=50). Tacrolimus whole blood trough concentrations were measured and doses required to achieve target blood concentrations and dose-adjusted trough concentrations (C/D ratios) were compared according to allelic status of ABCB1 and CYP3A5. Results were the tacrolimus C/D ratios were obviously lower in recipients carrying ABCB1 3435 CC genotype. For CYP3A5, recipients who received organs from CYP3A5*3/*3 donors had higher C/D ratios. Analysis of the combination of recipients' ABCB1 and donors' CYP3A5 genotypes revealed that the tacrolimus C/D ratios were significantly lower in the ABCB1 3435 CC carrying recipients, regardless of donors' CYP3A5 genotype. In conclusion, ABCB1 C3435T polymorphism is a major determinant of tacrolimus trough concentration and recipients with 3435 CC genotype will require higher dose of tacrolimus during the 1st month after transplantation.

Key words: tacrolimus; pharmacogenetics; liver transplantation

Acknowledgement: We thank our colleagues, Department of Hepatobiliary

P180003

An accurate and feasible approach for simultaneous detection of N-acetyltransferase 2 alleles in a Chinese population

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Objective: To establish a simplified PCR-RFLP method for detecting the polymorphism of NAT2. Methods: Genotypes in 150 healthy Han volunteers from 18 provinces of China were assayed by two-step PCR-RFLP method which is a new method. 20% of the samples were done by comparing phenotype status also by allele-specific amplification (ASA) method. And calculate the allele frequencies, using the Hardy-Weinberg equilibrium. Results: 20% of the samples were in complete agreement by both ASA and RFLP analysis and 100% correlation was achieved between the two methods. The NAT2 alleles frequencies in 150 Chinese (*4=63%, *5=4.3%, *6=18.3%, *7=14.3%) were different ($P<0.01$). The NAT2 genotype distribution for all detected combinations of NAT2 alleles in 150 Chinese subjects was consistent with Hardy-Weinberg equilibrium. Conclusions: The procedure is simple and suitable for clinical applications. The lower frequency of mutant *5 allele compared with that of Caucasians explains the low frequency of slow acylators in Chinese.

Key words: NAT2; PCR-RFLP; genotyping; polymorphism

This project was supported by the Nation Natural Science Foundation of China (No.30472055)

P180004

CYP3A5 and MDR1 genetic polymorphisms and correlation with tacrolimus pharmacokinetics in Chinese liver transplant patients

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Objectives We investigated the single nucleotide polymorphisms (SNPs) of CYP3A5 and MDR1 genes in mainland Chinese Han and Uygur, and genetic effects on tacrolimus concentration/dose (C/D) ratio in whole liver transplant patients. Methods Two hundred and four Chinese healthy subjects were genotyped using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. Tacrolimus concentration values were determined in 54 liver transplant patients with an automated microparticle enzyme immunoassay. Results An intermediate frequency of CYP3A5*3 (82.7%) was observed in Chinese Uygur, between Chinese Han (73.3%) and Caucasians (91.7%). Significantly higher tacrolimus C/D ratios were observed in patients engrafted with liver carrying CYP3A5*3/*3 genotype during 1-2 weeks post-transplantation. Conclusions The intermediate frequency of CYP3A5 SNP in Chinese Uygur might be due to the genetic admixture of Eurasians and Orientals. The genotype-phenotype analysis suggested that graft CYP3A5 genotype could contribute to the interindividual variability of tacrolimus pharmacokinetics in liver transplant patients.

Key words: CYP3A5, MDR1, SNPs, liver transplantation.

P180005

Single-nucleotide polymorphisms of the interleukin-18 gene promoter region in atopic asthma patients

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Atopic asthma is a chronic inflammatory disorder, which is associated with atopy and IgE mediated inflammation. Interleukin-18 (IL-18) is a proinflammatory cytokine postulated to play an important role in the regulation of TH1 as well as TH2 immunologic responses and thus in the development of chronic inflammatory diseases. Recently, it has been shown that the IL-18 protein expression is regulated by two single-nucleotide polymorphisms located at positions -607 (C>A) and -137 (G>C) in the promoter region of the gene. In the present study, we analyzed the IL-18 gene promoter region genotypes and combined genotypes (-607/-137) in 142 asthmatic patients and 185 unrelated healthy controls in association with disease susceptibility and severity. The genotyping was performed using PCR-RFLP method. The AC/AC diplotype was observed in 5.6% and 11.9% of asthmatic and healthy subjects, respectively ($P < 0.05$). No significant influence was found of IL-18 diplotypes on the FEV1. The results suggest that the AC/AC diplotype which is associated with low IL-18 expression seems to have the protective effect against atopic asthma development.

Key words: IL-18, polymorphism, athopic asthma

P180006

ABCB1 HAPLOTYPES DETERMINE METHADONE DOSAGE REQUIREMENTS

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This retrospective study investigated haplotypes of the ABCB1 gene, encoding P-glycoprotein, in opioiddependent subjects on methadone (MD) maintenance (MM, n = 60) and non-opioid-dependent controls (C, n = 60). Subjects were genotyped for five common SNPs; A61G, G1199A, C1236T, G2677T and C3435T, and frequencies of inferred haplotypes compared between groups. The relationship of haplotype to MD dose requirements (15-110 mg/day, n = 56) was also investigated. Chi-square analysis revealed a significant overall difference in haplotype frequencies between MM and C subjects ($p < 0.05$), with a significantly lower frequency of the AGTGT haplotype among MM subjects (33.3%) compared to controls (50.8%, $p < 0.01$). MM subjects homozygous for the AGCGC haplotype had significantly higher doses (mg/day) of MD (mean ± SEM, 98.3 ± 6.0) than heterozygous (59.7 ± 4.1, $p < 0.05$) and non-carriers (54.7 ± 4.9, $p < 0.01$). Also, MM subjects carrying the AGCTT haplotype had significantly lower doses of MD than non-carriers (38.0 ± 7.5 v 61.3 ± 3.4, $p < 0.05$). Therefore, it is possible that ABCB1 pharmacogenetics may influence MD dosage.

Key words: ABCB1, methadone, pharmacogenetics.

Acknowledgements: Royal Adelaide Hospital, University of Adelaide.

P180007

RELATIONSHIP BETWEEN THE ABCB1 GENETIC POLYMORPHISM AND CLINICAL OUTCOMES IN RENAL TRANSPLANT PATIENTS.

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We investigated the relationship between donor and recipient ABCB1 haplotypes and clinical outcomes following renal transplantation in patients receiving the P-glycoprotein substrates cyclosporin and tacrolimus for immunosuppression. Genotyping was performed from recipient blood and donor blood or tissues and clinical outcomes recorded from recipient case notes. There were no significant differences in the variant allele frequencies between recipients and donors at positions 61, 1199, 1236, 2677 and 3435 ($p > 0.05$), however, up to 40% of donor/recipient pairs had different haplotypes. Donor haplotype at position 61 was associated with changes in the plasma creatinine between 3 and 12 months: G61, -2.8 ± 6.4% (n = 6); A61, 11.9 ± 5.0% (n = 13), p = 0.036; and creatinine levels in recipients at one month G61, 123.7 ± 11.6 micromol/l (n = 12); A61, 149.5 ± 7.3 micromol/l (n = 34), p = 0.034. Clinical outcomes were not influenced by variability at other positions of ABCB1. Therefore, the G61 variant of ABCB1 may protect against nephrotoxicity.

Key words: ABCB1, renal transplant

Acknowledgements: Kidney Health Australia, Queen Elizabeth Hospital and University of Adelaide

P180008

Relationship between CYP2C8 and CYP2C9 genotypes and diclofenac metabolism in Spanish healthy volunteers.

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CYP2C8 seems to be involved in diclofenac 5-hydroxylation, while the 4'-hydroxylation and 3'-hydroxylation seems to be mediated mainly by CYP2C9 (1) in vitro. We have demonstrated the relevance of CYP2C9 genotypes for diclofenac 4'-hydroxylation in healthy volunteers (2). The aims of this study were to analyze the role of both CYP2C8 and CYP2C9 genotypes on the diclofenac metabolism. To determine the allelic frequencies of CYP2C8 alleles and its relationship with CYP2C9 variants was also aimed. A group of 142 (72 males/70 females) white Spanish healthy volunteers were studied. CYP2C8 and CYP2C9 genotypes were determined by allele-specific PCR-RFLP methods (2,3). The urinary concentrations of diclofenac and its main metabolites were analysed using a HPLC-UV method (4) after the administration of a single oral dose of 50 mg diclofenac (8 hours) as previously described in part of the population studied in here (2). The results showed that the urinary concentration ratio diclofenac/5-hydroxydiclofenac was higher in individuals carrying CYP2C8*3 or CYP2C8*4 allele than in subjects homozygous for wild-type allele CYP2C8*1 ($p < 0.05$). Moreover, approximately 93% of the subjects with a CYP2C8*3 allele also carried a CYP2C9*2 and 80% of the subjects that had CYP2C9*2 variant also carried a CYP2C8*3. In addition, the four individuals CYP2C9*2/*2 were CYP2C8*3/*3. In conclusion, this is the first study showing the influence of CYP2C8 genotypes on diclofenac metabolism in healthy volunteers. The linkage disequilibrium between CYP2C8*3 and CYP2C9*2 alleles was also confirmed in the Spanish population.

Key words: CYP2C8; CYP2C9; diclofenac; linkage disequilibrium; healthy volunteers.

Acknowledgement: supported by Consejería de Sanidad y Consumo, Junta de Extremadura (SCSS0575), and coordinated in the network Red Iberoamericana de Farmacogenética y Farmacogenómica (206RT0290). P.D. and M.C. are supported by grants from Junta de Extremadura, Consejería de Infraestructura y Desarrollo Tecnológico and Fondo Social Europeo REI05A003 and FIC04A096, respectively.

P180009

A Comparison of CYP2B6 Allele and Genotype Frequencies in Healthy Han and Uygur Chinese

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The purpose of this study was to investigate the frequencies of alldic variants of CYP2B6 in healthy Han and Uygur Chinese. Five non-synonymous mutation of CYP2B6-C64T, G516T, C777A, A785G and C1459T, were carried out in 193 unrelated Han Chinese and 91 unrelated Uygur Chinese by using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method. Allele frequencies for CYP2B6*2, *3, *4, *5, *6, *7 and *9 in Han and Uygur Chinese were 0.034 and 0.027, 0 and 0.011, 0.091 and 0.033, 0.003 and 0.049, 0.184 and 0.214, 0 and 0.022, 0.018 and 0.044, respectively; Ethnic variation in allele frequencies was observed for CYP2B6*4 ($P = 0.014$), *5 ($P = 0.010$), and *7 ($P < 0.001$). Our results showed that there were marked ethnic differences in the mutant frequencies of CYP2B6. These results may help to improve individualization of drug therapy and offer a preliminary basis for more rational use of drugs that are substrates for CYP2B6 in different Chinese population.

P180010

Naturally occurring variations in the human 5-HT3A gene profoundly impact 5-HT3A receptor function and expression

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Five naturally occurring single nucleotide polymorphisms leading to amino acid changes have been identified in the coding region of the 5-HT3A gene. We investigated functional effects of these on the serotonin (5-HT)-gated ion channel 5-HT3A using fluorescence-based cellular assays. Notably, three of the variant receptors displayed 5-HT-induced maximal responses of 4-60% of the wildtype (WT) response, whereas two exhibited WT-like function. Co-expression of WT subunits with each of the former subunits gave rise to 'mixed' receptors that displayed reduced maximal responses to 5-HT compared to WT. All variant receptors displayed WT-like ligand potencies. Total expression of variant and WT subunits was similar but surface expression of three variants was reduced to 28-43% of the WT level. All variants displayed Kd values similar to the WT receptor. In summary, three variations caused functionally impaired receptors. Three variant receptors were surface expressed at reduced levels in spite of WT-like total expression, implying that these variants affect receptor biogenesis/trafficking.

Key words: 5-HT3, polymorphism, 5-HT

The work was financed by Center for Pharmacogenomics/the Lundbeck Foundation

P180011

Thiopurine S-methyltransferase genotype predicts adverse drug reactions to thiopurine drugs in renal transplant recipients

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Objective: This study explored the association between Thiopurine S-methyltransferase (TPMT) genetic mutations and the occurrence of azathioprine adverse effects in 122 Chinese renal transplant population. Methods Clinical data were evaluated during the first year after renal transplantation. TPMT genetic polymorphism was determined using polymerase chain reaction-based assays in patients and control. Results Eight patients possessing a single TPMT nonfunctional mutant allele were identified: TPMT*3C (n=8). Among five patients who developed haematopoietic toxicity, four had one TPMT variant alleles (80%). Conclusions TPMT heterozygotes were associated with significant reductions in hematological indices and a significant decrease in cyclosporine plasma concentrations in the first month post-transplant. Genotyping for the major TPMT variant alleles may be a valuable tool to reduce the risk of toxicity and improve efficacy with thiopurines in renal transplant recipients.

Key words: Azathioprine; Thiopurine methyltransferase; Pharmacogenetics; renal transplantation

Acknowledgement A part of this study was carried out in Henan Key Laboratory for Molecular Medicine.

P180012

The Phe124Cys mutant of the 5-HT1B-receptor reduces the contribution of 5-HT2A receptors to 5-hydroxytryptamine-induced contraction of human temporal artery

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The recombinant Phe124Cys mutart of the human 5-HT1B receptor has a 3-fold higher agonist affinity than the wildtype Phe124Phe receptor. Agonist-induced contractions through coexisting 5-HT1B and 5-HT2A receptors were studied in arterial rings from 98 patients undergoing neurosurgery. Genotyping disclosed 3 Cys/Phe patients which probably yielded coexpression of both 124Phe and 124Cys 5-HT1B receptors. In 95 Phe/Phe patients only the 124Phe receptor was expressed. The contractile potencies of 5-hydroxytryptamine (5-HT) and sumatriptan did not differ in arteries from Cys/Phe or Phe/Phe individuals. The 5-HT1B receptor antagonist SB224289 was 5-fold more potent in blocking the effects of 5-HT in arteries from 3 Cys/Phe than from 30 Phe/Phe individuals ($P < 0.03$). The fraction of 5-HT effects via 5-HT1B receptors, related to the total contractile amplitude via 5-HT1B plus 5-HT2A receptors, was enhanced from 0.42 ± 0.03 in 88 Phe/Phe individuals to 0.75 ± 0.10 in 3 Cys/Phe individuals ($P < 0.05$). The contribution of 5-HT1B receptors to the mediation of the effects of 5-HT is increased in Cys/Phe compared to Phe/Phe individuals.

P180013

Correlation of methylprednisolone chemosensitivity in vitro with C3435T MDR1 polymorphism and clinical outcome in childhood acute lymphoblastic leukemia.

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Objective: Cellular drug resistance measured at initial diagnosis is associated with an increased relapse risk and unfavorable clinical outcome in childhood ALL. In addition, the presence of adverse clinical prognostic factors such as age, and pro-B and T-lineage immunophenotype have been shown to be associated with cellular resistance to drugs in children with ALL. P-glycoprotein (Pgp), the gene product of MDR1, confers multidrug resistance to a number of antineoplastic agents. A silent mutation in the exon 26 (C3435T) has been associated with altered expression and function of Pgp in tissues. Methods: MTT cytotoxicity assay, PCR alnalysis of C3435T polymorphism in MDR1 gene. Results: We compared the impact of C3435T polymorphism on in vitro chemosensitivity of leukemia cells to methylprednisolone. CC genotype carriers showed higher IC50 values in comparison with the carriers of CT or TT genotype. Statistical analysis Mann-Whitney U test showed $P = 0.035$. Conclusions: The group of patients with CC genotype seems to be more resistant to glucocorticoids, or at least methyprednisolone.

Key words: ALL, MDR1 gene polymorphism, glucocorticoid

Supported by grant VEGA 11/2266/05 and 21/3372/06

P180014

The effects of polymorphisms on the functions of CB2 cannabinoid receptor

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CB2 plays an important role in regulating immune functions. Two non-synonymous single nucleotide polymorphisms are found on human CB2 gene. Both Q and R are found at position 63 of the first intracellular loop, and H and Y at position 316 of the C-terminal tail. We hypothesized that these alterations may have functional significance on CB2 receptor. To test our hypothesis, Q63R and H316Y, and Q63R/H316Y mutations were made by site-directed mutagenesis. Ligand binding and functional assays were used to test these mutant receptors stably expressed in HEK293 cells. In ligand binding studies, all mutant and wildtype receptors exhibited similar affinities to cannabinoid ligands. In cAMP accumulation assays, three of the five compounds tested had similar efficacy on mutant receptors as compared to wildtype CB2, but WIN55212-2 and 2-arachidonoylglycerol exhibited reduced efficacy on mutant receptors. In cell migration assays, WIN55212-2 demonstrated reduced efficacy on mutant receptors. In conclusion, these data suggest that the presence of polymorphisms at both positions 63 and 316 produced a ligand-dependent alteration in CB2 receptor functions.

Key words: CB2 cannabinoid receptor, single nucleotide polymorphism.

P180015

Effects of CYP2C9 and MDR1 polymorphism on the pharmacokinetics of losartan

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Objects: We studied the frequencies of CYP2C9 and MDR1 variant alleles in Korean population and the effects of major polymorphisms of CYP2C9 and MDR1 gene on pharmacokinetics of losartan. Methods: 358 healthy Korean subjects were recruited and genotyped for the variant alleles of CYP2C9 (*1, *2, *3, *4, *5, *11 and *13) and MDR1 (exon 21 and exon 26). Genotyping was done using PCR-RFLP method or direct sequencing. A 50 mg oral dose of losartan was given to 27 Korean volunteers with different CYP2C9 and MDR1 genotypes. Results: In subjects with CYP2C9 *1/*3 or CYP2C9 *1/*13, C_{max} and AUC of losartan were significantly greater, the half-life of losartan sig-

nificantly longer and oral clearance significantly lower than those with CYP2C9 *1/*1. Significant differences could be observed among the subjects with different MDR1 genotypes (GG/CC, GT/CT and TT/TT; G2677T/C3435T) for the AUC and C_{max} of losartan and E-3174 (a metabolite of losartan). Conclusion: The CYP2C9*3 allele was shown to be associated with decreased formation of E-3174 from losartan and MDR1 variants were associated with the disposition of losartan and E-3174.

P180016

Large Differences in Testosterone Excretion in Asian and Caucasian men Associated with an UGT2B17 Polymorphism- Implications for Doping Tests

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Inter-ethnic variation in androgen disposition may be related to differences in prostate cancer rate and a confounder in certain anti-doping tests. UDP-glucuronosyl transferases have a key role in the metabolism of androgens. Recently a deletion polymorphism was detected in the UGT2B17 gene. Objective: We evaluated the contribution of the UGT2B17 deletion polymorphism to the inter-individual and inter-ethnic variation of androgen metabolism and excretion. Methods: and Results: Urine from 122 Swedish and 74 Korean healthy men were analyzed for several androgen glucuronides including testosterone. Distribution of the log concentrations of testosterone and several other androgens was bimodal in both groups, suggesting a monogenic inheritance. All UGT2B17 del/del subjects had no or negligible excretion of testosterone. The del/del genotype was 7 times more common in Koreans (67 %) than in Swedes (9.3 %). Swedish subjects had significantly higher levels of serum testosterone. Conclusions: We show that the UGT2B17 polymorphism is strongly associated with the bimodal distribution of the testosterone excretion as well as the large differences in androgen excretion between Koreans and Swedes.

P180017

Genetic Predisposition to Postsuccinyldicholine Apnea in the Armenian Population

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More than 30 years ago it was shown that the patients responded abnormally to the action of the muscle relaxant succinyldicholine (ditiline) were carriers of mutant form of butyrylcholinesterase (BuChE, E.C.3.1.1.8). Patients with a genetically inherited, mutant form of BuChE responded with prolonged apnea. In some developed countries the patients previously are tested to avoid the post surgery complications. This work reports the frequency of carriers of the mutant form of BuChE in Armenian population for the first time. The BuChE activity was measured in plasma samples from 1250 (48.56 % male and 51.44 % female) healthy persons by the colorimetric, modified automatic method of Dietz. The determination of dibucaine number was applied for phenotyping of BuChE. From tested patients only 0.08 % and 1.2 % can be considered as subjects who are homozygous and heterozygous for the atypical BuChE allele. One subject had less than 10 % of the normal activity and so classified as homozygous for silent BuChE. The data show that the frequency of mutant forms of BuChE in Armenian population does not exceed the average value (2 %) in Europe and USA.

Key words: butyrylcholinesterase, mutant form, dibucaine number

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P180018

Effects of CYP2C9 polymorphism on the pharmacokinetics of irbesartan in healthy Korean subjects

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Objects: CYP2C9 is the principal enzyme responsible for the metabolism of numerous clinically important drugs. Genetic polymorphism of this enzyme shows high ethnic varuations. Previous in vitro studies indicate that glucuronidation and oxidation are the major routes of metabolism of irbesartan and that the CYP2C9 is the primary pathway for oxidation. In this study, the effect of major polymorphism of the CYP2C9 on the pharmacokinetic of irbesartan was investigated. Methods: A 150 mg oral dose of irbesartan was given to 20 Korean volunteers with different CYP2C9 genotypes (CYP2C9 *1/*1, *1/*3 and *1/*13). Irbesartan was dwtermined by HPLC. Results: In subjects with CYP2C9 *1/*3 or *1/*13 genotype, the AUC and C_{max} were significantly greater than those in subjects with CYP2C9 *1/*1. Conclusion: The pharmacokinetics of irbesartan are significantly affected by genetic polymorphism of CYP2C9.

Key words: Irbesartan, CYP2C9, polymorphism, pharmacokinetics

Acknowledgment: This study was supported by KFDA research Fund.

P180019

Comparison of Pharmacokinetic Variability of Metformin in German Caucasian and Chinese subjects

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Purpose: To evaluate the inter- and intra-individual variability of kinetics of metformin, a representative substrate of organic cation transporter (OCT2) in Germans and Chinese. Methods: Metformin kinetic data following 2 bioequivalent formulations in healthy Germans (n = 24) or Chinese (n = 28) were evaluated. The inter- and intra-subject variances were estimated based on ANOVA. The genetic contribution (rGC) was calculated using a standard formula from these variances. Results: The mean metformin oral clearance (CL/F) was 1.35 and 1.05 L/h/kg ($p < 0.01$) (with inter-individual CV of 32.1 and 32.2 %) in Germans and Chinese respectively. Their mean drug elimination half-life ($T_{1/2}$) was 3.89 and 3.78 h and the respective rGC 0.76 and 0.72 ($p > 0.05$). Conclusion: The total exposure of metformin appeared to be greater and its elimination t 1/2 and variability were similar in German Caucasian and Chinese subjects. Data mining from the bioequivalence studies in different ethnic groups may provide a rapid approach for identifying potential differences of commonly used drugs in different ethnic populations.

Key words: pharmacogenetics, metformin, transporters

P180020

PRELIMINARY STUDY ON THE ASSOCIATON OF MDR1 GENE POLYMORPHISMS AND LUNG CANCER RISK

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The aim of this initial study was to find differences in the frequency of MDR1 (*ABCB1*) common polymorphisms between lung cancer patients and healthy subjects. The population of study consisted of 98 Caucasian patients and 44 controls matched for age and smoking exposure. Genomic DNA was amplified and the presence of the C3435T and G2677T/A mutations assayed by sequencing methods. An analysis of the haplotypes showed that the number of mutant homozygous carriers (*T*-T) was higher in patients than in controls (21.4 and 15.9 %, respectively). Allelic and genotype frequencies for the C3435T polymorphism were unaltered between both population groups. However, lung cancer patients showed a significantly higher frequency for the 2677T variant allele than did healthy individuals [0.67 vs. 0.45; $p < 0.0001$, OR: 2.4 (1.4-4.0)]. Of all histological types analyzed, subjects with epidermoid carcinoma showed the highest frequency for the T-allele [0.74, $p < 0.0001$ vs. controls, OR: 3.6 (1.9-6.7)]. These preliminary results suggest the G2677T polymorphism is associated with lung cancer risk, probably by affecting the expression and/or function of P-glycoprotein in lung tissue.

Key words: MDR1, polymorphisms, lung cancer

P180021

Endothelial nitric oxide synthase gene haplotypes associated with circulating concentrations of nitric oxide products in hypertensives patients.

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Objective: In this study we compared the distribution of haplotypes (HAP) involving three relevant eNOS polymorphisms (T-786C-promoter; b/a-intron 4 and Glu298Asp-Exon 7) in and hypertensives (HT) patients with low and high circulating NOx levels. Methods: We studied 68 HT. Genomic DNA was isolated from blood samples and genotypes were determined by PCR. Circulating NOx was determined by chemiluminescence. Results: HAPs frequencies were

compared in two groups in of participants: those with lower NOx levels (group L) and those with higher NOx levels (group H) than median. The HAP including the alleles C, 4b, and Asp was significantly more common in group L (23%) than in group H (6%) and the haplotype C, 4b and Glu more frequent in group H (26%) than L (6%). The frequencies of the remaining HAP were not different among group L and H. Conclusion: These results are very interesting because the HAP more frequent in L group is a marker of development of hypertension and the HAP more frequent in H group is a marker of protection to development hypertension (1).

P180022

Polymorphisms of CYP2D6 in the Czech population.

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CYP2D6 a member of cytochrome P450 enzymes metabolises over 25% of commonly used drugs.

Aim: The aim of this study was to validate the genotyping methods and to investigate the frequency of important variant alleles of CYP2D6 gene through the Czech population. Methods: DNA of 223 unrelated volunteers were analysed to detect the presence of CYP2D6 *6, *5, *4, *3, and gene duplication. Presence of CYP2D6 *5 and gene duplication was analysed by long range PCR, for other alleles PCR - RFLP was applied. Results: The variant allelic frequencies in our populatien were 0.22%, 3.14%, 22.87%, 1.12% and 3.14% for CYP2D6 *6, *5, *4, *3, and duplication, respectively. Fifteen subjects carried two variant alleles leading to predicted poor type of metabolism, 84 subjects were heterozygous extensive metabolizers. The distribution of variant alleles complies to the Hardy - Weinberg equilibrium. Conclusions: The frequencies of variant alleles of CYP2D6 in Czech population are in concordance with the other Caucasians. The methodology can be used in future pharmacokinetic studies.

Key words: CYP2D6, polymorphism, genotype, frequency

Acknowlegement: This work has been supported by a grant IGA No. 1A/8632 5.

P180023

Polymorphisms of MDR1 and CYP2C9 genes in the Czech population.

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Aim: The aim of this study was to investigate the frequency of functionally important SNPs of MDR1 and CYP2C9 genes in the Czech population. Methods: DNA was isolated from whole blood of 163 healthy, young and unrelated subjects. The genotypes of polymorphic positions C3435T, and G2677T/A of MDR1 and CYP2C9 *2 (C430T) were determined by PCRRFLP. Results: Observed allelic frequencies of MDR1 were 56.75%, 47.55%, and 0.61% for the alleles 3435T, 2677T, and 2677A, respectively. We have found 59 subjects homozygous for 3435T, and 40 for 2677 T alleles. The variant allelic frequency of CYP2C9 *2 was 14.4%. The frequencies of wild type homozygous in our population were 74.8%, of heterozygous 21.47% and 3.68% of variant homozygous The distribution of variant alleles complies with the Hardy - Weinberg equilibrium. Conclusions: Allelic frequencies of functionally important MDR1 and CYP2C9 *2 variants in Czech population are in concordance with the other Caucasian populations.

Key words: CYP2C9, MDR1, polymorphism, genotype

Acknowledgement: Supported by a grant GAUK18/C/2005

P180024

Angiotensin - Converting Enzyme Deletion (ACE D) Polymorphism and Ischaemic Stroke in Multi - Ethnic Malaysian Population

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Background: The ACE D polymorphism has been shown to be associated with ischaemic stroke in some population studies. We investigated the incidence of the ACE D polymorphism in multi - ethnic Malaysian ischaemic stroke patients. Methods: 117 ischaemic stroke patients and 189 controls were recruited from University Malaya Medical Centre. They were of Chinese, Indian and Malay ethnicity. The ACE D polymorphism was analysed by PCR. Results: The DD genotype was significantly more common in the stroke group ($\chi^2 = 7.59$, $p = 0.02$), with the stroke genotype frequency being 0.37, 0.43 and 0.21 for II, ID and DD genotypes respectively, while the control group frequency was 0.49, 0.40 and 0.11 respectively. When analysed by separate ethnic groups, we found that it was only significant in the Chinese ($\chi^2 = 6.48$, $p = 0.04$). The D allele distribution was also significantly higher in the Chinese ($\chi^2 = 4.36$, $p = 0.04$). Conclusion: The deletion polymorphism of ACE may be associated with increased risk for ischaemic stroke in the Malaysian Chinese population.

Key words: Angiotensin converting enzyme, ischaemic stroke, polymorphism

Acknowledgement: Universiti Malaya, for vote F research grant (F0355/2004A)

P180025

Dopamine D2 Receptor (DRD2) Gene -141C Insertion/Deletion Polymorphism In Schizophrenic Patients.

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Schizophrenia is a chronic and neuropsychiatric disease. An increase in dopamine and DRD2 receptor gene products has been well described in schizophrenic patients. Our objective was to determine the relationships among schizophrenic symptoms in schizophrenia subtypes and severity of symptoms in terms of DRD2 gene - 141C Insertion/Deletion (Ins/Del) polymorphism. Restriction fragment length polymorphisms at the dopamine D2 receptor gene (DRD2) locus for BstNI for the detection of - 141C Ins/Del polymorphism was investigated in 73 patients with schizophrenia and 60 control subjects. The allelic frequencies of the DRD2 gene - 141C Ins/Del polymorphisms in case and control groups were 79.5% and 77.5% for I allele; 20.5% and 22.5% for D allele respectively. There was no significant difference in frequencies of genotypes and alleles between the two groups. In schizophrenic and control subjects, there were no significant relationship in severity of the disease and schizophrenia types among the - 141C Ins/Del genotypes and alleles.

Key words: DRD2 gene, polymorphism, schizophrenia.

P180026

Ethnicity, genetics and tailored pharmacotherapy

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Ethnic differences exist in both pharmacodynamics and pharmacokinetics of many drugs that are well documented by the comparison studies of propranolol, atropine and morphine between Chinese and White normal subjects. Ethnic difference in drug metabolism and sensitivity exists not only between Caucasians and Chinese, but also between the different ethnic groups in Chinese. Such differences usually reflect differences in the distribution of polymorphic traits, which occur at different frequencies in different population. The different frequency for the mutant alleles results in variations in the frequency of subjects who are homozygous for the mutant allele among the extensive metabolizers in different ethnic populations. Therefore, the plausible biological justification for making racial differences in drug response is genetic polymorphism of drug metabolizing enzymes, transporters and receptors. For instance, many Asians metabolize CYP2D6 - mediated drugs more slowly than Caucasians, due predominantly to high frequencies of variants of 2D6 *10, a reduced function allele. However, the inter - ethnic differences do not seem to be larger than intra - ethnic variations which means variability within populations seems to be greater than differences between populations. Interindividual variation of drug efficacy and toxicity is determined by genetic polymorphisms of drug metabolizing enzymes, transporters and receptors. Evidence indicated that in codominant alleles, the more or less drug metabolizing enzyme activity is linearly related to the number of genes of one type substituted by another type. In most cases the changes in gene expression may accompany drug - metabolizing enzyme gene polymorphism and cause alteration in enzymatic activity showing a gene - dosage effect. For instance, the activity of CYP2C19 was higher in the homozygous extensive metabolisers (EMs) compared with that in heterozygous EMs, and the latter was higher than that in the PMs (homozygotes of mutant alleles). The variability of receptor sensitivity may also relate the number of functional alleles of the correspondent encoded genes. Since the genotype of drug metabolizing enzymes, drug transporters and receptor determine the drug metabolism and drug efficacy, the determination of genotype of such proteins plays an important role in optimization of therapy for the individual patient. Even though the additional larger and controlled studies are needed to justify changes of treatment strategies, the pharmacogenetics approach to individualize therapy in some patients is promising. The genetic approach based on gene analyses is de-

veloping as a valuable tool to design tailored pharmacotherapy. To translate pharmacogenetics knowledge to the treatment of patients, a Tailored Therapy Center was founded in October 2004 at the Third Xiang Ya Hospital, Central South University. The Clinic offers patient tailored hypertension therapy; The Center is pioneering the use of patient tailored hypertension therapy and will continually use state-of-the-art research facilities to perform advanced testing of a patient's genotype of hypertensive pharmacotherapy genotype to determine which medications are effective against it and what dosage levels are needed to treat it. The goal of this tailored approach is to deliver the most effective therapy, while minimizing possible side effects related to drug dosing. Over 1300 hypertensive patients were treated through the Central. We have demonstrated that patient tailored therapy improves quality of life and is a superior treatment model.

P180027

Liver dysfunction markedly decreases the inhibition of CYP1A2-mediated theophylline metabolism by fluvoxamine

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Objectives: To evaluate the effect of cirrhosis on the inhibition by fluvoxamine of theophylline metabolism, to assess whether liver dysfunction has any influence of drug interaction involving CYP1A2. Methods: The study was carried out in 10 healthy volunteers, 10 patients with Child A, and 10 with Child C cirrhosis, according to a randomized, double-blind, 2-phase, crossover design. Results: Fluvoxamine-induced inhibition of theophylline clearance decreased from 62%, in controls, to 52% and 12% in Child A and C cirrhotics, respectively. CYP1A2-mediated formations of 3-methylxanthine and 1-methyluric acid were totally inhibited in controls, but reduced by only one third in Child C cirrhotics. Inhibition of 1,3-dimethyluric acid formation decreased from 58%, in controls, to 43% and 7% in patients with Child grade A and C cirrhosis, respectively. Conclusions: Two mechanisms are proposed to explain the attenuating effect of cirrhosis on CYP1A2 inhibition: decreased sensitivity to fluvoxamine of CYP1A2-mediated biotransformations, probably due to reduced uptake of the inhibitory drug by the cirrhotic liver; reduced hepatic expression of CYP1A2, which makes its inhibition less important.

Key words: theophylline-fluvoxamine interaction, liver disease

This work was supported by a grant from the University of Padova.

P180028

Impact of Apo-E genotype on the response to donepezil therapy in patients with Alzheimer's Disease

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Aim of the present study was to evaluate the impact of genotype for apolipoprotein E (ApoE) on the response to donepezil therapy. ApoE genotype was investigated by TaqMan allelic discrimination in 73 patients affected by Alzheimer's disease (AD), evaluated by Mini Mental State Examination (MMSE) screening test, before and three months after starting the therapy with donepezil. Five patients (6.9%) carried two and 27 (37%) carried one APOE-ε4 allele, while 39 (53.4%) were homozygous for ε3, and 2 (2.7%) were heterozygous ε2/ε3. Subjects carrying two ε4 alleles showed a slightly, though not statistically significant, poorer response, as compared to subjects with other genotypes (mean changes in MMSE score: -1.8 vs -0.31, respectively). However, no statistically significant association was found between ApoE genotype and response to donepezil. Our data suggest that the ApoE genotype is unlikely to play a major role in the response to donepezil therapy in patients with AD.

P180029

CAFFEINE-BASED APPROACHES FOR ASSESSMENT OF CYTOCHROME P450 1A2 (CYP1A2), XANTHINE OXIDASE (XO) AND N-ACETYLTRANSFERASE 2 (NAT2) ACTIVITIES IN VIVO.

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To compare different approaches of the probe drug caffeine (1,3,7-trimethylxanthine) (137X) for assessment of CYP1A2, XO and the polymorphic NAT2 metabolic activities, 10 male healthy subjects performed a caffeine test at four time points with a wash-out period of two weeks. The volunteers received different sources of caffeine and doses: A, 360 ml (12oz) of Coca-Cola (approx. 45 mg); B, 150 ml (5oz) cup of brewed coffee (approx. 100 mg); C, a single 150-mg oral dose; and D, a single 300-mg oral dose. The molar urinary ratios (AFMU+1U+1X+17U+17X)/137X (5-acetylamino-6-formylamino-3-methyluracil+1-methyluric acid+1-methylxanthine+1,7-dimethyluric acid+1,7-dimethylxanthine)/137X; 1U/(1U+1X) and AFMU/(AFMU+1U+1X) were used as indices of CYP1A2, XO and NAT2, respectively. The ratios did not show significant differences between the four time points. CYP1A2 ranged from 195 to 289 ($p < 0.9$); XO ranged from 70 to 137 ($p < 0.5$) and, NAT2 ranged from 0.021 to 0.032 ($p < 0.9$). Our data indicate that any of these caffeine-based approaches, at least in the range of doses tested in our study, can be used for metabolic purposes. In adults, we use 100 or 150 mg of caffeine successfully.

P180030

Phenotypic-genotypic analysis of CYP2C19 in a Chinese population

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Aim: To evaluate the phenotype-genotype of CYP2C19 in A Chinese population. Methods: Omeprazole are acted as an probe drug of CYP2C19 phenotype; Mutations were identified by PCR and enzyme digestion. Results: 9 subjects (13.8%) are identified as poor metabolizers(PMS). Among the 130 alleles, *2 and *3 were found in 66 alleles(50.8%) and 3 alleles (2.3%), respectively. 8 subjects(12.3%) carried two defect alleles(*2/*2, *2/*3 or *3/*3), 33 subjects (50.76%) were heterozygous for a mutant(*2 or *3) and a wild type (*1) allele, and the remaining 26 (40%) homozygous for *1 allele. From a total 9 PMs, 8 were genotypically PMs by analysis of the *2 and *3 alleles and only one PM was found to be heterozygous for the *1 and *3 alleles. At present it can not be judged whether this subject has a defective allele with a so-far unidentified mutation or a true wild type allele. Conclusion: The frequency of PMS of CYP2C19 identified in the Chinese population was 13.8%. Of the 65 subjects, 98.5% concordance was noted between phenotypic and genotypic findings.

Key words: CYP2C19, Chinese, polymorphism

Acknowledgements: Omeprazole and its two metabolites are gifts by Sweden AstraZeneca R&D Mölndal.

P180032

Relationship of P450 2C9 Genetic Polymorphisms in Chinese and the Pharmacokinetics of Tolbutamide

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AIM: To study the relationship of P450 2C9 genetic polymorphisms and the pharmacokinetics of tolbutamide in Chinese. METHODS: Using tolbutamide as a probe of P450 2C9 activity, P450 2C9 phenotype in 63 healthy individuals expressing the P450 2C9 *1/*1, *1/*3 and *3/*3 genotypes were evaluated. After administration of 500 mg tolbutamide pill, plasma and urine samples were collected from each subject over a 24-hour period. RESULTS: Tolbutamide AUC$(0\rightarrow\infty)$ was significantly increased by 20% and 116%, and $T_{1/2}$ was increased 60% and 813%, respectively, in subjects expressing the P4502C9 *1/*3 and *3/*3 genotypes compared with *1/*1 subjects. Significant reductions in tolbutamide oral clearance (68% and 11%) and formation clearance (39% and 3%) were detected in the *1/*3 and *3/*3 individuals, respectively, compared with */*1 subjects. CONCLUSION: The P450 2C9 activity was significantly reduced in *1 heterozygotes compared with *1 homozygotes, and the metabolism of tolbutamide was more severely impaired in *3/*3 individuals compared with those expressing *1/*3. Using tolbutamide as a P450 2C9 probe, P450 2C9 genotype was the major determinant of P450 2C9 phenotype.

P180033

Effects of CYP2C9 and VKORC1 polymorphisms on fluindione anticoagulation status.

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Our objective was to assess whether there is an association between the presence of allelic variants of CYP2C9, VKORC1 and anticoagulation problems during the initial phase fluindione (FL) treatment compared to acenocoumarol (AC). Twenty four healthy volunteers participated in this 2 period crossover study in which the effects of FL versus AC were compared. CYP2C9 *3 genotyping was determined before the study to include 12 homozygous (CYP2C9 *1/*1) and 12 heterozygous (CYP2C9 *1/*3). VKORC1 genotyping (intron1, C1173T) was determined for all subjects. The pharmacodynamic effect (INR T48h) were significantly higher among subjects harboring the CYP2C9 *1/*3 compared with CYP2C9 *1/*1 genotype during AC administration compared to FL: 2.4 ±0.8 versus 1.7 ±b 0.3 ($p < 0.05$) and 1.6 ±0.4 versus 1.5 ±0.3, respectively. Pharmacodynamic of both OA were significantly influenced by VKORC1. The presence of at least 1 CYP2C9 *3 allele in fluindione users is associated with an increased of FL pharmacokinetic. CYP2C9 and VKORC1 genotyping may be of clinical value during the introduction phase of FL and AC, as means of preventing unstable INR and overanticoagulation in genetically susceptible patients.

P180034

Genetic polymorphisms and migraine

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Trombosis susceptibility genes are genic variants (single nucleotide point mutations at a single nucleotide) which seem to have an increased incidence in migraineur subjects. Our study analysed the incidence of a wide series of genetic vascular mutations in migraineurs. 19 consecutive patients (13-66 years, mean age was 34,42) suffering from migraine (15 migraine without aura, 4 migraine with aura, ICHD-II criteria) were genotyped with Polymerase Chain Reaction (PCR) for 1) Factor V Leiden (G1691A), 2) Factor V (H1299R), 3) Prothrombin (G20210A), 4) Factor XIII (V34L), 5) β-fibrinogen (-455 G → A), 6 e 7) MTHFR (C677T and A1298C), 8) PAI-1, 9) HPA-1 and 10) ACE. Are heterozygous respectively; 11% in 1, 16% in 2, 11% in 3, 100% in 4, 53% in 5, 79% in 6, 53% in 7, 16% in 8, 21% in 9, 53% in 10. Are mutated respectively 5% in 7, 5% in 8, 37% in 10. The results obtained confirms the association between migraine and some genetic polymorphisms, such as MTHFR and ACE. Moreover, in our survey, come out positivities (values over 50%) even for Factor XIII and β-fibrinogen. Therefore, it appears useful to confirm these evidences on larger and case-control surveys.

P180036

Gene expression of human epithelial cells by the cloned Omp38 of Acinetobacter baumannii.

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Outer membrane proteins (Omps) of Gram negative bacteria are known to be key players in bacterial adaptation and pathogenesis in host cells. The major band of Omp of Acinetobacter baumannii is a 38kDa porin (Omp38). Recently, there was a report that Omp38 induces apoptosis of HEp-2 human epithelial cell line. We developed the clone for Omp38 and purified protein with soluble form. In this study, HEp-2 cells were treated with 10 μg/ml Omp38 for 4 hours, 12 hours and 24 hours. RNA was isolated, and the expression of all known genes was analyzed using Affymatrix HG_U133A 2.0 arrays. The results showed that 230 genes at 4 hours, 239 genes at 12 hours and 257 genes at 24 hours were found to be differentially expressed at least two fold compared to untreated cells. In conclusion, the data demonstrates that Omp38 modulate gene expression in human epithelial cells.

Acknowledgement: This study was supported by a grant from the Korea Science and Engineering Foundation, Republic of Korea (Project No. R01-2005-000-10880-0).

P180037

Mechanism-Based Inactivation (MBI) of Recombinant CYP2C19 and CYP3A4 but not Human Liver Microsomal CYP2C19 and CYP3A4 by Nortriptyline

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Nortriptyline was evaluated as a mechanism-based inactivator of CYP2C19 and CYP3A4 by varying pre-incubation time and inhibitor concentration. Recombinant CYP (Escherichia coli-expressed) or human liver microsomes (HLM) were used as the enzyme sources. Spectral studies were conducted to elucidate potential mechanisms of inactivation. Nortriptyline caused time- and concentration-dependent loss of CYP2C19 and CYP3A4 activities employing recombinant preparations but not HLM. The inactivation of recombinant CYP2C19 and CYP3A4 was characterised by K_I and k_{inact} values of 4 μM and 0.19 min^{-1}, and 70 μM and 0.06 min^{-1}, respectively. Addition of either omeprazole or cyclosporine to pre-incubation mixtures partially protected CYP2C19 and CYP3A4, whereas inactivation rates were unaltered in the presence of trapping agents (superoxide dismutase and glutathione). Ultrafiltration failed to restore recombinant CYP2C19 and CYP3A4 function since nortriptyline formed quasiirreversible metabolite-intermediate complexes with these enzymes. These data suggest that recombinant CYP and HLM are not equivalent enzyme sources for assessing MBI caused by some drugs.

Key words: drug-interactions, cytochromes P450, inactivation, nortriptyline.

P180038

Developmental inhibition of fetal rats exposed to nicotine in utero: possible involvement of CYP1A1, CYP2E1 and p-glycoprotein

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The aim was to investigate whether prenatal nicotine exposure would interfere with the fetal development and alter cytochrome P450 (CYP) 1A1, 2E1 and p-glycoprotein (Pgp) expressions in maternal liver and placenta during pregnancy. Pregnant Wistar rats were given nicotine subcutaneously twice a day from gestational day 8 to 21. In nicotine treated groups, the fetal body weights, litter size and placental weights were significantly lower. The levels of CYP1A1 and 2E1 increased with advancing gestation, but decreased slightly in late pregnancy employing enzyme assay and real-time RT-PCR technique. Expression of placental Pgp was monitored using a combination of quantitative RT-PCR and immunohistochemistry, and there was a decreased tendency in mdr1a mRNA expression and came to the lowest at late-gestation. However, no remarkable difference was found in the protein expression of Pgp between the control and the nicotine groups. Our findings demonstrate that nicotine exposure in utero may lead to restraining the development of fetal rats and result in the increases of CYP1A1 and CYP2E1, and decrease of Pgp in mRNA expression.

Key words: Nicotine prenatal exposure; CYP1A1; CYP 2E1; P-glycoprotein.

P180039

Trp64Arg polymorphism of β3-AR and Gln27Glu polymorphism of the β2-AR are associated with obesity in Chinese male hypertensive patients

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Aim: The aims of the present study were to investigate the association between β3-AR Trp64Arg, β2-AR Arg16Gly and Gln27Glu polymorphisms and obesity in Chinese hypertensive patients. Methods: 437 Chinese subjects (250 males, 187 females) including 288 essential hypertensive patients (169 males and 119 females) and 169 healthy controls (81 males and 68 females) participated in this study. PCR-RFLP and AS-PCR assays were used to identify Trp64Arg and Arg16Gly, Gln27Glu polymorphisms, respectively. Results: The allele frequencies of 64Arg and 27Glu in the group of hypertension with obesity were 0.178 and 0.128, respectively. Both were significantly higher than those in the group of hypertension and in the group of controls ($P < 0.05$). Further study showed that the association between Trp64Arg and Gln27Glu polymorphisms and obesity existed only in male hypertensive patients, but not in females. Moreover, there was a weak association between β2-AR haplotype and obesity in male subjects ($P = 0.09$). Conclusion: These data suggest that β3-AR Trp64Arg polymorphism and β2-AR Gln27Glu polymorphism are associated with obesity in Chinese male hypertensive patients.

Key words: β3-AR, β2-AR, polymorphism, haplotype

P180040

Association of CYP3A5 genotype with the metabolic ratio (MR) of cyclosporine in Chinese renal transplant recipients

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To investigate whether the CYP3A5*3 polymorphism would affect CsA metabolism in renal transplant patients, CYP3A5*3 genotype was determined by PCR amplification of specific alleles (PASA) with the blood samples from Chinese renal transplant recipients, and concentrations of CsA and metabolite were measured by FPIA to obtain the metabolic ratio (MR) values. The result came out the MR values for the subjects with each genotype for CYP3A5*3 were respectively as follows: 0.92 ±0.62 in homozygous G/G genotype (n=14), 0.99 ± 0.51 in heterozygous A/G genotype (n=15), and 1.45 ±0.62 in homozygous A/A genotype (n=9). The result of statistics showed that the MR values between A/A group and G/G group or A/G group are significantly different ($P_{A/A vs G/G}$=0.0308, $P_{A/A vs A/G}$=0.0311), and the MR values between G/G group and A/G group are not significantly different ($P_{G/G vs A/G}$=0.3778). The mean MR was 36.03% smaller in G/G group compared to A/A group in practice. The results of this pilot study suggested that there is statistically significant influence of CYP3A5 genotype on CsA metabolism in renal transplant patients.

Key words: cyclosporine; CYP3A5 polymorphism; metabolic ratio (MR)

P180041

Changes of CYP isoforms of hepatic stellate cells during cell activation[1]

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The activation of hepatic satellite cells (HSCs) is the central event in hepatic fibrosis. We study the changes of CYP isoforms during HSC activation to explore their possible roles on HSC activation. Culture of HSCs isolated from rat livers on plastic dishes were used as a model of HSC activation. α-Smooth muscle actin was used to an activated marker of HSC. The expressions of CYP isoforms during HSC activation were determined by real-time RT-PCR. RT-PCR study revealed that the deactivated of HSCs (day 1) could express several CYP isoforms including CYP1A1/2, 1B1, 2B2 and 2E1. CYP1B1, 2B2 and 2E1 mRNA were expressed at the highest levels in HSCs at an early stage of activation (2 days after plating), particularly CYP1B1 and 2E1, and diminished upon further activation. However, the levels of CYP1A1, and 1A2 mRNA were constantly decreased during the whole activation of HSC. The significant variations of CYP isoforms during HSC activation indicate the regulation of CYP isoforms are closely related to HSC activation.

Key words: HSCs; activation; CYP isoforms;
[1]Supported by the National Natural Science Foundation of China, No. 30371666

P180042

Effect of CYP2C9 polymorphism on the pharmacokinetics of candesartan in healthy Korean subjects.

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Objects: CYP2C9 is the principal enzyme responsible for the metabolism of numerous clinically important drugs. Genetic polymorphism of this enzyme shows high ethnic variations. Candesartan is metabolized in the CYP2C9 to the inactive metabolite and is excreted as such through renal and biliary routes. In this study, the effect of major polymorphism of the CYP2C9 on the pharmacokinetic of candesartan was investigated. Methods: A 16 mg oral dose of candesartan was given to 22 Korean volunteers with different CYP2C9 genotypes (14, 6 and 2 carriers of CYP2C9 *1/*1, *1/*3 and *1/*13 genotypes, respectively). Results: In subjects with CYP2C9 *1/*3 or *1/*13 genotypes, the AUC ratio of candesartan significantly greater than that in subjects with CYP2C9 *1/*1. Conclusion: The pharmacokinetics of candesartan are dependent on CYP2C9 polymorphism.

Key words: CYP2C9, polymorphism, candesartan, pharmacokinetics
Acknowledgement: This study was supported by 2006 KFDA Research Fund.

P180043

GENETIC POLYMORPHISM OF OCT2 GENE AND HAPLOTYPE PROFILE IN THE CHINESE POPULATION

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Purpose: The human organic cationic transporter 2 (OCT2) plays an important role in the renal clearance of many drugs. At present the genetic polymorphism of OCT2 gene and haplotype profile are unknown in the Chinese population. Methods: To identify the single nucleotide polymorphisms (SNPs), all 11 exons including the surrounding introns and the promoter region of OCT2 were sequenced using genomic DNA from 112 healthy Chinese subjects. Based on the SNPs detected, haplotype analysis was subsequently performed using the expectation-maximization algorithm. Results: A total of 17 SNPs were identified in our population, with 3 in the exons, 9 in introns and 5 in the promoter region. Their frequencies ranged from 2.7% to 75.3%. From these SNP data sets, 19 haplotypes were inferred, and 5 of them were the most common with frequencies of 7.1% to 23.2%. Conclusion: Our study provided the new information of the genetic polymorphism of OCT2 gene in Chinese population. The functional importance as well as the phenotype-genotype relationship of these SNPs and haplotypes require further investigation.

P180044

Genetic Polymorphism of Cytochromes P450, CYP2D6, CYP2C9 and CYP3A5 in he Greek Population

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Objective: To determine the prevalence of most common polymorphisms of allelic variants CYP2D6, CYP2C9 and CYP3A5 of cytochrome P450 (CYP) and to predict genotype frequency in Greek population. Methods: DNA isolated from peripheral blood samples derived from 200 non-related Greek citizens was used to determine the frequency of most common polymorphisms of CYP i.e. CYP2D6*3, CYP2D6*4, CYP2C9*2 CYP2C9*3 and CYP3A5*3 allelic variants by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and CYP2D6*2 (gene duplications) by long PCR analysis. Results: For 200 volunteers genotyped for CYP2D6, CYP2C9 and CYP3A5, the allele frequencies of CYP2D6*3, CYP2D6*4, were 4%, 30.5% respectively while CYP2D6*2 were found at 7.5%. For CYP2C9*2, CYP2C9*3 alleles the frequencies were 22.5% and 17.5% respectively. The CYP3A5*3 allele was abundantly present in the Greek population with an allelic frequency of 94.25%. Conclusions: While CYP2C9 and CYP3A5 allelic variants are in accordance, the prevalence of allelic variants and predicted genotypes of CYP2D6 in the Greek population sample are slightly increased to those reported in other southern European populations.

Key words: Pharmacogenetics, CYP2D6, CYP2C9, CYP3A5
Acknowledgements: G.G. Ragia is a recipient of a graduate scholarship by IKY (State Scholarship Foundation of Greece).

P19. Developmental Pharmacology

P190001

Role of enzymatic and non-enzymatic antioxidant factors in elevation of total antioxidant capacity of plasma in developing and adult rats treated with acetaminophen

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Contribution of individual antioxidant factors on ferric reducing ability of plasma (FRAP) assay, as an index of total antioxidant activity has been studied. A surge in FRAP 1h after high dose (250 or 450 mg/kg B.W) drug administration was recorded in young as well as adults. Whereas, low dose APAP (25 mg/kg) failed to alter FRAP in both the age groups. Elevation in FRAP begin rapidly, reaching a maximum at 1h (>500%). Increased FRAP was associated with a marked increase (~14 fold) in plasma bilirubin 6 h after drug administration at 450 mg/kg only in suckling rats. Similarly, APAP-related increase in superoxide dismutase activity in erythrocytes was limited to young rats. Other factors measured viz., plasma uric acid, bilirubin and total protein together with catalase activity of erythrocytes remained unchanged in treated rats. During 12 h study, the concentration of hepatic lipid peroxidation products was unchanged. The end-point hepatotoxic effects of APAP was similar in both the age groups, suggesting that like adults, immature rats are resistant to APAP toxicity owing to their

drugdependent induction in certain antioxidant factors.

P190002

In vitro development of gut-like tissue demonstrating rhythmic motility from embryonic mouse intestinal cells

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The rhythmic motility of the intestine is regulated by interstitial cells of Cajal (ICC) and the enteric nervous system. Rhythmic motility is considered to occur after the differentiation of mesenchymal progenitor cells to ICC during the late embryonic period. In this study, we successfully reconstructed a gut-like tissue demonstrating rhythmic motility by culturing single cells enzymatically isolated from the mouse intestine during the middle embryonic period. These intestinal cells reconstituted into collagen gel at a high density, proliferated remarkably and grew up into gut-like tissue after 1 week of culturing. This reconstituted tissue showed rhythmic motility, and the immunostaining of PGP9.5 and c-Kit, the specific marker proteins each for neurons and ICC, demonstrated network formation by developing nerve cells and ICC. Moreover, in the presence of nifedipine, c-Kit positive cells in the reconstituted tissue showed spontaneous Ca oscillation, which is considered to be coupled to the electrical activity corresponding to slow waves. Therefore, this culture system may be useful for elucidating the developmental mechanism of gastrointestinal motility.

P190003

The study of the extraction of the flavonoids in the dogbane leaf and its protective effect to the liver

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Aim: Apocynum Venetem L. is the plant of the Apocynaceae Apocynum Venetem L. can be used for palpitation, insomnia and HBP. The dogbane leaf contains the flavonoids. we do some experiments to study the extraction of the flavonoids of the dogbane leaf and its protective effect to the liver. Methods: The extraction technics were selected with the orthogonal design. In the study of the protective effect to the liver, the method of CCL4 injured liver model in mice was used. The Bifendate was used as the positive control. There were there doses for the Apocynum Venetem L. Results: The optimum extraction process is as follows: adding eight times amount of 70% alcohol into Chinese traditional medicine, extracting three times and 2 h each time. The total flavonoid of the dogbane leaf can protect the injured liver, depress the ALT and AST of the blood serum, the protective of the total flavonoid depressed with the depression of the dose. Conclusion: The total flavones of the dogbane leaf can protect the injured liver and there were steady technology for its extraction. It will be used in the medcal treatment.

P190004

Preparation and characteristics of polysulfone/polyether blend membranes and the application to anti-hepatitis B virus drug Oenanthe Javanica

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Aim: Oenanthe Javanica (OJ), umbelliferate, has been widely used in traditional Chinese medicine for treatment of jaundice, hypertension, and polydipsia diseases for many years. Previous studies have shown that it was helpful in treatment of HBV infection. Methods: The present study aimed to seek the active part OJ of against HBV and investigate the anti-hepatitis activity of OJ Flavone (OJF). The content of total flavonoids of OJF extract is 56.90%. Results: The results demonstrate that OJF is a strong inhibitor of HBsAg and HBeAg secretion in 2.2.15 cells and DHBV-DNA levels in the infected duck model. Conclusion: At present, people's attention is gradually aroused as to the untoward effects and safe problems of TCM infections. Utrafiltration membrane separation technique is an effective method to solve the puzzle, remaining active component and getting rid of ineffective substance (impurity and pyrogen). Good effects were gained by using the PSF/polyether blend membrane to OJ injection and Drug granules.

P190005

TGFβ Signaling Is Required For Atrioventricular Cushion Mesenchyme Remodding During Cardiac Development

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Defects in septation and valvulogenesis are leading causes of human congenital heart diseases. Cushions are initially formed through epithelial mesenchyme-transformation (EMT) by some endocardial cells in the atrioventricular canal region invading into the extracellular matrix as the result of interaction between the myocardium and endocardium. The cellularized cushions undergo complicated remodeling processes to form the mature valves and septa. To reveal the roles of TGFβ signaling during cardiogenesis, we specifically inactivate Tgfbr2, which encodes the type II TGFβ receptor, in the myocardium or endothelium using a Cre/loxp system. TGFβ signaling in the myocardium is dispensable for cardiogenesis. Contrary to previous reports, disruption of endocardial TGFβ signal does not inhibit cushion mesenchyme formation. This study further reveals an essential role of TGFβ signaling in remodeling the AVC region, as perturbation results in a double-inlet-left-ventricle (DILV) defect. By characterizing this unique genetic model we propose for the first time a cellular mechanism for DILV.

Key words: TGFβ, Cardiogenesis, DILV

P190006

Mechanisms underlying the growth inhibitory effects of NSAIDs in human breast cancer

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Objective To characterize the effects and its mechanisms of Aspirin-DL-Lysine for Injection in inducing growth inhibition and apoptosis in human breast cancer cell line (MDA-MB-231). METHODS The inhibitory rate of cell growth was assessed by MTT spectrophotometric analysis, the apoptosis index of cells were measured by flow cytometry (FCM), Immunohistochemical staining was used to detect the expressions of COX-2 and caspase-3 in cells. RESULTS Aspirin-DL-Lysine for Injection inhibited MDA-MB-231 cell proliferation in a time- and dosedependent fashion, increased apoptosis cells number, decrease the expressions of COX-2 and activated caspase-3. Conclusion Aspirin-DL-Lysine for Injection could inhibit the growth of MDA-MB-231 cell obviously and induce apoptosis, the mechanism of them is correlated with downregulation of COX-2 expression and caspase-3 activation.

P190007

Differentiation of human bone marrow mesenchymal stem cells into cardiac phenotype in cardiomyocytes microenvironment

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Objective: In this study, the ability of hBMSCs to differentiate into cells with characteristics of cardiomyocytes in conditioned culture was investigated. Methods: Human bone marrow cells were collected from clinical patients. Myocytes were obtained from neonatal rat ventricles. hBMSCs were cocultured with rat myocytes in a rate of 1:10 by semipermeable membrane. Real-time RT-PCR, immunocytochemistry, western blotting, and whole-cell patch-clamp technique were used to evaluation. Results: After passage 3, the hBMSCs marker of CD29 and CD44 were highly expressed, however, leucocyte marker of CD34, CD45, and CD11b could hardly be identified. Following induction 1 to 3 weeks, some hBMSCs became sarcomeric α-actinin, cardiac troponin T (cTnT), and cTnI positive. hGATA-4 mRNA and connexin 43 protein expressiones were also upregulated. However, the c-kit, a stem cell marker, was expressed only before hBMSCs induction. After coculuring with rat myocytes, hBMSCs can be detected special cardiomyocyte current IK1, which didn't exist in untreated hBMSCs. Conclusion: BMSCs possesses the differentiation potential to cardiomyocyte in mimic heart microenvironment that was independent on cell-to-cell touch beteewn BMSCs and myocytes.

Key words: BMSCs, Cardiomyocyte, Differentiation.
Acknowledgments: This work was supported by NSFC (30271287, 30571850) and GDNSF (015015, 04102307).

P190008

Y118, S378, H310 three crucial acid residues that contribute to sterd 14a demethylase and inhibitor interaction in candida albicans

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CYP51 (sterol 14R- demethylase) is an essential enzyme in sterol biosynthetic pathways and is the only P450 gene family having catalytically same reaction in different biological kingdoms. As a result of their structural similarity, the natural substrates are often easily interchangeable in vitro. Although structural analysis of MT- CYP51(Mycobacterium CYP51) has been extensively examined, less is known about the structural basis of CACYP51(candida albicans CYP51) function. In this study, based on evolutionary trace method and relative solvent accessibility prediction of residues, a set of trace residues was selected for site- directed mutagenesis. A series of CA- CYP51 mutations was made, and Yeast12667 cell lines stably expressing different CA- CYP51 mutants were generated. According to the survival and differentiation responses of these stable Yeast12667 cells upon different azole stimulation and the MIC, GC- GS assay, residues Y118, S378, and HB10 in the CA- CYP51 central region were found to be critical for CYP51 binding to azole and natural substrate.

Key words: CYP51 candida albicans azole

P190009

The effect of 3- Daidzein Sulfonate Sodium on the level of gonadal hormone of mice

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Objective: To study the effect of 3- Daidzein Sulfonate Sodium on the level of gonadal hormone of mice. Methods: Models of Benign Prostatic Hyperplasia were established by subcutaneous injection testosterone propionate in mice, observed the effect of 3- Daidzein Sulfonate Sodium on the level of gonadal hormone of the control groups, model groups, and different dose groups. Results: 3- daidzein sulfonate sodium can obviously reduce the cotent of testosterone(T), estrogen (E2), T/ E2 in serum of mice, control the level of gonadal hormone of mice, inhibit Benign Prostatic Hyperplasia in mice induced by testosterone propionate. Conclusion: 3- daidzein sulfonate sodium can obviously reduce the cotent of T, E2, T/ E2 in serum of mice, control the level of gonadal hormone of mice, inhibit Benign Prostatic Hyperplasia in mice induced by testosterone propionate.

Key words: 3- daidzein sulfonate sodium; prostatic hyperplasia; gonadal hormone; mice

P190010

Contribution of enzymatic and non- enzymatic antioxidant factors in total antioxidant capacity of plasma in devdoping and adult rats treated with acetaminophen

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Contribution of antioxidant factors to ferric reducing ability of plasma (FRAP) assay, as an index of total antioxidant activity was assessed. A surge in FRAP 1h after drug administration(250 or 450 mg/ kg b.w) was recorded in young and adult rats. Low dose APAP (25 mg/ kg) failed to alter FRAP in both the groups. Timecourse studies show that elevation in FRAP begin rapidly, reaching a maximum at 1h (>500%). Increased FRAP was associated with a marked increase (~14 fold) in plasma bilirubin 6 h after drug administration at 450 mg/ kg only in suckling rats. Similarly, APAP- related increase in superoxide dismutase in erythrocytes was limited to young rats of both the age groups. Other factors measured during this period viz., plasma uric acid, bilirubin and total protein together with catalase in erythrocytes remained unchanged in treated rats. APAP- related depletion in liver glutathione was almost similar in both the age groups. Based on lipid peroxidation products and the endpoint hepatotoxic effects of APAP measured it may be concluded that, immature rats, like adults, are resistant to APAP toxicity owing to their drug- dependent induction in certain antioxidant factors.

P190011

NO CHANGES IN THE ACUTE AND CHRONIC GASTRIC MUCOSAL PROTECTIVE EFFECTS OF CAPSAICIN IN HEALTHY HUMAN SUBJECTS.

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Background: Small doses of capsaicin have gastric mucosal cytoprotective effect in animal and human observations. Aim: of the study was to approach the possible changes in the acute and chronic gastric mucosal protective effect of capsaicin (200 or 400 µg orally) on the indomethacin (IND) - induced gastric mucosal microbleeding in healthy human subjects. Materails and methods: The studies were carried out in 18 healthy human subjects (age: 39 ±5; average ±SD) in prospective, randomized manner, respected the Good Clinical Practice (GCP) and accepted by the Regional Ethical Committee. The gastric mucosal injury (microbleeding) was produced by IND (3x25 mg orally). The capsaicin was applied acutely (200 and 400 µg orally given) before and after 2 weeks (3x400 µg orally) capsaicin treatment. Results: The capsaicin- induced gastric mucosal protection remained dose- depently and same before and after the 2 weeks capsaicin treatment. Conclusion: No change exists in the acute and chronic gastric mucosal protective effects produced by capsaicin.

Key words: capsaicin; indomethacin; acute and chronic capsaicin treatment; gastric mucosal protection.

The study was supported by the grant of RET- II 08/ 2005.

P190012

Effective of Ginkgolides on the Expression of Apoptosis Related Gene during PC12 Cells Glucose Deprivation

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Objective: Ginkgolides have beneficial effects on Central Nervous System function. This study investigated the protective effects of ginkgolides on glucose deprivation- induced apoptosis in PC12 cells and the mechanism underlying the protective effect. Method: PC12 cells were treated under glucose deprivation, and the proliferation was determined by tetrazolium(MTT) assay. Furthermore, the mRNA levels of bcl- 2、bax、c- myc were measured by Fluorescence Quantitative PCR(FQ- PCR). Result: Ginkgolides could markedly inhibit the injury of glucose deprivation on PC12 cells and increase the cell proliferation compared with the model groups ($P < 0.01$). Ginkgolides can up- regulate bcl- 2 and down- regulate bax and c- myc at 12hr, respectively. There were no significant differences in the bcl- 2 and bax levels in both group at 24 hr, and ginkgolides only reduced the elevation of c- myc from 4.3- fold to 2.9- fold at this time. Conclusion: During the early period of glucose deprivation, bcl- 2、bax and c- myc were regulated to inhibit cell apoptosis by ginkgolides. After that, ginkgolides seems inhibit the apoptosis through attenuating the elevation of c- myc.

Key words: Ginkgolides; PC12 Cell Lines; Apoptosis

P190013

The effects of nicotine exposure on the expression of GAP- 43 in cerebral cortex of embryo rats

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Aim: To investigate a potential role of gestational nicotine exposure in expression of GAP- 43 for cerebral cortex during rat brain development. Method: Pregnant rats were treated with different doses (0, 1, 2, 3 mg/ kg/ day) nicotine from gestation day 1- 20. On the 20th day of gestation, we detected and analyzed the expression of GAP- 43 in cerebral cortex of embryo rats by immunohistochemistry method. Result: In control group, the expression of GAP- 43 was detected in cells and axon from cortex. The density of GAP- 43 immunoreactivity was significantly decreased in cortical cells and axons after nicotine treatment (1, 2 mg/ kg/ day). Furthermore, there was very faint expression in cortical cells without in axons in 3 mg/ kg/ day group. Conclusion: These findings indicate that the prenatal nicotine exposure delay GAP- 43 expression in neurons, which may be effect the establishment of neuronal connections and synaptogenesis during brain development.

Key words: Nicotine, GAP- 43, Development

P190014

Hormonal Regulation of the Human UDP- Glucuronosyltransferase- 1 (UGT1) Locus During Pregnancy and Lactation in UGT1 Transgenic Mice

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The UGT1 enzymes detoxify drugs, endogenous metabolites and environmental toxicants via conjugation to glucuronic acid, and are an essential part of drug metabolism and detoxification. A transgenic mouse expressing all nine functional UGT1 proteins in a tissue- specific and inducible manner was created in order to study the regulation of the human locus by circulating humoral factors and global

hormonal events. Regulation of the UGT1 locus was examined at days 7-20 of pregnancy and days 1-14 post-pregnancy in maternal, fetal, and neonatal organs. In maternal liver, UGT1A1, 1A4, and 1A6 protein is upregulated during pregnancy, and 1A4 and 1A6 remain highly upregulated during post-partum lactation. UGT1 regulation was also apparent in maternal sex organs. Downregulation of 1A1 and 1A6 in the uterus was observed during pregnancy, with a return to normal levels post-partum. 1A6 was expressed in the placenta and increased throughout pregnancy, whereas fetal 1A1 and 1A6 expression began one day prior to birth and increased during neonatal development. These results indicate that UGT1 regulation is dynamic during important hormonal events and may give insight into the in vivo hormonal regulation of the locus.

P190015

Neuropeptide substance-P promotes adult neural progenitor proliferation

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Neurogenesis continues throughout the life of mammals in the subventricular zone (SVZ) of the lateral ventricles. As enhancing neurogenesis can repair damaged brain, we tested the potential of substance-P acting via neurokinin-1 receptor (NK1R) in promoting the proliferation of cultured adult rat neural progenitor cells. Exposure to 10 to 1000 nM substance-P for 3 days significantly induced the proliferation of progenitors by 32%. 100 nM substance-P continuously increased proliferation between 6h to 5 days. NK1R antagonist L-703,606 prevented the progenitor proliferation by 95%. Furthermore, L-703,606 prevented the proliferation stimulated by 100 nM substance-P by 69%. The neural progenitors showed immunoreactivity for both substance-P and NK1R indicating that these effects are receptor-specific. A 5 day continuous i.c.v. infusion of substance-P (1000 nM) using Alzet osmotic minipumps resulted in a 6 fold increase in the number of BrdU & DCX (proliferating neural progenitor marker) double immunopositive cells in the SVZ of adult rats. These studies indicate that substance-P can promote neurogenesis and thus plasticity in adult brain. Funded by US NIH.

P190016

Effect of prenatal exposure to choline-deficient diet on brain total antioxidant status and enzyme activities of the offsprings, in rats.

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Choline is an essential nutrient, important in brain development.

Choline deficient (CD) diet causes accumulation of homocysteine which is known to cause oxidative stress. The aim of this study was to investigate how CD diet during gestation only could affect the total antioxidant status (TAS) and the activities of acetylcholinesterase (AChE), (Na^+,K^+)- and Mg^{2+}-ATPase (enzymes involved in synaptogenesis) in the brains of the offsprings. TAS and enzyme activities were measured spectrophotometrically at the 1st day and 21st day (end of lactation) of age. At 1st day in CD group brains, TAS and the activities of AChE and Na^+,K^+-ATPase were significantly reduced by 23%, 24% and 50% respectively compared to control group. At 21st day CD group showed a reduction of TAS (-27%, $P<0.001$) while the rest of the enzyme activities did not differ compared to control. Mg^{2+}-ATPase activity was unaltered. No differences were observed between female and male offsprings. Our data suggest that rat offsprings prenatally exposed to CD after 21 days of lactation continued to exhibit reduced TAS, while the enzyme activities were restored to normal, possibly due to novel synaptogenesis.

P190017

Extended tryptophan restriction during early postnatal stage produces depression-like characteristics: a study in rat.

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Serotonin transmission dysfunction plays an important role in mood disorders. In order to investigate whether low-tryptophan tortilla diet (TD) (80% less than commercial rat chow) during development could produce depression-like features, we established an animal model with rats fed a TD during early postnatal stages. Forced swimming test (FST), elevated plus maze (EPM) were used as behavioral tests. Experimental animals displayed significant increase of immobility in FST and anxiety-like behavior in EPM. Immunocytochemical reaction (IR) against 5-Bromo-2'-deoxyuridine (BrdU) showed a decrease of proliferation rate in the subgranular zone of dentate gyrus (DG). c-Fos expression after FST was found reduced in prefrontal cortex, dentate gyrus, CA1 and hilus of hippocampus and amygdala. Moreover, dendrite atrophy and decreased spine density were evident in Golgi-Cox impregnated CA1 pyramidal neurons. These findings indicate an involvement of hyposerotoninergia produced by diet tryptophan restriction during critical developmental stages in the emotional disturbance and suggest that neuroplasticity changes might underlie these observed alterations in the rats.

P190018

GABAERGIC NEURONS DERIVED FROM MOUSE EMBRYONIC STEM CELLS: A BRIEF CHARACTERIZATION

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Stem cell differentiation is central to the development of the nervous system. Directing embryonic stem cells to a desired cell fate will enable us to use these cells in regenerative medicine, or for toxicological and drug screening assays. Barberi and co-workers (2003) generated populations of mouse ES cells (mES) that possess some of the morphological and immunological features of GABAergic neurons. We have cultured mES cells using similar methodology and investigated, using immunocytochemistry, reverse transcriptase PCR (rtPCR), Ca^{2+} imaging and [^{3}H]-GABA release studies, the characteristics of these cells. Twenty four days after induction of differentiation, cultured cells were immunoreactive for MAP-2a, synaptophysin and GABA; rtPCR showed GABA receptors and uptake mechanisms. Cells also responded to stimulation with KCl (30 mM) and acetylcholine (30 uM) with elevation of intracellular Ca^{2+} and [^{3}H]-GABA release. Thus GABAergic neurons derived from mES cells appear to have some of the functional characteristics of GABAergic neurons in vivo. Baberi T et al. Nature Biotech. 2003, 21, 1200-7.

Key words: GABA, neuron stem, cell

Acknowledgment: Stem Cell Sciences, Australia for the mES cells.

P190019

Effect of cocaine on protein kinase C isozyme gene expression pattern in the developing heart.

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Cocaine abuse among women of childbearing age is associated with numerous adverse perinatal outcomes including cardiac dysfunctions. Our previous experiments have demonstrated that prenatal cocaine exposure leads to an increase in heart susceptibility to ischemic insults in offspring adult rats. The present study investigated potential epigenetic mechanisms of altered PKC gene expressions. Pregnant rats were administered subcutaneously either saline or cocaine (15 mg/kg) twice daily from day 15 to day 21 of gestational age, and fetal hearts were isolated at the end of treatment. Protein and mRNA levels of five PKC isoforms (α, β, σ, ε, ζ) were determined by Western blot and real-time RT-PCR, respectively. In cocaine-treated animals, the mRNA levels of PKC σ and ε in the heart were significantly decreased as compared to its saline treated counterpart, ($p < 0.05$). Correspondingly, protein levels of PKC σ and ε were also significantly decreased in cocaine-treated fetal hearts ($p < 0.05$). In contrast, cocaine showed no significant effects on other isoforms of PKC in the fetal heart. These findings suggest that chronic cocaine exposure during fetal development results in a selective down-regulation of PKC isozyme gene expression pattern in the fetal heart, which may present an epigenetic mechanism in the programming of the developing heart and increase heart ischemic vulnerability in adult offspring. (Support in part by NIH grant HL82779)

P190020

Effect of Fetal Anemia on Myocardial Ischemia-Reperfusion Injury in Adult Life

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Chronic fetal anemia initiates cardiovascular adaptations including increases in coronary conductance and cardiac output. The functional significance was revealed in later life by improved cardiac response to hypoxia stress. To further investigate whether in-utero anemia protects adult against ischemia-reperfusion (I-R) injury, we studied adult sheep at 7 month of age that were made anemia in utero while transfused to normal hematocrit (HCT) before birth. Infarct size was determined by tetrazolium staining. Isovolemic hemorrhage reduced ($p < 0.001$) HCT from 31.6 ±2.2% to 13.5 ±0.8% and carotid oxygen content from 7.98 ±0.69 to 2.24 ±0.10 ml/dl. The in-utero anemia group ($n = 5$) did not differ from controls ($n = 5$) with respect to age, body weight and HCT, either as newborn or as adult. Hemodynamic parameters were similar at baseline, 1-h coronary occlusion, and 2-h reperfusion in each group and between groups. However, infarct size markedly increased in the in-utero anemic animals (70.7 ±3.5% vs. 49.8 ±4.5%, $p = 0.006$). Thus, fetal anemia increases the susceptibility of adult heart to I-R injury. Fetal, Anemia, Ischemia-Reperfusion

Supported by American Heart Association Post-doc Fellowship Grant.

P190021

Role of eIF3 p170 in differentiation and its association with early development

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The expression of p170 has been found increased in several human tumors and thought to be a proto oncogene. We analyzed the expression of p170 during mouse development and in Caco-2 cells under differentiated and undifferentiated conditions. Method: Fetal small intestine, stomach, lung, kidney, liver, and heart were readily discernible when viewed under the dissecting stereomicroscope. Postnatal mice were also sacrificed on days 1, 2, 3, 10, 21, and 90 by decapitation. Western blot analyses were used to determine the expression of p170 protein in Caco-2 cell lysates and mice tissues. Alkaline phosphatase and sucrase activities were determined. Results: We found that the expression of p170 in intestine, stomach, and lung abruptly stopped on the 18th day in gestation while it persisted in liver, kidney, and heart. Knocking down the expression of endogenous p170 using siRNA promoted Caco-2 cell differentiation without the cells reaching confluence. Conclusion: These findings suggest that p170 plays an important role in mouse development and in cell differentiation and that the decreased expression of p170 is likely a pre-requisite of cell differentiation.

Key words: p170; differentiation; Caco-2

P20. Environmental Toxicology

P200001

IMPACT OF ENVIRONMENTAL LEAD POLLUTION ON PREGNANT FEMALES AND THEIR OFFSPRINGS

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Background: A childs lead burden begins before birth with lead transferred from maternal circulation. During pregnancy lead is liberated from maternal skeleton and transferred from mother to child in utero. Purpose: The purpose of this work is to determine umbilical cord blood lead levels in Cairo and its effect on newly-born. Methoddology: A total number of 65 was collected. The specified group was personally interviewed and examined during labour. Also assessment of the neonates was performed concerning birth weight, prematurity and complications during labour. Results: The mean umbilical blood lead level of the studied population was 24.2 Ug/dl. There was no significant association between cord blood lead level and mother's age, parity and complication of delivery, however there was a statistical association between increased cord lead level and prematurity and also reduced birth weight. Recommendation: Special concern should be directed to underprivileged groups as females to prevent the health impact on the newly born and children.

Key words: environmental lead, pregnant females.

P200002

Gynaecological disturbances among females engaged in the manufacture of sex hormones

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Introduction and objective: Numerous studies have established as association between exposure to sex hormones and many gynaecological troubles. The aim of this work is to investigate the different gynaecological disturbances which may affect female workers occupationally engaged in the manufacture of hormonal preparations. Materials and methods: The total number of female workers was 214, a control group of 220 subjects. All workers were subjected to a prepared questionnaire. Gynaecological examinations were carried out. Results and discussion: Hysterectomy was done to 11.2% of exposed workers. Our study showed a significant positive relationship between duration of exposure and the prevalence of hysterectomy. About 51% of married workers had reproductive disorders. Gynaecological examination showed that exposed workers suffered from vulvo-vaginitis, cervical erosion and leucorrhea ($P < 0.05$). About 12% of the exposed workers complained of some family health disturbances. Recommendations: We recommend health education and periodic medical examination.

P200004

Transplacental transfer of acrylamide in human placental perfusion

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Human placenta does not protect fetus from xenobiotics that may cause birth defects. Most drugs can penetrate the placenta but there are only a few studies on environmental toxic compounds. Human and animal placentas differ significantly. We used dual recirculating human placental perfusion to determine how neurotoxic and probably carcinogenic compound, acrylamide, behaves in placenta. Placentas were collected right after delivery and kept physiologically functional for 4-6 hours. Acrylamide concentrations used were 5 and 10 microg/ml. Acrylamide and its genotoxic metabolite glycidamide were measured by UV-HPLC method developed for this study. According to preliminary results acrylamide crossed placenta rapidly from mother to fetus. The consentrations of acrylamide were the same in fetal and maternal sides after 4 hours. Placentas metabolized acrylamide to glycidamide, which was secreted both to maternal and fetal circulations suggesting fetal exposure. Human placental perfusion is a useful and unique method for studying fetal exposure not only to drugs but also to environmental toxic compounds during pregnancy.

Acknowledgement: EU-project QLK4-CT-2002-02198

P200005

The Cyanide-Metabolizing Enzyme Rhodanese in Tissues of Human (Homo sapience)

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The enzyme rhodanese is widely distributed ni nature and is believed to plays a central role in cyanide detoxification. The purpose of this investigation was to determine and compare rhodanese activity in different tissues of human. The highest activity of rhodanese was found in kidney, followed by liver. Other tissues studied did not show significant rhodanese activity. The results obtained in this study was compared with the previously reported information on some domestic animals. Human liver contains lower rhodanese activity compared with ruminants and non-ruminants, except for dog which has comparable hepatic activity to human. Human kidney contains significantly higher activity than those found in domestic animals. The results of this study might indicate the involvement of rhodanese in cyanide detoxification in tissues which might be more exposed to cyanide, due to higher blood supply to these tissues.

Key words: rhodanese, human, kidney, liver, cyanide detoxification

P200007

CULTURE FILTRATE FROM *Shigella dysenteriae* AND ACUTE CELL INJURY ON CHICK EMBRYO SKELETAL MUSCLE TISSUE "ex vivo" and "in vitro"

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Excretion products of Shigella dysenteriae contain Shiga toxins (Stxs), potent cytotoxins which are responsible for widespread pathologies. Although many organs are commonly affected, it is not yet clear whether striated muscle tissue is a target of Stxs. The aim of this study was to evaluate the acute cell injury of culture filtrate from Shigella dysenteriae in both whole lower limb of chick embryo "ex vivo" and Hanging-drop cultures "in vitro". Acute cell injury was evaluated

through morphologic changes by images analysis techniques on histological sections. Mitotic and apoptotic index were estimate. Quantification of apoptotic cells was also measured by an enzyme-linked immunoassay. The percentage mitotic index decreased while the percentage of apoptotic index increased in response to excretion products. Membrane blebbing, vacuoles, small aggregates of chromatin and loss of cell adhesion were observed. Culture filtrate from Shigella dysenteriae injured striated tissue and had cytotoxic effect on cell of muscle fibers. Acute cell injury may include induction of apoptotic process.

Key words: S. dysenteriae, striated tissue, apoptosis, chick embryo.
Acknowledgement: CDCH. N-PG09-30-5409-2004. UCV

P200008

Effect of garlic during and before administration of lead acetate on lead content of some tissues in mouse

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Garlic ability to reduce lead in body tissues before and during chronic lead toxicity in mice was studied. 80 mature mice were divided into 8 groups. Group D (negative contrd) received placebo. Groups A1, A2 and A3 respectively received 500, 250 and 125 mg/kg/day garlic in first four weeks, and in second four weeks they received 5 mg/kg/day lead acetate and 500, 250 and 125 mg/kg/day garlic respectively. Groups B1, B2 and B3 respectively received 1/4, 1/8 and 1/16 garlic tablet/kg/day in first four weeks and in second four weeks received 5 mg/kg/day lead acetate and also respectively 1/4, 1/8 and 1/16 garlet tablet/kg/day. Group C (positive control) received a quarter of a placebo garlet tablet/kg/day in first four weeks and in second four weeks they received 5 mg/kg/day lead acetate and a quarter of a placebo garlet tablet/kg/day. Reduction in lead content of kidney, liver and bone as a result of administration of garlic or garlet tablet in studied groups was significant compared with group C. ($p < 0.05$) and reduction in lead content of blood in all groups was significant except group A3. Results showed that fresh garlic extract and garlet tablet had the same effects on lead reduction in tissues.

P200009

Pharmacological prevention of the induced mutagenesis

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The pharmacological techniques were used to determine and comprehensively evaluate in vivo the antimutagenic properties of a number of synthesized and naturally occurring substances of pharmacological and nutritional usage. Benzodiazepine derivatives, 2-mercaptobenzimidazole and 3-oxipyridine enter the first group, caratinoid colors, aspartam, ubikhinone, betuline and some others agents are included in the second group. Separate studies were carried out to assess changes in human cells sensitivity to the mutagenic exposure dependingly on nutrietns untake. The vitamin-mineral complexes of certain contents were shown to augment the resistance to mutagenic exposure in humans. The number of mutagens were employed in clinics, the actoprotector bemithyl was used to prevent the mutagenic effects of a antibacterial drug Dioxidine, flavanoid rutine was used as an agent able to reduce an abnormally high mutation level in Fankoni's anemia. Along with the above, the were designed and successfully tested the functional nutrients capable of increasing the resistance to mutagenic loads in human.

P200010

Antimutagenic and anticarcinogenic effects of afobazole

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Cancer chemoprevention is defined as the use of natural or syrthetic agents to reverse, suppress or prevent carcinogenic progression to invasive cancer. Afobazole, a new selective anxiolytic drug, produce a pronounced antimutagenic activity. Using chromosome aberration assay in the bone marrow cells of mice it was showed that the AF reduced by 45-100% of the clustogenic effects of prooxidant mutagen dioxidine and DNA-crosslinker cyclophosphamide in various regimens of treatment. In further experiments it was found that administration of AF can reduce DMBA-induced expression of the c-myc, H-ras and p53 gene in the liver, lung, kidney, lymph nodes and bone marrow of female CBA/CA inbred mice. In the long-term assay, AF getting continuously over one year reduced (DMBA)-induced tumor incidence from 80% to 30% in female and male inbred mice. Also among the mice treated with AF none developed kidney and hepatocellular malignomas. Thus, combined results obtained from the experiments in mammalian suggest that AF is effective agent to be used in either preventing or inhibiting cancer.

P200011

Effects of environmental accumulating industrial material, perfluorooctanesulfonate (PFOS) in isolated rat arteries

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Perfluorooctanesulfonate (PFOS) and its perfluoro-analogues are persistent in the environment and bioaccumulation of them in both human and animals was reported. The highest PFOS concentration reported in a fish blood from Tokyo bay was 1 micro M. Poor information on the bioactivities of PFOS-like compounds was available. In the present study, effects of PFOS on isolated rats arterial rings were compared. Cumulative concentration-dependent contractions for PFOS (1-100 micro M) were obtained in the thoracic aorta, common carotid artery (CA), femoral artery, pulmonary artery, renal artery and supramesenteric artery. The most sensitive region was CA and 10 micro M of PFOS showed significant contraction. This concentration is almost 10 fold less compared with so-called non-toxic concentration described previously. The maximum contraction on CA was larger than that of noradrenaline. PFOS was the most potent compared with perfluorooctanoic acid, octanesulfonate or octanoic acid, suggesting the importance of carbon-fluoride structure as well as sulfonate. Present results indicated the possible toxicity of PFOS as an environmental contaminant.

Key words: Perfluorooctanesulfonate; Environment; Toxicity; Vasculature

P200013

Use of HPLC-MS/MS combining precision-cut rat liver slices evaluating DNA oxidative damage

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The objective of our research was to construct a convenient and reliable method for the detection of 8-hydroxy-2'-deoxyguanosine (8-OH-dG), DNA oxidative damage marker, in precision-cut rat liver slices (PCLS) by HPLC-MS/MS and investigate isoniazid (INH)-induced oxidative DNA damage. Precision-cut rat liver slices (300 μm) were prepared, and incubated with INH (0.018 or 0.036 mol·L^{-1}) for 2 h after 1 h preincubation. DNA samples were extracted and digested into free nucleosides. After removed proteins, the samples were injected into a HPLC system with a triple quadrupole mass spectrometer. The extent of DNA damage was estimated using the ratio of 8-OH-dG to deoxyguanosine (dG). The limit of detection was 1 ng·mL^{-1} (S/N = 3) when using one production as quantifier and two further productions as qualifier and the relative standard variation was 3.38%. the linear range was from 2 to 20 ng·mL^{-1}, and the correlation coefficient was 0.9997. Isoniazid significantly increased 8-OH-dG level in PCLS at both doses. Results of the present work clearly demonstrate that PCLS-HPLC-MS/MS is a useful tool in estimating the DNA damage in the toxicity of environmental xenobiotics.

Key words: isoniazid; 8-hydroxyl-2'-deoxyguanosine; HPLC-MS/MS

P200014

Protective effects of nifedipine on vascular system against toxicity induced by mercuric chloride

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Aim: To explore the toxic effects of mercuric chloride ($HgCl_2$) on vascular smooth muscle as well as its relationship to calcium antagonist. Methods: Isolated vascular methods were used to study the effects of $HgCl_2$. Results: $HgCl_2$ (1-100 umol/L) produced a concentration-dependent contractile responses of rabbit aorta, which did not change with phentolamin or without endothelium. In KH solution with Ca^{2+}, the maximum contraction amplitude reduced by (61.2 ± 3.3) %. Nifedipine produced a concentration-dependent decrease of the maximum contraction amplitude. Conclusion: The results suggest that in vascular smooth muscles of rabbit aorta, contractile responses to $HgCl_2$ may be associated with influx of Ca^{2+} from outside of cells through nifedipine-sensitive calcium channel and release of stored Ca^{2+}, mainly with influx of Ca^{2+} from outside of cells, nifedipine has protective effects on vascular smooth muscle against damage induced by $HgCl_2$.

Key words: mercuric chloride; aortic rings; nifedipine

P200015

In vitro studies into the modes of action and the potential metabolic pathway/s activated by Norbormide (NRB)

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This study investigates the mode of action of NRB, the most selective vasoconstrictor so far known. It has previously been shown that NRB elicits a selective vasoconstriction of small arteries and vasodilation of large arteries in the rat, while dilating both small and large arteries of other species. The present study demonstrates that NRB has two further potential physiological pathways of activity leading to death. Using the Langendorff heart perfusion model, a potent coronary constriction was seen and using calcium fluorimetric assay, a deleterious effect on mitochondrial function was observed. HPLC analysis has revealed for the first time that NRB undergoes metabolism in the liver of several rodent species and that it is dependent on the co-factor NADPH. We suggest that there is potential for the metabolites to play a key role in the identified modes of action that ultimately causes lethality and that due to the unique tissue specific activity may be developed into powerful pharmacological tool(s) for the design of new drugs.

Key words: Norbormide, Coronary, Mitochondria, Metabolism.

Acknowledgement: This work is supported by Landcare Research

P21. Safety Evaluation Gastrointestinal Pharmacology

P210001

Proective effect of the ethanolic extract of Radix morindae officinalls on hypoxia/reoxygenation injury in cultutred neonatal rat cardiomyocytes

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Objective: To make a research into the protecting effect of Radix morinda officinalis(RMO) on the reputing injury of purified cultural hypoxia/reoxygenation of cardiomyocytes. Method: The models of purified cultural hypoxia/reoxygenation of cardiomyocytes were made and divided into five groups: normal cultural group, the group of hypoxia/reoxygenation of cardiomyocytes three RMO groups of high dosage, medium dosage and low dosage. The activities of cardiomyocytes SOD were measured by the method of xanthine oxidase and the contents of cardiomyocytes MDA by the method of thiobarbituric acid, The activities of LDH in culture were evaluated, The contents of cardiomyocytes NO were measured by he method of nitrifying ferment. Result: RMO could distinctively raise SOD and LDH, lower MDA and increase NO. Conclusion: Radix morinda officinalis has obviously protective effects on cultured neonatal rat myocardial cell injured by H/R.

Key words: the ethanolic extract of RMO; cultured cardiomyocytes; hypoxia/reoxygenation njury; lipid peroxidation

P210002

Gaultherin, a natural salicylate derivative from Gaultheria yunnanensis: towards a better non-steroidal antiinflammatory drug

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Gaultherin has been shown to have analgesic and anti-inflammatory effects and lack gastric ulcerogenic effect compared to aspirin in our primary study. The aim of the study was to investigate the mechanism of action of gaultherin, which may rely on its active metabolite, and the mechanism responsible for its non-ulcerogenic property. The results showed gaultherin (400 mg/kg) significantly inhibited acetic acid-induced writhings (33%) and croton oil-induced ear edema (39%) in mice. The metabolism characters of gaultherin in animals indicated that it could be converted to salicylate, which produced the pharmacological effects and provided effective concentrations for an extended period. In vitro metabolism study showed that gaultherin was metabolized by β-glycosidase produced by intestinal bacteria and esterases in vivo successively to release salicylate finally. The study suggested gaultherin did not cause gastric ulcer for the reason that it released salicylate in intestine slowly, not in stomach and it left the cyclooxygenase-1 unaffected, which was the source of cytoprotective prostaglandins in gastric epithelium.

Key words: Gaultherin; NSAIDs; Salicylate; Gastric ulcer

P210003

The anti-ulcer effect of Tibet medicine of gentiana macrophylla

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Objective: To study the anti-ulceration effect of gentiana macrophylla form Tibet, and the mechanism of action. Methods: We studied the effect of the gentiana macrophylla ethanol extract on aspirin and absolute ethyl alcohol-induced rat gastric ulcer models. Results: The results showed that the gentiana macrophylla ethanol extract could significantly dwindle the areas of the gastric ulcer models. It could reduce the total quantity of gastric juice and the secrete of gastric protein if the quantity of gentiana macrophylla ethanol extract were enough. There was no difference between them and control. Conclusion: The gentiana macrophylla ethanol extract could prevent gastric ulcer and the mechanism need further research.

Key words: Tibet medicine; gentiana macrophylla; gastric ulcer

P210004

Effect of Lysozyme Chloride on Insulin-Resistance Aggravated Gastric Oxidative Stress and Hemorrhagic Ulcer in Indomethacin-Treated Rats

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The influence of insulin-resistance (IR) on indomethacin-induced gastric injury is unknown. The aim was to study the aggravation of IR on indomethacin (IDM)-induced gastric erosions and its protection by lysozyme chloride in rats. Male Wistar rats were allowed drinking water with or without 30% (v/v) fructose for 21 days. Rats were fasted for 12 h before an oral glucose tolerance test (OGTT) was performed to assure IR. Six hours after OGTT, rat stomachs were irrigated for 3 hours with gastric juice or normal saline. Gastric parameters, including acid back-diffusion, lipid peroxide, glutathione, mucus and hemorrhagic ulcer were determined. Increased serum glucose level and decreased insulin sensitivity was achieved in rats after challenge of fructose. Aggravation of various gastric parameters also was observed in these rats challenged with IDM. Intraperitoneal lysozyme chloride (0-300 mg/kg) dose-dependently inhibited gastric parameters in IR rats treated with IDM. In conclusion, IR exacerbated gastric hemorrhagic ulcer in IDM-treated rats was associated with oxidative stress that was effectively ameliorated by lysozyme chloride.

Key words: Lipid peroxide, glutathione, mucus, stomach

P210005

Protective effects of THSG on acetic acid-induced ulcerative colitis in mice

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To evaluate the protective effect of THSG (2,3,5,4'-tetrahydroxystilbene-2-O-beta-D-Glucoside, purity: 99%) on acetic acid-induced ulcerative colitis (UC) in mice. 105 mice were randomized into 7 groups: normal group, model group, positive drug group (5-aminosaliylic acid, 5-ASA 10 mg/kg), THSG treated groups (10, 30, 60, 120 mg/kg). UC model was induced by 0.1 ml 5% acetic acid. Colon tissue structures were observed with HE stain. Nitric oxide (NO), Myeloperoxidase (MPO), Malondialdehyde (MDA) and Superoxided mutase (SOD) of colon tissue were measured with biochemical methods. Results show that Colon tissues in model group had the obvious congestion, edema and ulceration. NO, MPO and MDA contents in model group were higher than in normal group; SOD were lower than normal group ($P < 0.05$). 5-ASA significantly improved pathological states and biochemical indexes. Similarly, THSG alleviated pathological changes, decreased NO, MDA, MPO levels and increased SOD, vs model group ($P < 0.05$). Moreover, MPO level diminished and SOD enhanced in dosedependent manner. Overall, THSG has protective effects on UC in mice by inhibiting the production of NO.

Key words: THSG, UC, acetic acid

P210006

Cyclohexenonic long-chain fatty alcohol reverses diabetic induced dysfunction of ileum in the rat

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Objective: Diabetic neuropathy is associated with development of intestinal motility dysfunction and autonomic neuropathy. We studied the effects of cyclohexenonic long-chain fatty alcohol (FA) on isolated-ileum in the diabetes induced rats. Methods and Results: The rats divided into 5 groups. One is the non-diabetic group, others induced diabetes. 4 weeks after induction of diabetes, one group killed immediately, while other 3 groups were administered FA (0, 2 or 8 mg/kg) for more 4 weeks. The serum glucose and serum insulin levels were unchanged by FA. The contractile responses to carbachol and KCl were argumented in the isolated-diabetic ileum. Real-time PCR and histological study showed changes of muscarinic M2 and M3 receptors of the diabetic ileum. Treatment with FA improved the thickness of intestine wall and diabetic-induced hyperreactivity of the rat ileum. Furthermore, FA reversed the diabetes-induced upregulation of muscarinic mRNAs in the diabetic rat ileum. Conclusion: These results indicate that FA has therapeutic effects on hyperreactivity in the diabetic ileum by ameliorating over expression of muscarinic M2 and M3 receptors mRNAs.

Key words: FA, neuropathy, muscarinic receptor, ileum

P210007

Gastroprotective activity of pectins against acute indomethacin-induced gastric mucosal injury in rats

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The purpose of the study was to estimate preventive influence of low-esterified pectin and calcium pectate on development of gastric ulcers induced by administration of indomethacin in rats. Pectin preparations were given daily through gastric gavage in various doses for 8 days before single administration of indomethacin. Chemical structure of the preparations was strictly determined before experiments. Results showed that preliminary administration of the polysaccharides prevented profound injury of gastric mucous lining. In animals given pectin was registered 1.8-2.1-fold smaller amount of lesions in the gastric mucous than that in non-treated rats. Calcium pectate also contributed to 1.8-2.0-fold decrease of the ulcer quantity. General area of ulcerous injury in the gastric mucous was reduced due to advance use of low-esterified pectin by 40.6-58.7% dependent on the dose used, whereas in rats given calcium pectate this parameter was 39.2-39.4% lower than that in nontreated animals. The results of the study showed that pectin substances may be considered as protective agents against gastric lesions.

Key words: Gastric ulcer, indomethacin, non-starch polysaccharide, pectin

P210009

Effects of lipoxin A4 and lipoxygenase inhibitors on gastric mucosal defense

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Aspirin leads to formation of protective 15(R)-epi-lipoxin (LX) A4 via acetylated cyclooxygenase (COX)-2 and further metabolism by 5-lipoxygenase (LO) (Fiorucci et al., 2002). Serhan et al. (2000) have described that in the presence of indomethacin and acetaminophen arrays of anti-inflammatory lipid mediators are produced from mucosal eicosapentaenoic acid via COX-2-dependent oxygenations and 5-LO. Whereas in rats ischemia reperfusion alone induced minor gastric damage pretreatment with the COX-2-inhibitor celecoxib markedly increased injury. Low doses of indomethacin, acetaminophen, S- or R-flurbiprofen, before or after celecoxib protected against the damage-aggravating effect of celecoxib. The protective effects of the drugs were reversed by pretreatment with inhibitors of 5-LO (A63162), 12-LO (baicalein) or 15-LO (PD146176) or the LXA4/annexin 1-receptor antagonist BOC1. The findings show that the protection by these non-steroidal anti-inflammatory drugs is not mediated by COX-2 as it operates when COX-2 is inhibited, but is modulated by LO activities.

Key words: Lipoxygenases, cyclooxygenase-2, non-steroidal anti-inflammatory drugs, gastric injury

Acknowledgement: This study was supported by the DFG

P210010

Protective role of tissue factor (TF) in mesenteric ischaemia

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Tissue factor (TF) is a key protein in coagulation and the associated inflammation. Transgenic TF(low) mice appear normal, but develop cardiac fibrosis over time (Mackman 2002). To study the role of TF in a model of critical illness, we have explored the outcome of mesenteric ischaemia/reperfusion in transgenic TF(low) mice compared with normal wild type (WT). Anaesthetised mice were exposed to 45 min of mesenteric ischaemia. The TF(low) mice died within 1 hour after reperfusion, whereas WT survived for more than two hours. Both group of animals died from cardiac incapacitation and arrest. At autopsy the animals exhibited inflamed intestines. The haematocrit was increased from 44 (normal) to > 60. This was reflected by decreased wet/dry ratios of lung and heart. MPO activity in lung was doubled. At time of death the parameters were equally abnormal in TF(low) and WT mice. It can be concluded that TF expression level inversely determines the rate at which inflammation develops and fluid accumulates in the gut. In addition or alternatively myocardial TF may be a critical factor in determining the capacity of the heart to maintain viability in response to severe haemoconcentration.

P210011

Central mechanisms involved in gastric mucosal defense.

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Vagal nerve is likely to have a prominent role in centrally induced gastroprotection. However, different neuronal pathways project to dorsal vagal complex which may influence the vagal efferent activity. The present work analysed the chain of events involved in alpha-2-adrenoceptor-initiated gastric mucosal defense. Methods: Gastric damage was induced by ethanol. Gastric acid secretion was measured in pylorus ligated rats. Drugs were given either intracerebroventricularly (icv.) or intraperitoneally (ip.). Results: Rilmenidine-alpha-2/I1-imidazoline receptors agonist-inhibited the ethenol-induced lesion (ED50: 2 nmol/kg ip., 6 pmol/rat icv.). Yohimbine, prazosin, ARC 239 (icv.) (alpha-2B-adrenoreceptor antagonists) and the opioid receptor antagonist naloxone inhibited the gastroprotective effect. The gastroprotection was also blocked by NMDA receptor antagonist dizocilpine and NO synthase inhibitor L-NNA (icv.). Conclusion: Activation of central alpha-2B-adrenoceptor subtype initiates an opioid-excitatory amino acid-NO-mediated process resulted in gastric mucosal protection.

The work was supported by ETT 389/2003 and National Research and Technology, Hungary

P210012

CHARACTERIZATION OF THE PATTERN OF EICOSANOID PRODUCTION IN GUINEA-PIG COLON

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Our study aimed to investigate the effects of cyclooxygenase (COX) isoform inhibition on in vitro colonic eicosanoid production. Rings of guinea-pig distal colon were mounted under isotonic conditions in 5-ml organ baths containing warmed (37 °C) and gassed (carbogen) Krebs solution. Eicosanoids were measured by radioimmunoassay. During the basal 30-min collection fraction, total production of prostaglandin I2 (PGI2), PGE2, PGF2α, PGD2, thromboxane A2 (TXA2) and cysteinyl-leukotrienes was 39.3 ±4.3, 3.0 ±0.5, 2.8 ±0.4, 2.2 ±0.2, 1.6 ±0.2 and 0.6 ±0.1 pg/mg of tissue (n = 30). Indomethacin (3 μM), SC-560 (0.3 μM) and NS-398 (1 μM) (non-selective, COX-1- and COX-2-selective inhibitors, respectively) significantly reduced PGI2, PGE2, PGF2α and TXA2 total production (indomethacin to 5.1 ±1.8 %, 32.2 ±8.2 %, 13.5 ±1.5 % and 49.6 ±10.5 % of control levels (n = 6), respectively; SC-560 to 13.6 ±3.2 %, 50.2 ±6.1 %, 29.7 ±6.6 % and 53.0 ±9.9 % of control levels (n = 7), respectively; NS-398 to 26.1 ±6.3 %, 50.2 ±6.3 %, 37.0 ±7.1 % and e 53.7 ±9.7 % of control levels (n = 6), respectively). These data show that both COX isoforms produce significant eicosanoid amounts, with a slight predominance of COX-1.

Supported by MIUR COFIN 2003.

P210013

Gastroprotective researches of curcumin in solid dispersions with the polymers PVP-K30[1]

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This work was to assess gastroprotective effects of curcumin in solid dispersions with the Polyvinylpyrrolidone K30 (PVP). Curcumin-PVP solid dispersions (SDs) in different ratios were prepared by co-evaporation in ethanol solution. The best ratios of curcumin to PVP was ensured by dissolution test. The gastroprotective activity of curcumin-PVP SDs (1:8) was determined on gastric ulcer rat models induced by acetic acid, ligated pylorus, and reserpine. The effect on the healing of subacute gastric lesions in rats was also studied. The results of curcumin SDs by oral administration on gastric ulcer rat model induced by acetic acid indicated that the ulcer index was decreased significantly. The serum NO level was markedly increased and the plasma ET level was markedly reduced. Curcumin SDs could prevented ligated pylorus induced gastric ulcer by decreasing ulcer index, vdume and acidity of gastric juice and the level of pepsin output. Curcumin SDs also prevented reserpine induced gastric ulcer by decreased the ulcer index. Curcumin-PVP SDs could be applied its gastroprotective and ulcer healing activities

Key words: Curcumin; Polyvinylpyrrolidone; solid dispersions; gastric ulcer

[1] Project supported by the National Natural Science Foundation of China, No. 30170105; Supported Program of New Century Excellent Talent(No. NCET-04-0808); Supported Program of Fok Ying Tung Education Foundation(No. 91036)

P210014

Nicotine aggravates ethanol-induced gastric mucosal injury: role of asymmetric dimethylarginine

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Objective: To explore the involvement of asymmetric dimethylarginine, a major endogenous nitric oxide (NO) synthase inhibitor, in the intensifying effect of nicotine on ethanol-induced gastric ulceration. Methods: In vivo, male Sprague-Dawley rats were oral treated with nicotine (5 mg/kg/day) for 28 days, and then gastric mucosal injury was induced by oral administration of ethanol (75%, 1.5 ml). In vitro, human gastric epithelium cells (hGEC-1) were incubated with 8% ethanol for 1 h followed by 24h-pretreatment with nicotine (1-10 μM). Results: Chronic nicotine treatment significantly intensified ethanol-induced gastric mucosal injury (evaluated by ulcer index) associated with an elevated concentration of ADMA and a reduced content of NO in plasma of rats. As shown by MTT test, pretreatment with nicotine concentration-dependently aggravated the decreased viablity of hGEC-1 induced by ethnal, concomitantly with an increase in level of ADMA in culture medium. Conclusion: The intensifying effect of nicotine on ethanol-induced gastric mucosal injury may be related to increase of ADMA accumulation.

Key words: Asymmetric dimethylarginine; Nicotine; Gastric mucosa

P210015

Long-term Toxicity Study on tianchuan granule in rats

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To study long-term toxicity of tianchuan granule in rats, which will offer theory basis on clinical uses. Tianchuan granule of 100, 50, 25 $g \cdot kg^{-1} \cdot d^{-1}$ was given by repeated gastric infusion for 180 days in rats. During the experiment, the rats' physical conditions, body weight, hematological, hemotobiochemical parameters, coefficient and histomorphological figure of main organs were observed. After administering tianchuan granule, the rats' conditions were not so better in 45d and 75d. body weight grew slower than that of control. When the rats were given drugs for 60d. The cholesterol decreased markedly in the large and middle groups. There were no significant changes in other groups compared with control. No notable histopathological changes were observed. After withdrawaling tianchuan granule 15d, the abnormal index was restored. The results suggested that toxic dose of tianchuan granule for rats was about 100 $g \cdot kg^{-1} \cdot d^{-1}$. The safe dose was about 25 $g \cdot kg^{-1} \cdot d^{-1}$. The decreased cholesterol of two groups in 90d and 180d, which restored normal after withdrawaling of tianchuan granule for 15d, maybe the enlarged effect of tianchuan granule.

Key words: tianchuan granule; long toxicity; enlarged effect

P210016

Experimental pathdogical study on orally administration of Tx to rats

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To study the pathological change of Tx, 96 Sprague-Dawley rats were randomly assigned to 4-group, as referred to vehicle group and 3-dosing-group. Females were orally administrated Tx daily at doses of 1, 2 and 4 mg/kg, while males at 2, 4 and 8 mg/kg. Treatment continued for 4 weeks followed by 4-week recovery period after withdrawal. Organ weight measurements and gross and histopathologic examination were performed. Following the 4-week treatment, splenomegaly in the females of 2 and 4 mg/kg groups and males of 8 mg/kg group, and atrophy of epididymis and testes in males of all dosing groups were observed. The abscolute and relative weights of spleen increased, while that of testes and epididymis decreased. Histopathologically, enhancement of extramedullary hematopoiesis in spleen, degeneration of seminiferous tubules in testes, and decrease in sperm in epididymis occurred. All lesions developed in a dose-dependent manner. After withdraw, only lesion in spleen recovered. And no any other delayed pathological change developed. In conclusion, Tx could induce pathological changes in spleen in both sexes and in reproductive organs in males.

Key words: tripterygium wilfordii; experimental pathology

P210017

THE EFFECTS OF OMEPRAZOLE-LIKE COMPOUNDS ON GASTRIC ACID SECRETION AND INDOMETHACIN-INDUCED GASTRIC MUCOSAL DAMAGE IN RATS.

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Background: Omeprazole is a basic molecule of proton pump inhibitors having gastric acid inhibitory actions. Aims: 1. To procude new chemical compounds of omeprazole with its chemical modification having PARP-inhibitory and antioxidative properties [L-2279 ($C_{19}H_{29}N_3OS$), L-2243 ($C_{14}H_{12}N_4OS$), HO-3098 ($C_{17}H_{22}N_4OS$), HO-3215 ($C_{18}H_{24}N_4OS$) HO-3243 ($C_{20}H_{22}N_5OS$)]; 2. To study the compounds on the gastric acid secretion in 4 h pylorus-ligated rats and indomethacin (IND)-induced (20 mg/kg sc.) gastric mucosal damage. Materials and Methods: The observations were carried out in 4 h pylorus-ligated and in IND (20 mg/kg sc.) treated (without pylorus ligation) rats. Results: The omeprazole and omeprazole-like compounds (having antioxidative and PARP-inhibitory properties) dose-dependently decreased both gastric acid secretion and gastric mucosal damage. Conclusion: The gastric acid inhibitory and mucosal preventive effects can be combinated chemically by the PARP-inhibitory and antioxidative properties, representing a new pathway in the drug research.

Key words: gastric acid secretion; gastric mucosal damage; omeprazole; omeprazole-like comporents with PARP-inhibitory and antioxidative properties.

Grant: RET-II. 08/2005.

P210018

Protective effect of total glycosides of Zhizi on experimental gastric mucosal lesion induced by low dose aspirin

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Objective: To study the protective effect of total glycosides of Zhizi (TGZ) on experimental gastric mucosal lesion induced by low dose aspirin in rats. Methods: Used low dose aspirin(5.0 mg/kg) and TGZ continuous intragastric injection 14d to make the model, measured the lesion index of rats in TGZ (140, 70, 35 mg/kg) and control groups. The activity of Nitric oxide synthase (NOS) and Nitric oxide (NO) level in blood and the expression of intercellular adhesion molecule (ICAM-1) in gastric tissue were determined as well. Results Compared with control group, TGZ (140, 70, 35 mg/kg, ig, 14d) could obviously relieve gastric mucosal lesion index induced by low dose aspirin continuous intragastric injection 14d. TGZ increased NOS activity and NO content as well. Immunological

histology examination showed that ICAM-1 expression increased evidently in control group, and TGZ could degrade the expression of ICAM-1 in gastric tissue. Conclusion: TGZ can inhibite low dose aspirin-induced gastric mucosal lesion, the mechanism maybe related to increasing of NO level and reducing ICAM-1 expression in gastric tissue.

Key words: gastric mucosal lesion, total glycosides of Zhizi, Aspirin

P210019

Healing effect on gastric and oral ulcers of tannic extract obtained from Pinus caribaeae Morelet bark and predinical toxicology tests.

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The study examines the healing effect on gastric and oral ulcers of tannic extract obtained from Pinus caribaeae Morelet and preclinical toxicology tests. The protective effect on gastric mucous was evaluated in lesions induced by ethanol in Wistar rats at dose of 5 mg/kg. Oral mucous healing effect was evaluated in lesions induced by acetic acid in Golden Syrian hamster at 3 dose levels: 64, 80 and 100 mg/ml. Oral acute toxicity was conduced at dose of 2000 mg/kg and subchronic doses of 1, 2.5 and 5 mg/kg/day were used for 90 days exposure in Wistar rats. Tannic extract causes significant decrease of gastric lesions. Ucers number and lesions index decreased in 47 and 35% respectively. Oral mucous dosing causes significant acceleration of scaring. Epithelial regeneration and own sheet maturation were accelerate in treated group. There were no mortality and signs of toxicity in acute toxicity assay. In subchronic exposure signs of toxicity were observed and body weight gain was significantly increased. Some of the blood and biochemical elements were affected. The histopathological examination showed abnormalities in liver, kidney, stomach and nasal cavity organs at doses of 1.0 and 5.0 mg/kg/day.

Key words: gastric and oral mucous healing, oral acute toxicity, oral subchronic toxicity.

P210020

A Report of Oral Dose Toxicity Study of Triptolides (Tx) in Sprague-Dawley Rat

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Tx is a novel immunosuppressant derived from triptolides. To detect the no observed adverse effects level (NOAEL) and the toxic target organ of Tx, SD rats were orally administrated daily for 28 days following 28 days recovery period after withdrawal. 48 females and 48 males were randomly assigned to 4 groups respectively. The acute toxicity (LD50) of Tx in rats was 10 mg/kg in females and 23 mg/kg in males, indicating the different toxic sensitivity on rats in both sexes. The study was designed as for females at doses of 0, 1, 2 and 4 mg/kg, while males at 0, 2, 4 and 8 mg/kg. There were no drug-related changes in females at 1 mg/kg group. However, females at 2 and 4 mg/kg and males at 4 and 8 mg/kg groups showed obvious drug-related changes in the decreased body weight, hematology (decrease of erythrocyte count, hemoglobin and hematocrit, and increase of reticulocyte count and platelet), and histopathology (spleen, testes and epididymis). All toxic changes were dose-dependent. It is suggested that the NOAEL is 1 mg/kg in females and less than 2 mg/kg in males. The toxicity target organs of Tx were the spleen in both sexes and the reproductive system in males.

Key words: Triptolides NOAEL Toxicity

P210021

Comparative measurement of cyanide and paraquat mitochondrial toxicity using two different mitochondrial toxicity assays

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Cyanide (KCN) and paraquat (PQ) are very toxic to mitochondria. In this study the toxicity of KCN and PQ in the isolated rat liver mitochondria were determined using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and JG-B (Janus green B) assay by multiwell scanning spectophotometry. JG-B was used not only for the vital staining of mitochondria but also for mitochondrial viability assay and was compared to MTT assay. The rat liver mitochondria were first isolated by centrifuge in a mixture of 0.25 M saccharose solution and 0.05 M Tris buffer. Various concentrations of paraquat (0.001 to 100 mM) and KCN (0.0001 to 100 M) on the mitochondria isolated from the liver were investigated. The 50% lethal concentration of toxins were found for PQ (4.45 ±0.02, 49.69 ±0.01) and KCN (0.22 ±0.02, 4.95 ±0.02), as determined by these assay (JG-B and MTT respectively). Significant correlations were also observed among the two methods with a 95% coefficient interval ($r = 0.95$, $p < 0.0001$; $r = 0.91$, $p < 0.0001$; PQ and KCN respectively). These results suggest that both methods are reliable and are comparable for determining the mitochondrial assay. It is concluded that the JG-B assay may be preferable to MTT assay methods because of its simplicity, low cost, sensitivity and objectivity; in addition, this method is not time dependant.

Key words: Rat liver mitochondria. Janus green B. MTT. PQ. KCN. micro ELISA reader

P210022

Methylisogermabullone isolated from radish roots stimulates small bowel motility via activation of acetylcholine receptors

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We have previously reported that extract of radish roots exhibits an increase in gastroingestinal motility through the activation of muscarinic receptors. Based on the stimulatory activity-guides fractionation on rat ileal segments, this study isolated methylisogermabullone (MIGB) from methanol extracts of radish roots. MIGB caused a significart increase of the isolated rat ileal contraction in a concentration-dependent manner, and the pattern of MIGB-induced ileal contraction was different in the time course to that produced by ACh. MIGB (230 μM)-induced ileal contractions were enhanced by pretreatment of segments with ACh (0.1 μM). Ileal contractions produced by MIGB (230 μM) or ACh (0.1 μM) at submaximal concentration were partially inhibited by pretreatment of hexamethonium mice stimulated the small intestinal transit of charcoal in a dose-dependent manner, and MIGB-induced stimulation of small intestinal transit was significantly attenuated that MIGB stimulates the small bowel motility through the activation of ACh receptors. These findings suggest that MIGB may become a potential regulatory agent for therapeutic intervention in dysfunction of gastrointestinal motility.

Key words: Methylisogermabullone, Muscarinic receptors, Gastrointestinal motility, Rat

Acknowledgement: This study was supported by a grant from the Wonkwang University Research Fund in 2005.

P210023

The assessment of melatonin in intestinal ischemia reperfusion in rat

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The aim of this study was to determine the effect of melatonin, a hormone which secreted from pineal gland and is known as an antioxidant and free radical scavenger, on the protection of tissue damage mesenteric ischemia-reperfusion (i/r). a total of 36 young male wistar-albino rats (wighting 80-120 G) were divided equally in to 6 groups with variane concentration of melatonin (10, 20, 30 MG/KG) respectedly treatment. group 1 was control. group 2 was sham that surgical process was applied until superior mesenteric artery (sma) dissection and received vehicle solution only in equall volume also by intramuscular route. group 3 was i/r, group 4 was i/r plus melatonin 10 MG/KG, group 5 was i/r plus melatonin 20 MG/KG, group 6 was i/r plus melatonin 30 mG/KG. after laprotomy, a microvascular atraumtic clip was placed across the superior mesentric artery (sma) under general anaesthesia, and it was removed after ischemia for 30 minutes. the first dose of melatonin was applied intramuscularly just before reperfusion, the second dose was applied just after reperfusion, and the third dose was applied on the second day intramuscular route. on the third day of the expriment all of rats were killed, and their bowels were removed. histopathological analysis and melandaldehyde (mda) levels, as an index of lipid peroxidation were assayed. the levels of tissue mda were found to be significantly lower in group 4 with group 3 ($P < 0.05$). there was significant diffrence in histopathological analysis of group 4 with group 3 ($P < 0.01$). these results suggest that melatonin has antioxidant effect in preventing intestinal ischemia reperfusion (i/r) damage.

Key words: melatoin: antioxidant: oxidative damage: ischemia-reperfusion

P210024

Early Toxicity Screening on 3′,4′-Di-O-(-)-camphanoyl-(t)-cis-khellactone Serial Compounds

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3′,4′-di-O-(-)-camphanoyl-(t)-cis-khellactone (DCK) serial compounds, yielded by modification of suksdorfin, showed extremely potent inhibitory activity against HIV-1 replication. The study was undertaken to examine the potential toxicity of DCKs with a short-term toxicity screening system, including MTT assay, the up-down method, Ames-fluctuation test and micromass culture assay, so that eliminate as early as possible the compounds that are unfit for further development. The IC_{50} values of DCKs were used to estimate the LD_{50} value which can then be used to determine the in vivo starting dose. The LD_{50} values of DCKs were more than 2000 mg/kg in female mice. All compounds showed negative results of Salmonella (TA_{100}) mutagenicity test. 3-F-4-Me-DCK might be teratogenic as indicated by differential inhibition on embryonic cells in vitro, and the 3-CH_2NO_2-4-Me-DCK and 3-CH_2CN-4-Me-DCK have toxic effects on fetal cells, but there was no evidence of teratogenicity. On account of its high potency and low toxicity, 3-CH_2CN-4-Me-DCK was chosen as a candidate for further development. Supported by Grant D0204003041631 from BMSTC.

Key words: Discovery toxicology, DCK serial compounds, Early toxicity

P210025

Acute and subchronic toxicity of *Galega officinalis* **L. in rats.**

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In this study, acute and subchronic toxicity of Galega officinalis L. (Galega) have been evaluated. In order for the acute toxicity study, five groups of 10 rats (5 males, 5 females) received orally four different single dose of plant suspension and animals were kept under observation for 14 days. The acute toxicity study has indicated that LD50 of Galega is higher than 5 g/kg. For subchronic toxicity, the animals (24 males, 24 females) were divided into four groups (6 animal/sex/group) and were fed a diet containing rat standard food and 0, 0.15, 1.5 and 3% w/w of Galega. At the end of the study (90 days) blood samples were taken for hematological and biochemical parameters. The results show that Serum levels of cholesterol in both females and males (1.5 and 3%) has increased significantly ($p < 0.01$). The organ/body weight ratio determinations demonstrate a statistically significant increase in liver/body weight in the highest two dose levels in males ($p < 0.01$) and group 3% in females ($p < 0.05$). Present data suggests that male and female rats were sensitive to toxicity effects of Galega officinalis and that liver could serve as a target organ in oral toxicity of this plant.

Key words: Galega officinalis L., Acute toxicity, Subchronic toxicity, Rats.

P22. Drug Discovery-High Throughput Drug Screening

P220001

Lung Functions Studies on Workers in Two Iraqi Industries

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Lung function test in twenty four Iraqi welders and forty three workers in Iraqi tanning industries were investigated. The welders showed a decrease in dynamic lung functions and some showed a decrease in static lung functions. No significant changes in lung function tests were observed in workers of chromium tanning industries compared to controls. These results are discussed in relation to the concentrations of welding fumes and chromium respectively in the working environment. Short and long term animal studies were performed to support the results.

P220002

A novel method for screening nonsteroidal ligands by androgen receptor ligand binding domain microarrays

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This study was to develop a high throughput screening (HTS) method based on protein microarrays technology and identify nonsteroidal ligands for androgen receptor ligand binding domain (AR LBD). The initial work focused on expressing soluble proteins of AR LBD in E. coli, and preparing protein mircoarrays by immobilizing purified AR LBD on the silane polysaccharide surface. Binding assays were then performed to evaluate the function of AR LBD microarrays and the stability of the HTS method. 190 candidates of nonsteroidal compounds were also selected from 10,067 compounds library with computer aide Cscore program. Finally, The AR LBD microarrays were used to screen these candidate compounds and to demonstrate the novel method. Based on the results, the shape of the close dependence curve suggested a positive cooperative binding of Methyltestosterone with AR LBD microarrays. A Z factor of the HTS method was 0.69 which can meet the requirement of drugs screening. One active compound for AR LBD was identified with IC50 of 371 μM. In conclusion, AR LBD microarrays method was stable and sensitive, and suited for high throughput screening efforts.

Key words: microarrays, androgen receptor, nonsteroid

P220003

Application of enzyme chip and chemical arrays in screening elastase inhibitors

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A microarray assay was fabricated to screen elastase inhibitors. Firstly, A protease film was formed by uniformly distributing elastase solution on an agarose-coated slide. Secondly, Fabrication of substrate microarray was completed by arraying substrate solution on the protease film. The Km value was identified as 7.31 mM, which was consistent with the previous report. Z' value of this assay was 0.52(>0.5). At last, chemical arrays were integrated with substrate microarray by arraying compounds and substrate solution at the same sites on the protease film. After incubation for two hours, the slide was analyzed by determining blue intensity of each spot. The precision assay showed excellent reproducibility. The spotted density was 480 spots/cm2 and 11680 compounds were used to screen. After primary and secondary screening, two compounds, J7720 and J11740 were hit with the IC50 values less than 1 mM. The results showed that the microarray assay is miniaturized, sensitive and applicable for high throughput screening.

Key words: chemical arrays substrate microarray
Acknowledgement: This work was supported by the National High Research and Development Program of China.

P220004

High throughput screening method of identifying potential ligand for CCR4

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Objective: The chemokine CCR4 plays an important role in the pathogenesis of asthma, and CCR4 antagonist is the potential compound of anti-asthma. Thus in this paper, we established a functional cell line stably overexpressing human CCR4 and optimized the condition of high throughput screening method to study the interaction of CCR4 and its ligand. Methods: HEK293 cells transfected pcDI-CCR4 vector were selected by G418 and identified by Western Blotting analysis. The assay condition, such as cell number in each well, cytokine concentration and incubation time, were examined and optimized. Results: A steady cell line and a reliable method for CCR4 ligand screening methods were established. The incubation time was 50 minutes, the concentration of FITC-CKLF1 is 0.16 mg/ml, and the cell number per cell was 3,000. Conclusion: The CCR4 vector has been successfully transfected into HEK293 cells, and the high throughput screening method has also been successfully applied to identify ligand for CCR4.

Key words: CCR4; HEK293; FITC-CKLF1; high throughput screening
Acknowledge: This work was funded by the National Science and Technology Attack plans (200BA711A02-06)

P220005

Drug screening based on reporter gene and the signal transduction of interferon-alpha

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Aim: To develop a method of drug screening based on reporter gene and the signal transduction of interferon-alpha system in order to screen the small molecular compounds with interferon-alpha like activity. Methods: A recombinant vector pTAL-ISRE-SEAP was constructed then transfected into ECV304 cells. Stably transfected cell clones were isolated and used to screen 400 compounds. The compound NO.258 was studied in antiviral model in vitro, and the mechanism of possible anti-HBV were explored by RTPCR. Results: The expression of SEAP was induced by IFN-alpha in dose-dependent manner. The Z-factor value was 0.8. The signal transduction of IFN-alpha. can be activated by compound NO.258, DNA copies of HBV in HepG2.2.15 cells were treated by this compound were lower than cell controls, OAS3 gene in Stably transfected cells was not influenced by this compound. Conclusion: The cloned cells can be used to screen for compounds with IFN-alpha like activity. Compound NO.258 can activate the signal transduction of IFNalpha and has antiviral activity in vitro.

Key words: ISRE; IFN-alpha; drug screening; reporter gene

Acknowledgement: We are particularly grateful to Ye Qi-nong for the generous guidance of PCR.

P220006

The expression of recombinant human LOX-1 and identifying its mimic ligands by fluorescence polarization-based high throughput screening

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LOX-1 was identified as a major receptor for oxLDL in endothelial cells. It critically mediates the endothelial dysfunction and the progression of atherosclerosis (AS) by oxLDL stimulation. To obtain human LOX-1 and identify its mimic ligand, a recombinant plasmid was structured and expressed hLOX-1. Western blot analysis ensured the expressed recombinant hLOX-1 protein and a receptor-ligand binding assay showed that it had a high binding affinity with oxLDL. A competitive fluorescence polarization (FP)-based high throughput screening (HTS) method was established to isolate the ligands of hLOX-1. The evaluating parameter Z' value of 0.72 for this method showed that FP-based HTS assay was robust and the results had a high reliability. A total of 20 316 chemicals were screened, and 2 chemicals were identified that they have a high affinity with hLOX-1. Uptake assay further confirmed that two chemicals block the uptake of hLOX-1 to DiI-oxLDL. And the preliminary results indicated that isolated mimic ligands may act as a function of antagonist. The discovery of hLOX-1 mimic ligand would benefit to further study the function of LOX-1 and identify a novel avenue for prevention and treatment AS.

Key words: LOX-1; FP; HTS; AS

P220007

HT Screening of MurA Inhibitors from Microorganism Metabolites Library

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MurA catalyzes transfer of enolpyruvate from phosphoenolpyruvate to uridine diphospho-N-acetylglucosamine, which is the first committed step of bacterial cell wall biosynthesis. MurA is highly conserved across different bacterial strains. No mammalian homologue of MurA so far has been found. A high throughput screening assay was developed to screen MurA inhibitors from a microorganism metabolites library composed of 20000 extracts from 10000 actinomycetes and10000 fungi strains collected from China. Four active compounds were identified. One compound showed an IC_{50} of 60 μg/ml against MurA. It also had moderate antibacterial activity against Enterococcus faecalis, Escherichia coli and Staphylococcus aureus. Structure elucidation showed that it was identical with a previously reported compound: Citrinin. Our results therefore suggest that the molecular mechanism of Citrinin for its mild antibacterial activity could be interference with bacterial cell wall synthesis by inhibiting MurA..

P220008

Establishment and its application of a reporter gene-based screening cell model for discovering new agonists of estrogen receptor beta subtypes

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Aim: To establish a reporter gene-based screening model and use it to screen compounds for discovering agonists of estrogen receptor beta subtype. Methods A recombinant vector pTAL-ERE-SEAP was constructed then transfected into HEK293 cells. The speciality, stability, time-effect relationship, dose-response relationship and the immunocytochemistry staining were tested. 400 compounds were screened. Results The expression levels of SEAP was induced by E2 in a dose-response relationship and time-effect relationship manner. The Z-factor value was 0.7, the result of immunocytochemistry staining showed the expression of ER beta. E2 had no proliferation effects on stably transfected clones. Conclusion The positive clones can be used to screen compounds for discovering agonists of estrogen receptor beta subtype. 7 compounds were screened out.

Key words: estrogen; ERE; drug screening; reporter gene

Acknowledgement: We are particularly grateful to Satoshi Inoue for the generous gift of pCXN2-hER beta.

P220009

Screening the specific intercellular proteins interacted with opioid addiction

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Aim: To screen the specific proteins that physically interacted with the intracellular domains of opioid μreceptor. Methods: C-terminus of μreceptor was selected as bait, and the rat brain cDNA library was constructed as prey. With total RNA as template, the rat brain ds cDNA was amplified by RT-PCR method. The ds cDNA and vector were then transferred into strain AH109. The library host strain was mated with bait strain Y187, to select the positive colonies, the mating mixture were spread on SD/-Ade/-Hs/-Leu/-Trp plates. And the positive clones were characterized by colony-PCR method and DNA sequencing. Results: About 60 positive clones were sequenced and analyzed, three of them were encoded functional proteins, which were choline acetyltransferase (ChAT), a secretory protein, and a proline-rich polypeptide. Conclusion: Based on some data reported by several references, it is likely that ChAT and the secretory protein may be the putative μreceptor partners, and the biological relevance of these interactions remains to be established.

Key words: opioid receptor; two hybrid system; morphine-dependence; specific intercellular protein; choline acetyltransferase (ChAT)

P220010

Equipotent Molar Ratios to Determine β-Adrenoceptor Subtype Selectivities of β2-Agonists

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Objective: In vitro assays provide first-line tools in drug discovery for the identification of potent and selective compounds that warrant further study. Selectivity of a compound is commonly assessed by determining potencies or affinities for the different receptors, and calculating the ratios thereof. Our goal was to develop in vitro cell-based assays for the three β-adrenoceptors that allow for determination of subtype selectivities of β-adrenoceptor agonists, which are predictive for the expected selectivities in vivo. Methods: The three cloned human β-adrenoceptor subtypes were heterologously expressed in cell lines, and potencies of different agonists in mediating cAMP accumulation were measured using a radioimmunoassay. For each compound, equipotent molar ratios (EPMRs) relative to isoproterenol were determined. EPMR values were then used to calculate compound selectivities between the three β-adrenoceptor subtypes. A statistical method was developed that allows for determination of 95% confidence intervals of the derived selectivities. Results & Conclusion: We developed assays and a statistical method to accurately quantitate selectivities of agonists for the three β-adrenoceptor subtypes.

P220011

The platform for quick discovery of natural lead compounds

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To establish the platform for quick discovering nature lead compound, and search for anti-diabetic lead compound from nature product by this platform. 1000 nature extracts from 200 plants were obtained by quick automatic separate technology

and screened by hypoglycemic drugs screening model. The result showed that four extracts could evaluate glucose consumption significantly. Then the four positive extracts were separated in 40 components by HPLC. The 40 components were screened again. After that we found one objective compound as anti-diabetic lead compound. The compound (0.1ug/ml) could accelerate glucose consumption by 53.27%. More study illustrated that the compound could reduce blood glucose level in diabetic mice, but there was no effect on blood glucose level in normal mice. By this platform, we have researched 200 plants and find several lead compounds and a candidate for anti-diabetics.

Key words: nature product, lead compound, quick discovery

Acknowledgement: The project was supported by the National High Technology Research and Development Program Foundation of China (863program) (No. 2004AA2Z3782) and the Tradtional Chinese Medical Technology Research (No. 02-03ZP08)

P220012

Identification of Type 1 Inosine Monophosphate Dehydrogenase as an Anti-angiogenic Drug Target

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To rapidly discover clinically useful angiogenesis inhibitors, we created and screened a library of existing drugs for inhibition of endothelial cell proliferation. Mycophenolic acid (MPA), an immunosuppressive drug, was found to potently inhibit endothelial cell proliferation in vitro and block tumor-induced angiogenesis in vivo. Inhibition and cell cycle arrest are overcome by addition of guanosine, suggesting that the de novo nuclectide synthesis pathway, and more specifically, inosine monophosphate dehydrogenase (IMPDH), as the target of MPA in endothelial cells. Using RNA interference, we found that knockdown of one of the two known isoforms of inosine monophosphate dehydrogenase (IMPDH-1) is sufficient to cause endothelial cell cycle arrest. As IMPDH-1 is largely dispensable for T cell development and function in mice, this isoform may be an attractive target for developing specific inhibitors of angiogenesis.

P220013

Determination of tanshinone IIA in rat plasma by liquid chromatography-tandem mass spectrometry method

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Objective: A rapid and sensitive liquid chromatography/tandem mass spectrometry (LC/MS/MS) method to determine tanshinone IIA in rat plasma was developed and well validated. Method: After a single step liquid-liquid extraction, tanshinone IIA and loratadine (internal standard) was subjected to LC/MS/MS analysis using positive electro-spray ionization (ESI) under selected reaction monitoring (SRM) mode. Chromatographic separation of tanshinone IIA and loratadine was performed on a Hypersil BDS C_{18} column. Results: The method had a chromatographic running time of 2.0 min and linear calibration curves over ranges of 1-1000 ng/mL for tanshinone IIA. The intra- and inter-day precision (RSD %) was less than 8.4%. The lower limits of quantification (LLOQ) of the method were 1.0 ng/mL for tanshinone IIA. The extraction recovery of the method was found to be 63.7-67.3%. Conclusion: Detailed validation following FDA guideline indicated that the developed method had high sensitivity, reliability, specificity and excellent efficiency with a total running time of 2.0 min per sample.

Key words: tanshinone IIA; liquid chromatography/tandem mass spectrometry

P220014

Validation Of Established Non-Animal HERG Testing Systems Using A Rubidium Assay.

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The HERG gene encodes the α-subunit of the I_{kr} in human cardiac cells. The channel is an unintended target for a wide range of drugs causing cardiac toxicity. Whilst HERG transfected cells have been used as screens for safety testing, we have assessed a native human neuroblastoma cell line (SH-SY5Y) as a screen. The assay involved Rb^+ loaded cells challenged with 50mM K^+ and Rb^+ efflux measured by atomic absorption spectrometry. The kinetics of the release process was optimized and the assay had a signal to noise ratio greater than 10-fold. A range of K^+ channels inhibitors were tested to isolate HERG channel function in SH-SY5Y cells and 10mM tetraethylammonium was selected. In the presence of 10mM TEA, classical inhibitors of HERG currents such as pimozide (10μM) completely abolished Rb^+ efflux. The IC_{50} values for 10 different, structurally unrelated HERG inhibitors were obtained and these were comparable to those obtained using patch clamping with a correlation coefficient of 0.97445. These results suggest that the channels in SH-SY5Y cells are similar to cardiac channels and that the method is a suitable HERG screening tool able to be adapted for medium throughput assays.

P220015

Novel approach for GPCR drug discovery: Indirect identification of S1P receptor agonists in antagonist screening using calcium measurements.

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To further elucidate the role of sphingosine 1-phosphate receptors 1-3 (S1P1-3) we aimed to identify selective agonists and antagonists using recombinant expression in mammalian cells. S1P2 and S1P3 are coupled to Gq, and are therefore linked to the calcium signaling. S1P1 is solely coupled to Gi, and was artificially linked to calcium signaling using co-expression of Galpha16. All three receptor subtypes desensitized upon challenge of the cells with an agonist, i.e. agonists caused desensitization of the calcium signal and appeared as antagonists in a second calcium measurement. We screened a compound library for inhibitors of S1P-stimulated calcium signals, and could identify with this single measurement technique agonists and antagonists. Agonism and antagonism was confirmed in a second screening cycle by measuring compound- and S1P-induced calcium signals from the same assay well. At all three S1P receptor subtypes, we found a reciprocal correlation of agonism and "apparent" antagonism of compounds. In addition, agonists indirectly discovered by desensitization of the target receptor signal are not inducing calcium signals through endogenous GPCRs coupling to Gq or G16.

P220016

Microdialysis - A State of the Art Drug Discovery Technique

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Introduction: Microdialysis (uD) is a cutting-edge sampling technique that has evolved from application within neurophysiology to pharmacological research. Method: uD involves the use of a probe implanted in the tissue sites of interest in animals or human subjects. The diffusion of substances across a probe membrane is dependent on several physical and chemical factors. The key structure is the semi-permeable probe membrane made from special polymer that allows certain molecular cut-off <=100 kD. Results: During a slow perfusion (typical range 0.2-10 microL/min) of a uD probe whereby no fluid is removed from the sampling media, the concentration gradient of the drug across the probe membrane is the driving force. uD is performed under non-equilibrium conditions, therefore the drug concentration in the microdialysate is not equal to, or mostly less than the probed tissue sites. The ratio of the concentration difference is a constant at steady state under same flow rate and within a certain period. This ratio is also termed the uD recovery. Conclusion: As a relatively innovative technique for sampling tissue extracellular fluid, uD is gaining popularity in pharmacokinetic and pharmacodynamic studies.

P220017

Expression and detection of Human Phosphodiesterase 3B gene in baculovirus

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Aim: To investigate the expression of recombinant human phosphodiesterase 3B (HPDE3B) using baculovirus expression system in Tn cell line. Methods The HPDE3B cDNA was recombined with baculovirus, and then the recombinant was transfected into Tn cell line. The expression of HPDE3B in Tn cell line was detected and identified by the RT-PCR, SDS-PAGE, Western-blot and RIA. Results The recombinant HPDE3B protein was stable expressed in Tn cell line and detected by the distinct morphological changes of Tn cell, RT-PCR, SDS-PAGE and Western-blot using polyclonal antibody. The MW of the recombinant protein was about 120 kDa. Conclusion Recombinant HPDE3B can be expressed in Tn cell line using the baculovirus expression system, and thus provided the basic material for studying its bioactivity and application in screening for PDE3B inhibitor.

Key words: HPDE3B; Tn cell line; baculovirus expression system

P220018

Non-invasive Profiling of Endogenous G Protein-Coupled Receptors in Living Cells with Optical Biosensors

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Dynamic redistribution of cellular contents, equivalent to dynamic mass redistribution (DMR), is common to many cellular processes including the signaling through G protein-coupled receptors (GPCRs) in response to stimulation. The DMR can be manifested by resonant waveguide grating (RWG) biosensors, and the resultant DMR signal offers a novel and integrated readout for sensing living cells under real physiological conditions. Upon investigating the DMR signals of quiescent A431 cells mediated trough the activation of endogenous GPCRs using the RWG biosensors in combination with a panel of GPCR agonists, a unique DMR sinature was identified for each class of GPCRs, based on the G protein(s) with which the receptor is coupled (i.e., Gq, Gs and Gi). The DMR signals were dependent on the doses of agonists and the expression levels of endogenous receptors. The dose-dependent switching from one type of DMR signal to another was observed for a small set of GPCR agonists. Together with its ability to screen GPCR modulators using endpoint measurements, the labelfree and non-invasive biosensors hold great potentials for GPCR drug discovery and deorphanization.

P220019

Identification of Novel Inhibitors for Cathepsin B by High-throughput Screening with Fluorescence Polarization and Fluorescent Intensity Assays

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Aim: Cathepsin B (ctsb) expression is up-regulated in various pathological conditions. Two high-throughput screening (HTS) assays for ctsb inhibitors, fluorescence polarization (FP) and fluorescent intensity (FI), have been developed and compared. Methods: Both formats involved incubation of the recombinant human ctsb with the specific fluorescent substrate FSE-casein (for FP) or Z-RR-AMC (for FI), respectively. Assay signals were detected by changes in molecular size of substrates in the FP format and by changes in fluorescent intensity of hydrolytic products in the FI format. Reaction conditions including substance concentrations, reaction time and temperature were optimized. Results: 10,000 library compounds were screened and 45 initial hits were identified. Six of them specifically inhibited ctsb activity in vitro and suppressed TNFα mediated HepG2 cell apoptosis. The Z' factor was 0.58 ±0.07 in FP and 0.61 ±0.05 in FI. Both assays have been miniaturized to a 384-well format, and automated by automated pipeting stations. Conclusion: The homogeneous proximity nature allowed these assays to be simple, robust, reproducible and well applied.

Key words: cathepsin B, HTS, FP, FI

P220020

A HIGH-THROUGHPUT IN VITRO COCKTAIL METHOD FOR SCREENING THE INHIBITORY EFFECTS OF CYP ISOZYMES

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We have previously developed a cocktail method for assessing CYP isozyme activities and for studying potential drug interactions in human subjects. The purpose of this study is to evaluate if our previous cocktail can be utilized as an in vitro screening tool. Six substrates representing markers of CYP1A2, 2C9, 2C19, 2D6, 2E1 and 3A4 activities were included. These substrates either alone or in combination were incubated with human liver microsomes, and their metabolite formation quantified using LC-MS-MS. To validate the lack of potential interactions among the substrates, specific inhibitors for each isozyme were incubated with each substrate alone or the cocktail, and their respective IC50 determined from both sets of experiments were compared. The LC-MS-MS method was able to determine the 6 metabolites simultaneously, with assay precision less than 10% and accuracy of 89-112%. The IC50 value of each inhibitor determined in the presence of the cocktail was also consistent with that obtained from the individual substrate. This in vitro cocktail together with the rapid LC-MS-MS method would provide a reliable high-throughput approach for screening potential CYP inhibition and drug interactions.

P220021

High throughput chemiluminescent method for detecting superoxide anion activity

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This work was to develop a high throughput screening (HTS) assay based on chemiluminescent method for detecting superoxide (O_2^-·) anion activity. The luminol-dependent chemiluminescent assay detects the presence of superoxide anions with a higher sensitivity than other assays, but it couldn't be used as a HTS assay because of the test signal persistence time is too short. In this study the activity of superoxide anion was dectected based on the change of luminol concentration in the reaction system by the courts per second (CPS) density. Some factors which would affect the test signal persistence time such as the concentration of phenazine methosulphate (PMS) and β-Nicotinamide adenine dinucleotide reduced Disodium salt Hydriate (NADH) were optimized in different conditions. The reaction performed in white 96 well micro-plate with a final volume of 100 μL. The results show that the ideal system contains 75 μM PMS, 300 μM NADH, 100 μM luminol. In this condition, the signal persistence time can be prolonged and the stable data can be got. So, after modulation the luminol-dependent chemiluminescent assay is economical, easily operated, and can be performed by HTS.

Key words: Superoxide anion (O_2^-·), High Throughput Screening (HTS), Chemiluminescence

Acknowledgement: This work was supported by the National High Technology Research and Development Program Foundation of China (863) (No. 2004AA2Z3782).

P23. Drug Discovery-New Drug Design

P230001

Comparison between novel μ-opioid antagonists and naltrexone of the central and peripheral μ-opioid receptor

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NKP-10513 and NKP-9494, newly synthesized μ-opioid antagonists, have an anti-pruritic effect in several mouse models of itch. We examined the effects of these compounds on the central and the peripheral action using animal models; 1) Randall Selitto and 2) gastrointestinal transit. Both NKP-10513 and NKP-9491 did not inhibit the morphine-induced analgesia at the dose of 10 mg/kg, orally. On the other hand, naltrexone exerted fully anti-analgesic effect at a dose of 0.3 mg/kg, orally. We evaluated the inhibitory effects of morphine-induced depression of gastrointestinal transit of a charcoal in mouse. All of three μ-opioid antagonists antagonized counteracted morphine-induced depression of motility at the similar doses for anti-pruritic effects. The concentration of NKP-10513, NKP-9491 and naltrexone was measured in rat cerebrospinal fluid one hour after oral 10 mg/kg administration. Considering together, both NKP-10513 and NKP-9491 may be different from naltrexone in the manner of action on μ-opioid receptor.

P230002

A New Thrombus-Specific Ultrasound Contrast Agent Based on Sulfur-Hexafluoride-Filled Gas Microbubbles Prolonged the Ultrasound Signal Enhancement

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This study was to develop new microbubbles based on lipids and sulfur hexafluoride (SF6) for targeting thrombi as an improved ultrasound contrast agent. A bioconjugate ligand designed specifically was synthesized for insertion into lipid-coated membranes and to recognize and bind to GPIIb/IIIa receptors. SF6 gas microbubbles' physicochemical properties and diagnostic efficacies were determined. Suspension of lyophilized powder were reconstituted by injecting saline containing 3.0×10^8 SF6 microbubbles/mL with a mean diameter of 4.4 μm. More than 90% are between 1 and 10 μm. After reconstitution, the echogenicity and microbubble characteristics were unchanged for 8 hours. The targeted microbubbles increased the echogenicity of thrombi significantly, and provided a longer period of optimal signal enhancement than nontargeted microbubbles. Our thrombus-targeted microbubbles contrast agent exhibits a high echogenicity and stability, and thereby both enhances the visualization of thrombi and prolongs the diagnostic window.

Key words: Thrombi, targeted microbubbles, SF6, signal enhancement

Acknowledgements: This study was supported by the National Natural Science Foundation (Nos. 30300325, 30470633)

P230003

Three Dimensional Quantitative Structure Activity Relationship of a new type of Acetylcholinesterase Inhibitors

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Acetylcholinesterase (AChE) inhibitors are an important class of medcinal agents useful for the treatment of Alzheimer's disease. A screening model of AChE inhibitor was established, and the activities of a series of phenyl pentenone derivatives were detected, the result showed that some compounds displayed higher inhibitory activities. In order to study the relationship between the biological activities and the structures, 27 compounds with the scaffold were analyzed. A 3D-QSAR model were constructed using the method of Comparative molecular field analysis (CoMFA). The result of cross-validated $R_{cv}^2 = 0.613$, non-cross-validated $R^2 = 0.952$, SE = 0.301, and F = 73.286, indicates that the 3D-model possesses an ability to predict activities of new inhibitors, and the information of CoMFA model can offers an approach to designing new AChE inhibitors.

Key words: Acetylcholinesterase (AChE), Comparative molecular field analysis (CoMFA), phenyl pentenone derivatives.

P230004

The antipsychotic properties of neurotensine dipeptide analog Dilept

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Dipeptide N-caproyl-prolyltyrosine methyl ester (Dilept) designed by the imitation of the structures of atypical neuroleptic sulpiride and beta-turn conformation of neurotensine main metabolite NT(8-13) was developed as a potential antipsychotic. Dilept ability to bind NT-receptors was revealed in the binding experiments. Dilept demonstrates the signs of antipsychotic activity in dopamine-dependent tests in doses range 0.4-4.0 mg/kg i.p. and 6.0-24.0 mg/kg p.o. It causes the selective increase of DA turnover in nucleus accumbence without concomitant changes in striatum. Even in doses 500 times higher than those provoking antiapomorphine effect Dilept fails to cause the catalepsy, miorelaxation, sedation. Besides, in contrast to the known antipsychotics, Dilept demonstrates positive nmemotropic effect in several cognitive tests. DA-negative effect of Dilept allows predicting its effectiveness against positive schizophrenia symptoms, while choline-positive and glutamate negative activities hint at putative effectiveness against negative schizophrenia symptoms and cognition deficit.

Key words: dipeptide, neurotensine, antipsychotic.

P230005

Evaluation of the potency of aminopeptidase N inhibitor, using Met-enkephalin induced twitch inhibition in guinea pig ileum preparation, in vitro

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It is well known that aminopepidase N (APN), a zinc-dependent ectoenzyme, plays an important role in the inactivation of Met-enkephalin (Met-enk), endogenous opioid peptide. In this study, we evaluated the potency of APN inhibitors using the myenteric plexus-longitudinal muscle preparation of guinea-pig ileum. The enkephalinase inhibitor (phosphoramindon, 1 micro M), dipeptidyl carboxypeptidase inhibitor (captopril, 1 micro M) were added in Krebs solution before application of Met-enk. The % inhibition of electrically evoked muscle twitch response by Met-enk with each concentration of APN inhibitors was plotted against the log concentrations of Met-enk to calculate its IC50. Then, we calculated the concentration of APN inhibitor, which decreased the IC50 of Met-enk to be half value (pA1/2). The newly synthesized compound and amastatin enhanced the effect of Met-enk with pA1/2 of 83.17 nM and 16.32 nM, respectively, indicating that the potency of the new compound is five times lower than that of amastatin. These results suggest that this system is useful for the evaluation of the potency of APN inhibitor.

Key words: Met-enk; APN inhibitor; pA1/2

P230006

Novel anti-alopecia agents, extracts of pleurotus cornucopiae, Tamogi-take, by proliferation activity in dermal papilla cells

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As a result of the worsening living environment and stressful social interaction, hair loss and white hair are pressing matters, but the effects of many anti-alopecia agents are controversial. In this study, we describe how extracts of pleurotus cornucopiae, (Tamogi-take) possess proliferation activity in dermal papilla cells. Extracts of Tamogi-take (Tamogi) were obtained by a six-step process; 1st. Soaking, 2nd. Steaming, 3rd. Homogenization, 4th. Boiling, 5th. Filtration, and 6th. Sterilization. These extracts were used directly or as powders after drying with dextrin. The anti-alopecia effects were estimated as follows: 1. Proliferation of the dermal papilla cells, 2. Growth of rat vibrissae from isolated follicles, 3. Growth of mouse hair by oral administration of the extracts. 1. Tamogi strongly enhanced the proliferation of dermal papilla cells more than the reference agents. 2. Rat whiskers grew quickly after Tamogi administration, whereas vibrissal growth was delayed with minoxidil. 3. The oral administration of Tamogi stimulated the growth of mouse hair significantly. The application of these extracts will be determined soon. Tamogi are natural food-derived novel anti-alopecia agents.

P230007

Protective Rde of Heme Oxygenase-2 against Apoptosis in LLC-PK1 Cells: Effects of Non-porphyrin, Imidazde-based Heme Oxygenase Inhibitors

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Heme oxygenase (HO) isozymes are involved in the biotransformation of heme to biliverdin/bilirubin, iron and carbon monoxide (CO). HO-1 is induced by oxidative stress while HO-2 is constitutively expressed. To enhance our understanding of the physiological roles of HO isozymes, we have developed novel imidazole based HO inhibitors. Unlike the metalloporphyrins, these compounds are selective for the inhibition of HO with minimal effects on most other heme-dependent enzymes. In the current study, we examined the effects of the imidazole-based HO inhibitors on tumour necrosis factor (TNF)-induced apoptosis in wild type and HO-2 stably transfected, LLC-PK1 cells. TNF-alpha caused significant cytotoxicity in wild type cells ($P < 0.05$), but not HO-2 overexpressing cells, and this decrease in cell viability was significantly enhanced by a sublethal dose (2-25 μM) of the imidazole-based HO inhibitors ($P < 0.05$). Pretreatment with hemin (10 μM) increased HO-1 expression but was not cytoprotective. These data are consistent with a cytoprotective role of HO-2 in LLC-PK1 cells. (This work was supported by Canadian Institutes of Health Research Grant MOP 64305).

P230008

Isozyme-Selective Heme Oxygenase Inhibitors: Design, Synthesis, and Biological Evaluation

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Several imidazole-containing compounds were synthesized and evaluated as novel inhibitors of heme oxygenase (HO). A number of these compounds showed enhanced activity for HO over other heme-dependent enzymes (such as NOS and sGC). In addition, some of these compounds were highly selective for the inhibition of HO-1 (inducible isozyme) compared with HO-2 (constitutive isozyme). One of the compounds, QC-13, exhibits an IC50 value of 0.8 ± 0.2 mM for HO-1 (rat spleen) and approximately 305 mM for HO-2 (rat brain). Over 100 compounds have been synthesized, and structure–activity relationships amongst these analogues with respect to the inhibition of HO and other enzymes will be presented. These drugs are anticipated to become useful tools in elucidating the physiological/pathological roles of HO/carbon monoxide in mammalian and other biological systems.

Key words: heme oxygenase, imidazoles, selective inhibitor.

Supported by the Canadian Institutes for Health Research, grant MOP 64305.

P230009

Two Novel Methods for Computer - Aided Drug Design

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Prediction of protein - ligand binding affinities is a central challenge in structure - based drug - discovery, especially during the process of lead - compound optimization. Recently, the second generation Mining Minima algorithm (M2) yielded binding free energies accurate to within 1 kcal/mol for various host - guest systems. The calculations account for changes in solvation on binding, and for flexibility (or preorganization) of both ligand and binding site, and associated entropy changes. We describe here implementation of and promising early results for this approach to protein - ligand modeling.

It is difficult for experimentalists to take advantage of ligand design software. We have therefore developed desktop software that guides users through docking and scoring calculations for proteins of known structure. This Windows application easily handles up to 40 candidate ligands; an add - on enables efficient screening of large compound databases.

Key words: Mining Minima, Computer - aided drug design, Lead optimization.
Acknowledgement: Made possible by Grants GM062050 and GM075350 from the NIH. Contents do not necessarily represent the views of the NIH.

P230010

Investigating the Conformational Preference of Constrained Homocholine Ligands for Neuronal Nicotinic Receptors

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Neuronal nicotinic acetylcholine receptors (nAChRs) are a class of ligand gated ion channels found in many cognitive areas of the CNS. nAChRs have been implicated in a number of debilitating neurodegenerative diseases, fuelling the search for agents selective for individual nAChR subtypes. Constrained analogues of the endogenous ligand acetylchcholine represent key leads in the development of such selective ligands.

Methyllycaconitine (MLA) is a highly potent and selective antagonist at the alpha - 7 nAChR with a highly constrained polycyclic structure. Simplified analogues of MLA that retain the azabicydic [3.3.1] nonane core motif, and possess an embedded acylated homocholine residue are active nAChR ligands. In this study, a series of novel azabicydic ligands were synthesized incorporating a constrained homochline motif with a different topology to that of MLA and previous analogues.

These ligands have been evaluated for functional activity at recombinant nAChR expressed in Xenopus oocytes using two electrode voltage clamp electrophysiology. All of the ligands tested possessed activity at nAChR, including a positive modulator, agonists and with the majority acting as antagonists.

P230012

Effects of novel μ- opioid antagonists on the scratching models in mice and on the general behavior in rhesus monkeys.

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We investigated the effects of NKP - 10513, NKP - 9491 and naltrexone on the scratching models induced by substance P or serotonin in mice. The number of scratching behavior for 30 min was inhibited by these NKP - compounds and naltrexone in a dose dependent manner. This inhibitory effect of NKP - compounds was similar to that of naltrexone in potency. In rhesus monkeys, we observed the side effect of NKP - 10513, NKP - 9491 and naltrexone on the general behavior. A dose of 2.0 mg/kg of naltrexone showed the retching behavior, whereas NKP - 10513 and NKP - 9491 did not show the retching behavior at the same dose. And more, a dose of 50 mg/kg of NKP - 10513 showed neither the retching nor the vomiting behavior in rhesus monkeys. These results suggest that both NKP - 10513 and NKP - 9491 can be useful compounds for the treatment of pruritic patients without any side effects related to μ- opioid receptor antagonists.

P230013

Synthesis and antimicrobial activity of some thiazolyl - pyrazdine derivatives

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Some 1 - (4 - aryl - 2 - thiazolyl) - 3 - (2 - thienyl) - 5 - aryl - 2 - pyrazoline derivatives (C1 - 28) were synthesized by reacting substituted 3 - (2 - thienyl) - 5 - aryl - 1 - thiocarbamoyl - 2 - pyrazolines (B1 - 7) with phenacyl bromides in ethanol. The structures of the synthesized compounds were confirmed by IR, 1H- NMR and MS - FAB+ spectral data. Their antimicrobial activities against Escherichia coli (NRRL B - 3704), Staphylococcus aureus (NRLL B - 767), Salmonella typhimurium (NRRL B - 4420), Bacillus cereus (NRRL B - 3711), Listeria monocytogenes (Ankara Uni. Fac. of Veterinary), Aeromonas hydrophila (Ankara Uni. Fac. of Veterinary), Candida albicans and Candida glabrata (isolates obtained from Osmangazi Uni. Fac. of Medicine) were investigated and in this investigation, a significant level of activity was illustrated.

Key words: 2 - Pyrazoline ; Thiazole ; Antimicrobial activity

P230014

Design, Synthesis and Pharmacological Evaluation of Novel N - substituted Benzamides as Antipsychotics Agents

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Substituted benzamides could represent the first class of atypical antipsychotics employed for both depressive states and schizophrenia. Our objective was to synthetise new N- (2 - dialkylaminoethyl) - N- (3 - chlorophenyl) - benzamides, to confirm their chemical structures by spectral methods (IR, UV, 1H- and 13C - NMR), and to test their potential antipsychotic activity. LD50 after intraperitoneally (i.p.) injection was determined on mice, in order to establish the subsequent testing dose. Subacute toxicity was evaluated after three weeks of daily i.p. injection of 1/20 LD50. We determined locomotor activity using an actometer Autotrack type, motility in rotarod test and traction test, hypothermic, cataleptic and antinociceptive effect. I.p. injected dose was 1/20 LD50. Results showed a slight reduction of locomotor activity with 11.52 % ($p < .05$) for compound I5C and no significant influence on motility. I5C reduced rectal rat temperature with 2.31oC ($p < .05$). Higher doses produced hypertonia and movements disorders. I5C and II5C showed antinociceptive effect: 19.99 % and 23.44 % ($p < .05$). The relationship between chemical structures and pharmacological effects was established.

Key words: benzamides, antipsychotic

P230015

NEW PKC - TARGETED COMPOUNDS INHIBIT PKC TRANSLOCATION IN LIVING CELLS

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Protein kinase C (PKC) isoenzymes are important regulators of cell proliferation and malignant transformation. The objective of this study was to investigate the effects of a series of locally synthesized PKC ligands on phorbol ester binding activity and translocation of PKC. We used the X - ray structure of PKC delta C1b domain with bound phorbol ester as a template for molecular modelling to design ligands that compete with phorbol esters for binding and thus modulate PKC activity. The best ligands competed with phorbol ester binding to PKC with IC50 values under 20 μM. HeLa human cervical cancer cells transfected with PKC - green fluorescent protein (GFP) constructs were pretreated with the ligands and stimulated with phorbol 12 - myristate 13 - acetate (PMA). The translocation of PKC - GFP was visualized with confocal microscopy and quantified from confocal microscopic images captured during the experiments. Three out of eight hydrophobic compounds tested inhibited PMA - induced translocation in micromolar

concentrations. In conclusion, these PKC translocation inhibitors could be used as lead molecules in drug development. This work was supported by EU (Pro-Kinase Research project no. 503467).

Key words: PKC, translocation, drug discovery

P230016

Targeting the protease activity of Dengue virus NS3

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Dengue virus is a member of the flaviviridae family and causes dengue fever and dengue hemorrhagic fever in millions of people each year in tropical and subtropical regions of the world. Currently, there is no vaccine or effective antiviral therapy for the four known serologically related virus types. Non-structural protein, NS3 serine protease is essential for viral replication, hence serves as an attractive therapeutic target for the dengue virus infections. In order to develop potent small molecule inhibitors of the dengue serine protease, we sought to capitalize on the substrate information of NS3 protease. Substrate-based tetrapeptide inhibitors with various warheads were designed, synthesized and evaluated against the Dengue virus NS3 protease. Aboronic acid has the highest affinity, exhibiting a Ki of 43nM. Additionally, we systematically synthesized and evaluated a series of tetrapeptide aldehydes based on lead aldehyde (Bz-Nle-Lys-Arg-Arg-H, Ki = 5.8 uM). Structural studies of NS3 protease identify the key residues for substrate recognition and mode of binding of the inhibitor. The design, synthesis and biological activity of these potential dengue NS3 protease inhibitors will be presented.

P230018

Pharmacological properties of novel μ-opioid antagonists with antipruritic effect.

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Novel five μ-opioid antagonists (NKP-6630, NKP-7048, NKP-9491, NKP-10363 and NKP-10513) having fentanyl moiety were found to show 50% inhibition of the number of scratches on the mouse models induced by substance P at a dose of 10mg/kg, intraperitoneally. Those compounds also showed inhibitory effects on the same model at a same dose, orally, whereas they had no apparent inhibition of general behavior in mouse up to 100mg/kg, orally. In physicochemical study, those compounds except for NKP-9491 showed low crystallinity and high hygroscopicity. We solved this problem by changing their salt forms. The study of single dose oral toxicity and repeated dose oral toxicity in rat were studied. The bioavailability in pharmacokinetics study in rat was also studied and NKP-10513 was estimated at 36%, the highest of the five compounds. All of these results lead to the conclusion that NKP-10513 and NKP-9491 can be the good candidates for the treatment of pruritic patients.

P230019

Sytheses and antispazmodic effects of some 2-aryl-4,5,6,7-tetrahydro-(1H)-benzimidazoles

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Previous studies indicate that benzimidazole derivatives have important pharmacological effects such as analgesic and antispasmodic. We decided to prepare some tetrahydrobenzimidazole (THBI) derivatives and investigate their antispasmodic activities. Four THBI derivatives prepared by the reaction of cyclo-1,2-dione with some aldehydes in the presence of ammonium acetate and acetic acide. The structures were elucidatied by spectral methods. The Lorke's method was used to determine lethal toxicity of the compounds. LD50 values were found to be greather than 100 mg/kg (i.p.) for all compounds. Antispasmodic activity of the compounds were examined by using rat ileum in isolate organ bath. Rat ileums were treated with 10-4 M dose of THBI derivatives in isolate organ bath. The differences of acetilcolin response with the tested compounds were recorded. Three of the synthesized compounds (1, 2 and 3) showed antispasmodic activity and compound 4 was found ineffective in the series.

Key words: THBI, antispasmodic activity, Lorke's method, rat ileum

P230020

Synthesis and Antituberculosis Activity of Some N-[4-(indan-5-yl)thiazol-2-yl]-N'-(1-phenylethylidene) hydrazine derivatives

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Some N-[4-(indan-5-yl)thiazol-2-yl]-N'-(1-phenylethylidene) hydrazine derivatives were synthesized by reacting acetophenone thiosemicarbazones with 2-bromo-1-(indan-5-yl)ethanone in ethanol. The structures of the synthesized compounds were confirmed by ^{1}H-NMR and MASS spectral data. The tuberculostatic activity is determined by TAACF (Tuberculosis Antimicrobial Acquisition and Coordinating Facility Birmingham, AL 35255, USA). Rifampin, isoniazid and thiacetazone are used as reference tuberculostatic agents for comparing the activities of compounds under investigation. Primary screening is conducted at 6.25 mg/ml against Mycobacterium tuberculosis H_{37}Rv (ATCC 27294) in BACTEC 12 B medium using a broth microdilution assay the Microplate Alamar Blue Assay (MABA).

Key words: Indan; thiazole; antituberculosis activity, MABA

P230021

Hypolipidaemic activity of new compounds with the synergistic structural properties of α-asarone and fibrates.

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In an effort to develop new hypolipidaemic agents, a novel series of nine bioisosteric analogs has been previously prepared. These compounds were constituted by a phenoxyacetic acid scaffold, including its methyl and ethyl esters, which was substituted by an ethyl side chain at the different positions of the benzene ring. They were evaluated in a model of hyperlipidaemia induced by a high cholesterol diet in mice. Because of the significant activity and aiming at expanding the pharmacological profile of these innovative derivatives, herein we describe their activity in a different model of experimental hyperlipidaemia induced in ICR male mice by a single 400 mg/kg intraperitoneal injection of Tyloxapol. Mice were treated with the drugs by gavage 1 h before and 22 and 48 h after the Tyloxapol injection at doses of 0, 25, 50, or 100 mg/kg. The derivatives exhibited potent hypolipidaemic activity, lowering the mice serum cholesterol up to 6.4% and low-density protein cholesterol levels up to 33.2%. These results support the idea that the phenoxyacetic frame and the ethyl side-chain can be considered as potent pharmacophores for the preparation of potential hypocholesterolaemic drugs.

Key words: α-asarone; fibrates; hypocholesterolaemia. Conacyt contract grant 38431

P230023

Role of isopropyl group on the inhibitory actions of carvacrol and ortho-cresol.

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Carvacrol is a isopropylated cresol derivative found in nature especially as a constituent of many plant essential oils. Carvacrol was suggested as the principle and active compound of some plant extracts (1) whereas it was shown to be the principle but inactive compound in recent reports (2). The aim of this study was to investigate the role of isopropyl group on the pharmacological actions. Carvacrol (10^{-4} M) and o-cresol (10^{-4} M) was tested on the isolated rat ileum preparations against acetylcholine (ACh), potassium chloride (KCl) and calcium (CaCl2) induced contractions. As a result, carvacrol was shown to inhibit ACh-induced contractions whereas o-cresol was inactive and carvacrol exhibited

more inhibitions on KCl and CaCl2 tests. It is concluded that the presence of isopropyl group gives and/or enhances inhibitory actions, thus isopropyl group can be regarded as a pharmacophore.

Key words: essential oil, carvacrol, isopropyl group, pharmacophore.

P230024

GPCR NMR Structural Proteomics: CB2 Receptor Structure for In-Silico CB2 Ligand Design

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The recent discovery of the endogenous cannabinoid (CB) system, i.e., the CB receptors (CB1 in brain and CB2 in spleen) and endogenous ligands, has triggered intensive pharmacological research into the CB receptors and the therapeutics of cannabinergic ligands. However, the CB drug design has been hampered by the lack of 3D CB structures. Actually, due to their intrinsic membrane properties and large size, very few high-resolution structures have been reported for GPCRs, which were attributed to: i) lack of high-quality crystals for X-ray studies; ii) limited protein expression systems satisfactory for producing the functional GPCRs and with sufficient yields for biophysical studies. We have developed a recombinant membrane protein-engineering and NMR structural proteomics approach. The CB2 receptor (39.7 kDa) was engineered into fragments or helix bundles. They were cloned and over-expressed in a preparative scale. The proteins were purified via Ni-columns/FPLC, confirmed by SDS-PAGE, MS, and were characterized by 3D $^{1}H/^{15}N/^{13}C$ NMR. The NMR-refined CB2 structure is a trustworthy 3D model for the receptor-based in-silico virtual screening for CB2 ligand design (NIH R01 DA15770: Xie).

Key words: recombinant CB2 protein, cannabinoid receptor, NMR computer modeling

P24. Drug Discovery-Potential New Drug Targets

P240001

DISTRIBUTION OF PROLYL OLIGOPEPTIDASE IN THE RAT BRAIN

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Prolyl oligopeptidase (POP) is a serine endoprotease that hydrolyses small peptides at the carboxyl end of the proline residue. It is of pharmaceutical interest, since POP inhibitors have had antiamnesic properties and been involved in inositol 1,4,5-triphosphate (IP3) signaling. However, very little is known about the distribution of POP protein.

We used immunohistochemistry to localize POP in the rat brain tissue. The highest POP densities were found in substantia nigra, hippocampus and cerebellum and the lowest in hypothalamus. Myelinated fiber bundles like corpus callosum were devoid of POP-immunoreactivity.

The distribution and size of POP-immunoreactive cells suggest that POP is localized largely in the projection neurons in the hippocampus, cerebellum and nigrostriatal system. The distribution of POP also follows the distribution of IP3-receptors in the rat brain. These findings support a role of POP in cognition, IP3 signaling and movement regulation.

Key words: Prolyl oligopeptidase; immunohistochemistry; IP3 signaling

P240002

Inhibitive Effect of Genistein on Hypoxia-Induced basic Fibroblast Growth Factor Expression in Human Retinal Pigment Epithelium Cells

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The time course changes of basic fibroblast growth factor (bFGF) expression induced by hypoxia and the effects of genistein on hypoxia-induced bFGF expression in the human retinal pigment epithelium (RPE) cells were studied. Hypoxia significantly increased bFGF mRNA expression. The maximal level detected at 24 h was about two times of that at the start of treatment. With pre-treatment of genistein for 30 min, the elevated expression of bFGF mRNA was suppressed in a concentration-dependent manner. bFGF mRNA expression was reduced to 30.4% by 200 uM genistein when compared with that untreated with genistein. Hypoxia treatment also remarkably increased the expression of bFGF protein. At 24 h after hypoxia, the highest expression of bFGF protein was observed, it was about two times as much as that at the start of treatment. Genistein could also suppress bFGF protein expression in a concentration-dependent manner. The highest suppression was observed when exposed to 200 uM genistein, which was 43% of control. These results suggested that suppression of bFGF expression in RPE cells might partly account for the inhibitive effect of genistein on retinal neovascularization in vivo.

P240003

Permeability transition pore, AQP8 and mitochondrial water transport

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Although movement of water into and out of the mitochondrion is central for its shape and activity the molecular pathways of mitochondrial water transport remain mostly elusive. By stopped flow light scattering we found striking high water permeability of isolated rat liver mitochondria and low activation energy characterizing the related osmotic transport. Experiments with mitochondria using cyclosporin A (CsA), an inhibitor of the opening of the permeability transition pore (PTP) acting as a mitochondrial coordinator of pro-apoptosis, and Hg^{++}, an ion blocking AQP8, the aquaporin water channel located in the inner mitochondrial membrane, indicated major roles for PTP and AQP8 in mediating the mitochondrial water transport. Targeting of these two water conductive pathways may be instrumental to act on the mitochondrial volume, a function that could be used to modulate cell death in an innovative therapeutic perspective.

Key words: Mitochondria, apoptosis, PTP, aquaporin.

Acknowledgements: funding from Italian PRIN and CEGBA is gratefully acknowledged.

P240004

Ubiquitin ligase gp78 increases solubility and facilitates degradation of the Z variant of alpha-1-antitrypsin

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Alpha-1-antitrypsin (AAT) is the most abundant circulating proteinase inhibitor. AAT deficiency, caused by the mutations of AAT gene that lead to AAT retention in the endoplasmic reticulum (ER), is widely recognized abnormality that causes liver injuries and lung disease. Mutant AAT is subject to ER-associated degradation (ERAD). To investigate the effects of gp78 (a ubiquitin ligase) on ATZ (the classic variant of AAT) degradation, HEK 293 cell line and lipid-mediated transfection were used. It was found that gp78 ubiquitinates and facilitates degradation of ATZ. gp78 over-expression also significantly increases ATZ solubility. Additionally, ubiquitinated ATZ is preferentially localized in the insoluble fraction where the degradation appears to occur. Expression of the E3-inactive form of gp78 increases ATZ. p97/VCP is involved in gp78-mediated degradation of ATZ. ATZ increases cell viability when over-expressed in cells, which can be alleviated by gp78 over-expression. These data indicate that gp78 has unique quality control roles over ATZ by facilitating degradation and inhibiting aggregation of ATZ, which is expected to be a target for the treatment of AAT deficiency.

Key words: ATZ; gp78; ERAD; ubiquitination.

P240006

The Superiority of Thienorphine As a New Partial Opioid Agonist to Buprenorphie

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Thienorphine (Thie) is a new derivative of buprenorphine (Bup), which was synthesized by our institute. It is superior to Bup in several aspects, in vitro studies Thie showed higher affinity for binding and potenter stimulation of [^{35}S] GTPγS binding to μ- opioid receptor than Bup in membrane preparation of CHO cells stably expressing the rat μ- opioid receptor. in vivo test Thie exhibited a greater antinociceptive effect with ED50 value of 0.25 mg/kg (s.c.), and more potent anti- morphine effect with ED50 value of 0.64 mg/kg ig, relative to Bup. Moreover, the bioavailability of Thie is greatly higher than that of Bup given orally. More importantly, Thie demonstrated a much longer antinociceptive effect (more than 8h), and antagonism of morphine toxicity (more than 15 days), compared to Bup. These results, along with others, indicate that Thie is a potent, long- acting partial opioid agonist with high bioavailability, and may have possible application in treating addiction.

Key words: thienorphine; partial opicid agonist; buprenorphine

P240007

Protective Effects of Novel Drugs in AZT- induced Cardiopathology in Mice

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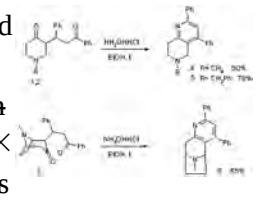
AZT (zidovudine), the most commonly used antiretroviral drug in AIDS treatment, induces severe deterioration of mitochondrial processes leading to cardiopathology. We suggest that cardiac cells may be protected by mildronate (aza- butyrobetaine class), cerebrocrast and glutapyrone (novel 1,4- dihydropyridine compounds), the mitochondria- targeted drugs. In present studies these compounds were administered i.p. for 2 weeks in mice by combining them with AZT (50 mg/kg, i.p.). Cardiac tissue ex vivo was examined morphologically and immunohistochemically (assessment of NF- kBp65 expression). All tested drugs (mildronate in particular) significantly prevented AZT- induced morphological changes (e.g. perivascular edema, diffuse leukocyte infiltration) and reduced nuclear NF- kBp65 expression. The data demonstrated a high activity of mildronate (100 mg/kg), cerebrocrast (0.1 mg/kg) and glutapyrone (1 mg/kg) to prevent inflammatory processes in cardiac tissue caused by AZT, indicating rational therapeutic combinations of these drugs with AZT for beneficial application in AIDS treatment.

Key words: AZT, NF- kB, cardioprotective drugs
Acknowledgements: Latvian Council of Science Grant Nr.05- 1418, ESF grant ESS2004/3

P240008

Synthesis and Pharmacological evaluation of 5,6,7,8- tetrahydro- 1,6- naphthyridines as potent analgesic agents.

Kostochka M.L.*, Vatsadze S.Z., Lezina V.P.*, Klodt P.M.*, Zyk N.V. Department of Chemistry, M.V. Lomonosov Moscow State University, 119899, Lenin hills, Moscow, GSP- 2, Russia *Institute of Pharmacology, RAMS, Baltijskaya St.8, Moscow 125315, Russia Opiate analgesics are highly effective in relieving acute pain, but have limited efficacy in the treatment of chronic and neurophatic pain. It has previously been shown, that condensed 4,5,6,7- tetrahydro- 1H- pyrazolo[4,3- c] pyridine derivatives exhibit analgesic activity[1]. 5,6,7,8- Tetrahydro- 1,6- naphthyridine compounds, similar to 4,5,6,7- tetrahydro- 1H- pyrazolo[4,3- c] pyridines, have recently been predicted to be an analgesic drug candidate, and this has led to synthesis and development such systems. We have recently developed a new approach to the synthesis of 5,6,7,8- tetrahydro- 1,6- naphthyridines: condensation of 1,5- dicarbonyl N- substituted piperidine and tropan derivatives 1- 3, obtained previously[2], with hydroxylamine hydrochloride. Compounds 4- 6 were obtained with good yields.(scheme 1).



The effects of synthesized compounds 1- 6 were investigated in the field- stimulated mouse vas deferens preparation- isolated organ. In the mouse vas deferens, all injected compounds were found to possess an agonist effect in 6.65×10^{-5} mol/l concentration. Under the incubation conditions used in these experiment, compounds 1- 6 interact and display selectivity to μ, δ- and k- subtype opiate receptors and shown to have analgesic activity.

P240009

The effect of Daxx on the cholesterol homeostasis of hepatic cells

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To evaluate the effects of Death- associated protein (Daxx) on the cellular cholesterol homeostasis, we transfected HepG2 cells stably with or without pEGFP- C1 or GFP tagged full- length Daxx vector (pEGFPC1/Daxx). Cellular free cholesterol (FC) and cholesteryl ester (CE) were determined by HPLC. RT- PCR was used to detect the mRNA expression of Daxx and SREBP. Immunofluorescence and western- blot were respectively used to measure the protein expression. Compared with control groups, FC and CE were significantly reduced in Daxx- overexpression cells. SREBP mRNA expression was unaffect, but active SREBP protein was down- regulated obviously in HepG2 cells transfected with pEGFP- C1/Daxx. Concomitantly, caveolin- 1 protein was upregulated. We concluded that overexpression of Daxx in hepatic cells inhibited SREBP activation and cholesterol production. Meanwhile, the caveolin protein promoting cholesterol efflux of hepatocyte was increased by Daxx.

Key words: Daxx; cholesterol; SREBP.
This work was supported by grants- in- aid from the National Natural Science Foundation of China (30470719) and the Health Department of Hunan province (B2004- 078).

P240010

Ability of prolyl oligopeptidase (POP) inhibitors to prevent glyceraldehyde- 3- phosphate dehydrogenase translocation in 6- hydroxydopamine treated cells

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We studied ability of POP inhibitors (Z- Pro- Prolinal and JTP- 4189) to prevent translocation of glyceraldehyde- 3- phosphate dehydrogenase (GAPDH) and formation of reactive oxygen species (ROS), in 6- hydoxydopamine (6- OHDA) and cytosine arabinoside (Ara- C) treated monkey fibroblasts (CV1- P) and human neuroblastoma (SH- SY5Y) cells. The cells were treated with POP inhibitors (30 min) before adding toxicants. GAPDH was analyzed by Western hybridization, ROS by fluorescent 2'7'- dichlorodihydro- fluorescein diacetate, and viability by MTT- method. Both toxicants induced GAPDH translocation to the particulate fraction containing mitochondria and nuclei. Z- Pro- Prolinal was able to inhibit translocation in 6- OHDA- exposed CV1- P cells. In SH- SY5Y cells and in JTP- 4189 pretreated cells, prevention of translocation was not seen but the intensity of cytosolic fraction was increased. Both inhibitors reversed 6- OHDA- induced ROS- production to the control level only in CV1- P cells although the viability of either cell line was not changed. As a conclusion, GAPDH translocation does not always lead to apoptosis and POP inhibitors are able to prevent part of cell stress indcating factors. GAPDH, 6- OHDA, POP- inhibitor, ROS

P240011

Establishment of Fluorescent Real Time Quantitive PCR for Detecting HBV cccDNA

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Objective: Accurate determination of HBV ccc DNA (HBV closed circular DNA) is very useful in prognosis of HBV infected patients and in assessment of drug for therapy of HBV patients. A novel approach to quantitative HBV ccc DNA using real time PCR has been developed. Methods: In order to establish a quantitative method in detecting HBV ccc DNA, HepG2.2.15 cell line and a recombinant plasmid was used as the source of HBV ccc DNA and external references, respectively. The PCR products were labeled with the fluorescent DNA dye SYBR green I. The amount of HBV ccc DNA was measured by ABI7000 Sequence Detection System. Result: The fluorescent real time quantitative PCR possesses very good specificity, sensitivity and duplication. Conclusion: This method provides a convenient and high- throughput format for detecting HBV ccc DNA. This may be a useful method in evaluating a drug on eradicating HBV virus from infected cells

in drug discorvery.

P240012

CELL CYCLE- TARGETED CANCER THERAPY BY NATURAL PRODUCTS

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Cell cycle machinery and components of cell cycle checkpoint have provided a wealth of target for novel anticancer drugs. In our study, we investigate key points of cell cycle control in order to focus on promising targets of new agents. We found that mdm2-siRNA and lidamycin induced cell cycle arrests in various cancer cells. The cell cycle arrests were associated with regulations of cell cycle components. Mdm2-siRNA, a RNA interfering agent, could increase p53 expression by specific down-regulation of mdm2, induce cell cycle arrest and apoptosis, and inhibit tumor growth in vivo. Moreover, mdm2-siRNA could synergically improve antitumor activity of DNA-damaged drugs. Lidamycin, an anticancer antibiotic, induced G2 arrest through Chk1/Chk2 pathway and at least partially activated by MAPK in p53 mutant cancer cells. lidamycin induces G1 and G2 arrests in wild-type p53 breast cancer cells through integrative mechanisms, including induction of p53, p21, activation of Chk2 and down-regulation of cyclin B1/cdc2. Taken together, cell cycle regulators are important molecular targets for cancer therapy.

P240013

Optimizing pH to Enhance Drug Transport Across Mucosal Membrane: Application To Propranolol

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Objective: To develop a method in predicting the optimal pH that improve transmucosal absorption of ionizable compounds Methods: Using propranolol as a representative ionizable compound, and based on its solubility, pKa and partition coefficient, an equation was derived in predicting the optimal pH (pHmax) that can lead to maximal transmucosal absorption. The predicted results were then compared to the experimental data obtained from excised porcine sublingual mucosal transport studies. Results: The experimental pH-solubility/permeability profile of propranolol fitted very well to that generated by the theoretical equations ($R2 = 0.9991$). The pHmax from the experimental work was 7.4, as compared to the theoretical value of pH7.62 and at pHmax highest transmucosal transport at was also verified. Conclusion: The validation of pHmax as shown with propranolol, provides a new approach to enhance transmucosal delivery of such ionizable compounds.

Key words: transmucosal, ionizable, sublingual

Acknowledgments: Supported by Direct Grant (No. CUHK 2041010) and ITF Gant (No. ITS/174/00) from Hong Kong Government.

P240014

Identification of soluble Thrombomodulin binding low density lipoprotein of acute coronary syndrome patiens

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Soluble thrombomodulin (sTM) is reportedly derived from injured or inflamed endothelial cells including arteriosclerotic disease, but clinical cross-sectional studies analyzing the association of plasma TM levels and arteriosclerotic disease have remained ambivalent at best. 96 subjects were divided into four groups. Coronary heart disease (CHD) included stable angina (SA), unstable angina (UA) and acute myocardial infarction (AM) with 24 patients respectively. 24 healthy controls group as comparison. Density gradient ultracentrifugation was used to separate plasma lipoprotein and sTM was measured by enzyme link immunosorbent assay (ELISA).

Results showed that the plasma levels of sTM were significantly higher in patients with CHD than normal controls ($p < 0.05$); but there no difference between the three groups of patients with CHD ($p > 0.05$). There was a marked increase of sTM in low density lipoprotein (LDL) from CHD patiens, sTM binding LDLs were significantly increased in patients with UA and AMI than that of SA. These data suggest that the binding of sTM to LDL may be plays an important role in atherosclerotic disease, especially in acute coronary syndrome.

P240015

Anion exchangers expression in cardiomyocyte anoxia and ddelayed preconditioning and possible mechanisms

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To determine if anion exchangers (AEs) are involved in myocardial protection of delayed preconditioning (DP), we measured AEs mRNA and protein expression in rat cardiomyocytes, and investigated if the K+-ATP channel, extracellular signal-regulated kinase (ERK)-dependent pathway and NO synthesis were involved in. The primary cultured neonatal rat cardiomyocytes were subjected to anoxia-reoxygenation injury. Myocardial biochemical indicator, cardiomyocyte ultrastructure and AEs expression were examined. Our results showed that LDH activity significantly decreased, myocardial cell pulse rate and viability increased, moreover, cardiomyocytes remained in good pulse rhythm and ultrastructure in DP. Additionally, AE1, AE3 mRNA and protein expression were up-regulated. PD98058, glibenclamide and L-NAME, however, completely or partly abolished the delayed preconditioning. The findings suggested that AE1 and AE3 participate in delayed protection, and the mechanisms are associated with ERK pathway, NOS and K-ATP channel.

Key Words: Anion exchanger; Ischemia Preconditioning; Cardiomyocyte

Acknowledgement This work was supported by a grant from Natural Science Foundation of China (№.30560049).

P240016

Role of AE2 Protein in the Myocardial anoxia-reoxygenation Injury and Ischemic Preconditioning

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AIM: To examine anion exchanger 2 (AE2) expression in myocardial anoxia-reoxygenation (A/R) injury and ischemic preconditioning (IPC), and explore the relationship with nitric oxide synthase (NOS), KATP channels and MAPK pathway. METHOD: RT-PCR and Western blot were used to measure the AE2 mRNA and protein expression respectively in primary neonatal cardiomyocytes which simulates acute myocardial A/R injury and IPC model. L-NAME, Glibenclamide and PD98059 were administered as the antagonist of NOS, KATP channels and MAPK pathway correspondingly. RESULT: Expression of AE2 was up-regulated in A/R injury, while IPC could abolish it. Inhibition of NOS, KATP channels or MAPK pathway could reverse the IPC mediated reduction of AE2 mRNA and protein. CONCLUSION: AE2 may participate in the myocardial injury and IPC can inhibit AE2 expression to protect myocardium against A/R injury, which depends on NOS, KATP channels or MAPK pathway.

Key words: Anion exchangers; Ischemia-reperfusion; Ischemia preconditioning; Cardiomyocyte

Acknowledgement: This work was supported by a grant from Natural Science Foundation of China (№.30560049).

P240017

Pentadecapeptide BPC 157 (PLD116, PL14736, Pliva) influences ATP energy system and antagonizes 0.6 MHCl- and 96% ethanol-gastric lesion in rat

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Stable gastric pentadecapeptide BPC 157, studied for IBD (PLD116, PL14736, Pliva), influences NO-system, protects endothelium, promotes angiogenesis, both internal and external wounds healing (Eur J Pharm 332, 23, 1997; Burns 29, 323, 2003). BPC effect on ATP energy system was so far not studied. Methods. ATP, ADP, ATP/ADP, AMP, ATP+ADP+AMP, ATP+0.5ADP/

ADP + AMP, cAMP were assessed in rats 0.6 M HCl 1 ml i.g.- and 96 % alcohol 1 ml i.g.- gastric lesion as described (J Clin Gastr 14, S135, 1992) 0, 1, 5, 15, 30, 60 min; BPC 0, 1, 10 ug/kg i.g. at 30 min before injury. Results. ATP tissue level decreased, and ADP increased parallel severe gastric lesion in controls. BPC along with 0.6 M HCl and 96 % ethanol stomach lesion inhibition also antagonizes energy breakdown, leading to more ATP, ADP, AMP and cAMP than in controls (Fig. 1).

Conclusions. Together, an influence on ATP energy system is along with this pentadecapeptide BPC 157 as an agent known to protect mucosa, endothelium, and to modulate NO-system. Likewise, with respect to virtually no toxicity in clinical studies, these findings could be likely relevant for further therapy applications.

P240018

The antithrombotic agent, bp5250, a novel potent cyclic nucleotide phosphodiesterase 5 inhibitor

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In present study, we investigated the effects of a newly synthetic YC-1 analogue, bp5250, on platelet function in vitro and platelet plug formation in vivo. Bp5250 concentration-dependently inhibited platelet aggregation caused by collagen and thrombin. Bp5250 inhibited intracellular Ca^{2+} mobilization and P-selectin expression of human platelets stimulated by thrombin, and thromboxane A2 formation caused by collagen. However, bp5250 did not block fibrinogen binding to $\alpha_{IIb}\beta_3$ of fixed elastase-treated platelets. Bp5250 markedly potentiated the platelet-inhibitory effect of nitroglycerin, and markedly increased cyclic GMP levels and potentiated the elevated cyclic GMP by nitroglycerin. Phosphodiesterase 5 was inhibited by bp5250 with IC50, 4.21 μM. Bp5250 significantly prolonged the latent period in triggering platelet plug formation in mesenteric venules of fluorescein sodium-pretreated mice, as it was intravenously given at a dose of 9 μg/g, whereas bp5250 at the same dose had no significant effect on the tail bleeding time of mice. In conclusion, promising antithrombotic profile of bp5250 provides a lead compound for developing antiplatelet drugs.

Key words: Antiplatelet agent; Phosphodiesterase 5; cGMP

P240019

Microsphere embolism-induced protein tyrosine nitration mediates the disruption of blood-brain barrier in the rat brain

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Brain ischemic injury elicits cerebral microvascular injury and results in blood-brain barrier (BBB) disruption, which exacerbates the postischemic edema. The precise molecular mechanisms underlying ischemia-induced BBB disruption remain unclear. We here determine whether peroxynitrite formation in the vascular endothelial cells (ECs) mediates BBB disruption after microsphere embolism (ME) ischemia in rat. The present study indicated that eNOS expression was significantly up-regulated in the brain microvessels 2-48 hours after ME, preceding disruption of BBB. In the vascular ECs, ME-induced eNOS expression was closely associated with protein tyrosine nitration. Leakage of rabbit IgG was also evident around nitrotyrosine-immunoreactive microvessels. To support the idea of undesirable roles of eNOS overexpression, a novel calmodulin-dependent NOS inhibitor, DY-9760e, significantly inhibits protein tyrosine nitration after ME. Taken together, ME-induced eNOS expression and subsequently peroxynitrite formation in the vascular ECs likely accounts for the ischemia-induced BBB disruption.

Key words: DY-9760e; peroxynitrite; ischemia; blood-brain barrier

P240020

Assay of serum antibody to rat spinal sensory protein annexin V in patients with peripheral neuropathy

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Rat spinal sensory protein annexin V was purified and identified for studying the distribution of serum antibody against annexin V in patients with peripheral neuropathy. Rat spinal sensory protein annexin V was purified through anion-exchange chromatography and HPLC. The expression of anti-annexin V autoantibody was studied by Western blot analysis with sera from patients and normal controls as primary antibody. A positive signal was detected around 35 kDa in the Western blot analysis with anti-annexin V antibody. Serum IgM or IgG against annexin V was negative in the normal controls, but positive in patients with Guillain-barré syndrome (GBS). In this study, we further proved that the 35 kDa rat spinal sensory protein was annexin V and we also found that serum antibody to annexin V was detectable only in patients with immune-mediated neuropathy. This result indicated that immune response to annexin V may play a role in the pathogenesis of autoimmune mediated sensory neuropathy and sensory neuronopathy.

Key words: Sensory nerve; Annexin V; Peripheral neuropathy Acknowledgment This study was supported by National "211 Project" in Peking University

P240021

Development of PACAP derivatives with improved metabolic stability

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PACAP (pituitary adenylate cyclase-activating polypeptide) was described as a potent neuroprotective factor in various pathophysiological models, thus illustrating its therapeutic potential in some neurodegenerative diseases.

Since PACAP exhibits a poor metabolic stability, the synthesis of PACAP analogs with lower susceptibility to proteolysis represents the first step towards the development of useful clinical applications. Therefore, derivatives of both PACAP27 and PACAP38, containing specific chemical modifications, were produced by targeting peptide sites recognized by peptidases. Results showed for instance that N-terminal capping and modifications in position 2 of the sequence contributed to improve the stability against dipeptidyl peptidase IV, the major enzyme involved in PACAP degradation. All modified peptides were able to decrease PC12 cell proliferation and to induce guinea pig trachea relaxation. This study demonstrated the possibility of increasing the metabolic stability of PACAP without inhibiting its biological activity.

Financial supports from the NSERC and the Ministere de l'Education du Quebec.

Key words: PACAP, metabolic stability, neuroprotection, neurodegenerative diseases.

P240022

An agent improving ischemia-reperfusion injury to the rat myocardial tissue—antisense oligodeoxynucleotide against tissue factor

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In order to investigate the effects of antisense oligodeoxynucleotide against tissue factor (AS/TF) on ischemia reperfusion injury, 50 male Wistar rats were randomly divided into 5 groups, in which 3 groups were given AS/TF, sense oligodeoxynucleotide (S/TF) and scrambled oligodeoxynucleotide (Sc/TF) respectively, another 2 groups were given Saline and served as Sham and ischemia-reperfusion (I/R) injury group. Myocardial ischemia reperfusion was achieved in I/R, AS/TF, S/TF and Sc/TF group, while blood sample and ischemic myocardial tissue were collected. Results showed that after myocardial ischemia-reperfusion, cardiac troponin I (cTnI), thrombin-antithrombin complex (TAT), granule membrane protein 140 (GMP-140) in blood, TF, Ag, interlrukin-6 (IL-6), interleukin-8 (IL-8) and the transcription and expression of TF in ischemic myocardial tissue of the rat increased obviously, while in AS/TF group, they rose less than those in I/R, S/TF and Sc/TF group respectively. From the study, we think that AS/TF strongly suppresses the transcription and expression of TF and thereby improves ischemia-reperfusion injury to the rat myocardial tissue by inhibiting inflammation and activation of blood coagulation.

P240023

Cocaine esterase: Proficient blockade of cocaine toxicity and potential immunogenicity in the mouse

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Cocaine esterase (cocE) has superior catalytic efficiency for cocaine. We investigated the in vivo potency of cocE in blocking cocaine toxicity in mice by measuring the occurrence of convulsions and lethality (n = 6/condition). I.v. injection of cocE (0.1 - 1 mg) 1 min prior to cocaine injection dose - dependently produced rightward shifts of the dose - response curve for cocaine toxicity. I.v. cocE 1 min after the occurrence of convulsions also dosedependently shortened the recovery time from convulsions. CocE 0.32 mg retained its effectiveness against cocaine (320 mg/kg) - induced toxicity in mice with single prior exposure of cocE (0.1 - 1 mg), and these mice displayed a weak antibody response. CocE also retained similar effectiveness in mice with triple prior exposures of cocE (once/week x 3), and these mice displayed a 10 - fold higher antibody titer. In contrast, cocE lost some effectiveness in mice with four prior exposures of cocE (once/2 weeks x 4), and these mice displayed 100 - fold higher antibody titers. Thus, cocE produced robust prevention and reversal of extreme cocaine toxicity and only extensive repeated exposures of cocE increased the risk of immunologic effect (Supported by USPHS Grant DA21416).

P240024

Synergistic facilitation of Bryostatin - 1 and Vitamin E on classical conditioning of the rabbit (Oryctolagus cuniculus) nictitating membrane response

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Our previous work has demonstrated that protein kinase C (PKC) is involved in classical conditioning. This study was to investigate if PKC modulator, Bryostatin - 1, was capable of facilitating rabbit conditioned NMR; and if there was a synergistic effect between Bryostatin - 1 and Vitamin E. Bryostatin - 1 showed a dose - dependent increase in conditioned eyeblink responses from the fifth trace day. Compared to the paired rabbits receiving vehicle, Bryostatin - 1 alone, paired animals receiving both 10 μg/kg Bryostatin - 1 and Vitamin E exhibited significantly more conditioned eyeblink responses. Bryostatin - 1 did not alter the reactivity to airpuff (US) and tone (CS). These findings demonstrate a strong synergistic effect on rabbit conditioned nictitating membrane responses between Bryostatin - 1 and Vitamin E and suggest Bryostatin - 1 and Vitamin E may be an optional treatment for learning disabilities and memory deficits in clinics.

P240025

Application of RNA Interference (RNAi) Technology for Target Validation in Cultured Human Tissue Explants.

Song Lily*, Tortorella Micky, Malfait Anne - Marie, Arner Elizabeth, Griggs David W. Pfizer Inc

RNA interference (RNAi) is a powerful technology to silence expression of specific genes and is being increasingly used to validate targets for drug programs. We have employed this technology to suppress the expression of several proteinases that are elevated in osteoarthritic cartilage and in some tumor types: ADAMTS - 4 (Aggrecanase - 1), ADAMTS - 5 (Aggrecanase - 2) and PACE - 4. Human chondrocytes and cartilage explants were efficiently transfected with small interfering RNA (siRNAs), and expression of each gene was specifically decreased. Suppression of each enzyme, but not negative controls, significantly attenuated the ability of catabolic cytokines to stimulate glycosaminoglycan release and aggrecan neoepitope formation in normal cartilage.

Reduction in aggrecan degradation was also observed following siRNA - mediated knockdown of each gene in osteoarthritic cartilage. These data support ADAMTS - 4, ADAMTS - 5, and PACE - 4 as validated targets for the design of drugs to prevent cartilage destruction. Furthermore, they illustrate the potential of RNAi for analysis of the roles of these and other genes in ex vivo models of any disease process.

P240026

EP 80317 a prototype of a new class of anti - atherosclerotic agents

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EP 80317, a synthetic hexapeptide derived from the growth hormone - releasing peptides family, as a selective ligand of CD36, was shown to exert anti - atherosclerotic effect in apoE - null mice. Hypothesis: EP 80317 exerts its effect by modulating cholesterol trafficking in macrophages. Methods: ApoE - null mice fed with an atherogenic diet received daily sc injections of EP 80317 (300 μg/kg) starting at 6 weeks of age until sacrifice at 18 weeks. Results: En face analysis of oil red - O- stained aortas revealed that EP 80317 induced a significant reduction in lesion areas (51%) and a hypocholesterolemic effect (30%). A significant reduction (23%) of labeled - macrophages accumulation to lesion - prone in EP 80317 treated mice and endothelial VCAM- 1 expression at lesion sites as well as a selective upregulation of LXRα and ABCG - 1 at the macrophage level was found. These beneficial effects of EP 80317 were CD36 - dependent and reversible upon cessation of the treatment. Conclusion: EP 80317 exerts a CD36 - dependent atheroprotective effect in regulating both cholesterol metabolism and macrophage trafficking to lesion sites and might be a novel prototype for the treatment of atherosclerosis. Supported by the Canadian Institutes of Health Research and Ardana Bioscience.

Key words: CD36, atherosclerosis; growth hormone - releasing peptides; LXRα.

P240027

Calcineurin mediates delayed neuronal death through NFAT activation in mouse brain ischemia.

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Ca^{2+}/calmodulin (CaM) - dependent protein phosphatase, calcineurin (CaN) is composed with A and B subunits with 60 and 19 - kDa, respectively. Calpain, a Ca^{2+} - dependent cysteine protease, in vitro converts it to constitutively active forms with 45 and 48 - kDa by cleaving out the autoinhibitory domain in the A subunit. In mouse middle cerebral artery occlusion model, calpain converted CaN A subunit to the constitutively active form with 48 - kDa in vivo. We also confirmed an increased Ca^{2+}/CaM- independent CaN activity in brain extracts. The generation of constitutively active form and Ca^{2+}/CaM- independent activity of CaN was peaked at 2 hours after ischemia in brain extracts. The generation of constitutively active CaN was accompanied with translocation of nuclear factor of activated T- cells (NFAT) into nuclei in the hippocampal CA1 neurons. In addition, a calmodulin antagonist, DY- 9760e blocked the generation of constitutively active CaN by calpain, thereby inhibiting NFAT translocation into the nucleus. Together with previous studies indicating that NFAT plays a critical role in apoptosis, we propose an idea that calpain - induced CaN activation mediates in part delayed neuronal death in the brain ischemia.

P240028

Galphai1 - adenylate cyclase system: receptor - independent activation

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An altered functionality of the inhibitory subfamily of G proteins (Gi) was involved in disease states. Compounds able to activate Gi proteins, in a receptor - independent manner, would be useful to treat these pathological conditions. Aimed to study Gi protein direct activation we have reconstituted a recombinant transductor - effector complex cloning both the human Galphai1 subunit and adenylate cyclase (AC). The myristoylation of Galpha, fundamental for interaction with AC, was obtained in the prokaryotic expression host E. Coli transformed with a single plasmid containing both the coding sequences for $Galpha_{i1}$

and myristoyl transferase. Activity of AC was significantly reduced in the presence of Gi, activated by incubation with both GTPgammaS or reference activator compounds Mastoparan and ML250. A new synthesized 4-aminopiperidric derivative, named BC5, was able to activate isolated Gi proteins with higher potency and efficacy.

This functional transductor-effector system provides a new tool to give a better insight into G protein signalling pathways, moreover BC5 is a suitable candidate for receptor-independent Gi protein activation.

Key words: G protein, direct activators, adenylate cyclase

P240031

Molecular Mechanism for Colorectal Cancer Chemoprevention with Mesalamine (5-ASA)

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Our aim was to determine the effect of mesalamine on cancer-related genes. Colon cancer Caco-2 cells were treated with vehicle or mesalamine (4 mM or 40 mM) for 2 and 5 hours. Isolated RNA was used as templates for hybridization with a cancer pathway gene array. Studies: 1) mRNA expression by gene array, 2) protein expression by Western blot analysis, 3) localization by immunohistochemistry, 4) apoptosis detection by Annexin V. 2-hour treatment with mesalamine 4 mM and 40 mM downregulated expression of genes encoding transcription factors and signaling transduction molecules: Akt (61% and 158%), c-Ets2 (74% and 77%), and c-Myc (50% and 89%). Apoptosis regulator Bcl-x was decreased by 34% and 89%. 5-hour treatment with mesalamine 40 mM significantly decreased protein expression of c-Myc 3 fold ($p < 0.05$) compared to cells treated with mesalamine 4 mM or control. Mesalamine increased apoptosis. To conclude: 1) Mesalamine dose dependently downregulates genes encoding anti-apoptotic and transcription factors, and signal transduction molecules involved in survival and proliferation in human colon cancer cells. 2) c-Myc protein expression is significantly reduced by high dose mesalamine.

P240032

Molecular-Targeted Antitumor Agents: Discovery of Natural Product-Based PPAR-gamma Activators

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors. Ligands of PPAR-gamma have been shown to inhibit growth, promote terminal differentiation, and induce apoptosis in human breast tumor cells. A MCF7 cell-based reporter assay was developed to examine extracts of terrestrial and marine organisms for the ability to activate PPAR-g. Bioassay-guided isolation of active extracts from the marine sponge Pseudoceratina rhax and a member of the tomato family Physalis angulata yielded the histone deacetylase (HDAC) inhibitor psammaplin A and a group of highly oxygenated seco-steroids known as physalins, respectively. Psammaplin A and physalins were shown to activate PPAR-g and induce apoptosis in MCF-7 breast tumor cells. Molecular modeling studies suggest that psammaplin A and physalins may interact with binding sites within the PPAR-g ligand-binding pocket and activation of PPAR-g-regulated gene expression may play a role in the ability of these natural products to induce apoptosis in tumor cells.

Key words: PPAR-gamma, breast cancer, drug discovery, molecular target

Supported by DOD/2000-BCRP DAMD17-00-1-0686 and NOAA NURP/NUST NA16RU1496.

P240033

The study of anti-LPS material basis and biological activity within Allium Sativum L.

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Objective: We screened the active components extreated from Allium Sativum L., and studied their biological activities against sepsis。Methods: (1) The active fractions were isolated by biosensor technique. (2) Observing the inhibition of TNF-αand IL-6 release in RAW264.7 cells induced by LPS. (3) Observing the protection of the fraction for mice from lethal challenge of LPS. (4) Isolating the active monomes from ASLA, studing their LPS-neutralizing effect. Results: (1) The active fraction with the best affinity was separated and benamed of Allium Sativum L. fraction A (ASLA). (2) ASLA could markedly inhibite TNF-αand IL-6 release in RAW264.7 cells induced by LPS; It also protected mice from lethal challenge of LPS. (3) There were two main components in ASLA. Both of them had significant biological activities against sepsis. Conclusions: (1) The ASLA had significant activity against sepsis. (2) The main components of ASLA were two monomers. And they had significant activities against sepsis.

Key Words: Allium sativum L.; biosensor; Lipopolysaccharide; Lipid A; sepsis

P240034

Akt activation and inhibition of forkhead transcription factors mediate vanadium compound-induced neuroprotection in the brain ischemia

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Phosphatidylinositol-3-kinase (PI3K)/Akt pathway has central role in the cell survival. We recently documented that brain ischemia-induced reduction of Akt activity mediates delayed neuronal death in the gerbil and rat hippocampus (1-3). However, the downstream targets underlying the Akt-mediated neuronal survival have not been defined. We here documented precise spatial and temporal profiles of Akt inactivation and dephosphorylation of forkhead transcription factors such as FKHR, FKHRL1 and AFX following mouse transient middle cerebral artery occlusion model. Akt inactivation during brain ischemia mediated dephosphorylation of all these members of forkhead transcription factors and in turn promoted their DNA binding activities in the nuclei. Fas-ligand was expressed under control of the forkhead transcription factors 24 hours after brain ischemia. Finally, Akt activation, and inhibition of forkhead transcription factors and Fas-ligand expression mediated vanadium compound-induced neuroprotection in mouse brain ischemia. (1) Kawano et al. (2002) J. Cereb. Blood Flow & Metab. 22: 926-934; (2) Hasegawa et al. (2003) 23: 1040-1051; (3) Hashiguchi et al. (2004) 24: 271-279

P240035

Linalyl acetate as a major ingredient of lavender essential oil (LEO) relaxes vascular smooth muscle through dephosphorylaton of myosin light chain

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Aromatherapy is widely known as an alternative treatment with essential oils. Among them, LEO has been reported to be effective for hypertension and atherosclerosis. Thus, the present experiments were designed to investigate whether lynalyl acetate (LA) as a major ingredient of LEO relaxes vascular smooth muscle, if so to analyze the mechanisms. Transverse strips of rabbit carotid arteries were used for isometric tension measurements and Western blotting to assess the phosphorylation ratio of myosin light chain (MLC). LA exerted a sustained and progressive relaxation during the contraction caused by phenylephrine. Pharmacological analyses revealed that relaxation with LA was resulted from partially activating endothelial NO-cyclic GMP pathway and partially reducing the MLC phosphorylation ratio in smooth muscle layer. The reduced MLC phosphrylation ratio and relaxation with LA were reversed by calyculin A as an inhibitor of MLC phosphatase, but remained unaffected by ML9 as an inhibitor of MLC kinase, suggesting the possible involvement of activation of MLC phosphatase in causing relaxation with LA. Taken together, our results seem to be providing a new possibility on approach for vascular diseases.

P240036

14-3-3 η protects rat's cardiomyocytes against acute anoxia-reoxygeneration injury

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14-3-3 proteins represent a family of acidic intracellular protein and the roles that they protect cardiomyocytes against acute anoxia-reoxygeneration(A/R) injury are unknown. The present study attempted to investigate the roles of 14-3-3 η protein in A/R injury. The primary cultured neonatal rat cardiomyocytes with the acute A/R injury were used. Liposome-coated pEBG 14-3-3 η wild-type construct and 14-3-3 η dsRNA were transfected into the myocardiocytes. 14-3-3 η mRNA and its protein, viability, ultrastructure of myocytes, and LDH activity in medium were examined. The results showed transfection of pEBG 14-3-3 η wild-type construct induced up-regulated expression of 14-3-3 η mRNA and protein and decreased acute myocardial A/R injury. In contrast, transfection of dsRNA resulted in down-regulated expression of 14-3-3 η mRNA and protein and aggravated acute myocardial A/R injury. The findings well demonstrate a cytoprotective role of 14-3-3 η in acute rat myocardial A/R injury.

Key words: 14-3-3 protein; cardiomyocyte; RNA interference

Acknowledgement: This study was supported by the Natural Scientific Foundation of China, Research Grant 30460048.

P240037

NF449 INHIBITS NERVE-MEDIATED CONTRACTIONS OF GUINEA-PIG PROSTATIC SMOOTH MUSCLE

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This study compares the efficacy of NF449 with suramin and alpha,beta-methylene ATP in antagonizing the P2X1-purinoceptors mediating fibromuscular contraction in the guinea-pig prostate. Electrical field stimulation (60 V, 1 ms, 0.1-20 Hz) elicited frequency-dependent contractile responses in isolated prostatic preparations. The P2 receptor antagonist suramin (100 mcM) had no inhibitory effect on field stimulation-induced responses ($P = 0.97$, $n = 6$). alpha,beta-methylene ATP (10 mcM) considerably reduced contractile responses by 37% at 5 Hz.

Administration of alpha,beta-methylene ATP (10 mcM) and the alpha1-adrenoceptor antagonist prazosin (0.3 mcM), inhibited contractile responses by 49% ($P < 0.001$, $n = 6$, 5 Hz). The P2X1 receptor antagonist NF449 (10 mcM) attenuated contractile responses to field stimulation ($P < 0.001$, $n = 6$, 5 Hz) to 52% of control. NF449 (10 mcM) and prazosin (0.3 mcM) reduced electrically-evoked contractions ($P < 0.001$, $n = 6$, 5 Hz) by up to 75% with residual levels comparable to those observed in the presence of tetrodotoxin. These results further demonstrate the importance of adenosine 5'-triphosphate in nerve-mediated contractile responses of the guineapig prostate.

Key words: prostate, ATP, NF449

P240038

Anti-epileptogenic Effect of β-carotene and Vitamin A in Pentylenetetrazde-kindling Modell of Epilepsy in Mice

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Vitamin A and its derivatives have recently been reported to be implicated in synaptic plasticity. In this study, the possible effect of Vit A and its precursor, β-carotene on acute seizures and kindling, induced by pentylenetetrazole (PTZ), was assessed. Vit A and β-carotene were evaluated for: (1) Elevating the threshold of clonic seizures induced by I.V. infusion of PTZ; (2) anticonvulsant effect; (3) anti-epileptogenic effect. Diazepam was employed as positive control. All of the drugs showed anti-epileptogenic effect against PTZ-induced tonic seizures and lethality in kindling mice. β-carotene had neither any effect on clonic seizures threshold nor any anti-convulsant effect; Vitamin A increased the clonic seizures threshold but, had no anti-convulsant effect.

Non-genomic and genomic mechanisms might be involved in the anti-epileptogenic effect of Vit A and β-carotene and anti-convulsant effect of Vitamin A.

The expense of this study was supported by Tehran Pasteur institute and Tehran Shahed medical university.

P240039

Overexpression of Sorcin gene induces a low level of multidrug-resistance in human leukemia cells

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Objective To confirm the contribution of sorcin gene to drug resistant phenotype in human leukemia cells. Methods The contribution of sorcin by itself to drug resistant phenotype was dissected out by gene transfection in K562 cells and sorcin-targeting small interfering RNA. The expression of sorcin was measured by Western blot or RT-PCR. The sensitivity of those cells to chemotherapeutic agents were measured by MTT assay. Results The sorcin expression level in K562/A02 cells was higher than in K562 cells significantly.

Overexpression of sorcin by gene transfection in K562 cells resulted in increased drug resistance, from 4.1- to 22.5-fold, to a variety of chemotherapeutic agents. On the other hand, inhibition of sorcin expression in both MDR K562/A02 and the sorcin-transfected K562 cells with sorcin-targeting small interfering RNA led to varying the extent of reversal of drug resistance. Conclusion: Sorcin was concerned with MDR in K562/A02 cell line, and it is an important gene associated with the development of MDR in leukemia cells and may be a potential target for leukemic MDR modulators investigation in the future.

Key words: Multidrug resistance; Sorcin; Leukemia

P240040

Drug targeting to colon: Effect of inulytic enzymes on the indomethacin release from pellets coated with Eudragit RL containing inulin.

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Targeting of drugs to colon has several therapeutic advantages. Drug release may be controlled by the gastrointestinal pH, transit time or intestinal flora. The latter appears to be more interesting with regard to the selectivity. The aim of this work is to assess the suitability of such an approach for achieving specific delivery of indomethacin to colon using pellets coated with Eudragit RL aqueous dispersions containing inulin as a polysaccharide. Indomethacin was selected as a model drug because it has good indications for colonic delivery.

Indomethacin loaded pellets were coated with formulations containing different ratios of Eudragit RL and inulin. The indomethacin release was evaluated at different pH in absence or presence of inulytic enzyme (inulinase).

It was shown that in absence of inulinase drug release was low, but in presence of enzyme, drug release markedly increased. The results of this study revealed that inulin has potential for colon delivery and incorporation of inulin in Eudragit RL films is suitable for colonic delivery of indomethacin pellets.

Key words: Colon delivery; Indomethacin; Inulytic enzyme

P240041

BRAIN ISCHEMIA INDUCES CHANGES IN THE PATTERN OF Na+/Ca2+ EXCHANGER GENE EXPRESSION IN THE ISCHEMIC CORE, PERI-INFARCT AREA, AND INTACT BRAIN REGIONS

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Dysregulation of sodium and calcium homeostasis plays a pivotal role in the pathophysiology of cerebral ischemia. The sodium-calcium exchangers NCX1, NCX2 and NCX3 couple the movement of these ions across the cell membrane. To determine if NCX gene expression is regulated after cerebral ischemia, we used NCX specific probes to analyze, by radioactive in situ hybridization, the pattern of NCX transcripts in the ischemic core, peri-infarct area, and remote regions, after 6 and 24 h of permanent middle cerebral artery occlusion (pMCAO) in rats. In the focal region, comprising prefrontal, somatosensory and insular cortices, all NCX transcripts were downregulated. In the peri-infarct area, comprising part of the motor cortex and the caudate putamen, NCX2 mRNA was downregulated, whereas NCX3 mRNA was upregulated. In remote regions such

as the prelimbic and infralimbic cortices, and teniatecta, NCX1 and NCX3 transcripts were upregulated, whereas in the caudate-putamen only NCX3 mRNA increased. In these regions, NCX2 signal decreased. These results indicate that NCX gene expression is regulated after pMCAO in a differential manner, depending on the exchanger isoform and region involved in the insult

P240042

Effects of Pentadecapeptide BPC-157 on Transosseous Rat Mandibular Defects Healing In Vivo.

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The efficacy of local and systemic delivery of pentadecapeptide BPC-157 to promote bone healing was evaluated in transosseous rat mandibular drill defects. Insufficient or absence of bone healing is a frequent problem within all surgical fields. Based on the previously recognized positive osteogenic results of gastric pentadecapeptide BPC-157, the aims of the present study were to further develop a possibility of osteopromotion by various routes.

Transosteal defects were performed proximal to the entry of the inferior alveolar artery in the left rat mandibular ramus using extraoral approach. Rats received agents (i) BPC 157 10 microg, 10 ng/kg intraperitoneally immediately after the injury, or (ii) BPC-157 2 microg, 2 ng/ml (1 ml bath) locally at the injury site. The effects were assessed at 3rd or 10th day post injury using densitometric and histopathological assessment. Results indicate that gastric pentadecapeptide BPC-157 given either systemically or by local application significantly improves transosseous mandibular defect healing.

Key Words: Pentadecapeptide BPC 157, peptide treatment, bone, rat

P240043

The expression of CYP4Z1 in the human breast carcinoma and its role in regulating breast carcinoma cell growth

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To investigate the relationship between cytochrome P450 4Z1 (CYP4Z1) and carcinogenesis. Expression of CYP4Z1 in 15 cases of non-cancerous mammary gland tissues and 64 cases of human breast carcinoma tissues was detected by using RT-PCR. The effect of cell growth was evaluated by MTT methods. Apoptosis was detected by using flow cytometry. CYP4Z1 was over-expressed in 57% of breast carcinomas with no significant difference in breast tumor type. The expression of CYP4Z1 was correlated with differentiation and postoperative TNM staging of breast carcinoma tissues, but not with lymph node metastasis. CYP4Z1 was expressed in the human breast carcinoma cell lines (T47-D and MCF-7). Treatment with progesterone (a CYP4Z1 inducer) could increase the expression of CYP4Z1 (10 fold), promote cell growth and decrease activity of Caspase-3. Progesterone-induced cell growth contol was prevented by CYP4Z1 short interfering RNA. Our results demonstrate that overexpression of CYP4Z1 is correlated with carcinoma cell growth, which may be a new target for therapy of breast carcinoma in the future.

Key words: CYP4Z1; progesterone; breast carcinoma; growth control

P240044

Structure-activity relationship analysis of a series of diterpenoids from rubescens and their anticancer mechanism

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Objective: to test the structure-activity relationship of a series of diterpenoids and to explore their anticancer mechanism. Methods: Flow cytometry assay, caspase activity measurement, etc. Results: We found that differential structure exerts differential cytotoxity in HL-60 cell line. By2 (5μg/ml) induced significantly apoptosis(77.6%) in HL-60 cell after 24 hours. And it blocked HL-60 progression from G2/M to S phase in a time- and dose-dependent manner. By2 could induce mitochondrial membrane potential to lose and cytochrome c to release. The antioxidant NAC could decrease the degree of the cell growth inhibition and the quantity of the apoptosis cells. By2 also could induce caspases-3 to activate and the apoptosis was completely prevented by cotreatment of cells with the general caspase inhibitor Z-VAD-fmk. Conclusions: These results, suggested a possible structure-activity relationship of the diterpenoids and that diterpenoids-induced cell apoptosis was associated with oxidative stress and caspase activation.

Key words: diterpenoids, structure activity relationship, anticancer mechanism

Acknowledgement

We thank Dr. Zhao in Kunming Institute of Botany for extraction and isolation of diterpenoids.

P240045

Berberrubine inducing anti-proliferating effects in Human Colorectal Carcinoma cell line HT-29 in vitro.

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Aim: To investigate the inhibitory effects on the proliferation by using Berberrubine comparing with azidothymidine(AZT) in human colon cancer cell line HT-29 cultured in vitro. Methods: Human colon cancer cell line HT-29 was cultured and then exposured to different concentrations of Berberrubine and AZT for 24, 48 and 72 hours in order to screen the optimal concentration and exposed time. The proliferation of the cells was measured by cell counting kit-8 assay. Results: Berberrubine, the optimal concentration of which was 105umol/L, inhibited effectively the proliferation of human colon cancer cell line HT-29(the rate of inhibition was 34.17%) at 72h. While AZT was125umol/L(22.54%) at 72h.

Cnclusion: Our data indicated that Berberrubine can inhibit the proliferation of human colon cancer cell line HT-29. Moreover its effect was more than that of AZT.

Key words: Berberrubine, HT-29 colon cancer cell line, AZT

P240046

Steroid Receptor RNA Activator as a New Target to fight breast cancer.

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Estrogen receptors (ER), which activity is regulated by coregulators, play crucial roles in breast cancer development and progression. So far, most known ER coregulators are proteins, but one exception, the steroid receptor RNA activator (SRA) has been found to activate ER mediated transcription as an RNA molecule. Although the first described SRA was non-coding, we have identified coding SRA isoforms encoding for a SRA protein (SRAP), by virtue of an extended exon 1 that contains a methionine start codon. Interestingly, preliminary data suggest that SRAP, in contrast to SRA RNA, acts as an ER repressor. We are therefore facing a system that regulates ER signal pathway oppositely at the RNA and protein levels. We have also identified other SRA RNA isoforms containing full or partial intron 1. Intron 1 sequence retentions introduce a shift or a stop codon in the SRAP reading frame, making these isoforms non-coding for SRAP.

We have now characterized co-expression of coding and non-coding SRA transcripts in breast cancer cells as well as breast cancer tissue, and showed that their relative proportion varies. We hypothesize that in breast cancer, the balance between coding and non-coding SRA, regulated through alternative splicing, determines the equilibrium between SRA coactivator (SRA RNA) and co-repressor (SRAP) of ER signaling pathway.

Down-regulating ER activity has already been proved an effective strategy to design breast cancer therapeutics. We therefore plan to develop approaches aiming to specifically promote SRA intron 1 splicing in breast cancer cells in order to tip the balance toward an increase of coding SRA isoforms, and ultimately of SRAP, to inhibit ER signaling pathway in these cells.

Key words: steroid receptor RNA activator, SRAP, alternative splicing

P240047

Aquaporin-1 mediated the inhibitory effects produced by XJ-6-A on tumor growth and metastasis

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XJ-6-A is a newly designed and synthesized compound as an inhibitor of Aquaporin-1(AQP1). In this study, we investigated the role of XJ-6-A on tumor growth and metastasis and its potential mechanism. XJ-6-A(20 and 40 mg·kg^{-1}·d^{-1} for 20 d, ig) was found to inhibit the growth and metastasis of tumor cells in Lewis lung carcinoma bearing mice. The inhibition rate of lung metastasis at the dose of 20 mg·kg^{-1}·d^{-1} was up to 80%. Concurrently, XJ-6-A could mitigate the damage of lung alveolar caused by metastatic tumor deposits and obviously decrease AQP1 protein expression. In cell-based assays, XJ-6-A inhibited dramatically migration and invasion of human prostate cancer cells(PC-3M) at the concentrations of 0.1μM, 1μM and 10μM, whereas without showing cytotoxicity or anti-proliferative action. Simultaneously, the expression of AQP1 protein was obviously decreased by the observation of immunohistochemistry. These results indicate that XJ-6-A can inhibit tumor growth and metastasis, which partly depends on inhibiting the expression of AQP1 protein.

Key words: XJ-6-A; aquaporin-1; tumor metastasis

P25. Drug Discovery - Pharmacoinformatics

P250003

Inhibitory effects of separations from bamboo-root on Allergic Reactions

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objective: To observe effects of the separations from chloroform and acetic ether extractive from bamboorooton immediate type allergic reactions in order to elucidate its mechanism. Methods: Passive cutaneous anaphylaxis(PCA), an experimental model of type I allergic reaction, was induced by intradermal injection of ratanti-ovalbumin antiserum into rars or mice and Schultz-Dale reaction. Results: separations from chloroform andacetic ether extractive po significantly inhibited homologuos PCA and degranulation of mast cells of calvarial periosteum in rats and the tension of ileum in cavia cobayas. Conclusion: the separations from chloroform and acetic ether extractive can inhibit immediate allergic reaction.

Key words: bamboo-root; passive cutaneous anaphylaxis; mast cell; Schultz-Dale reaction

Acknowledgement: thanks professor xu peng for offering the directions.

P250004

Experimental Study of Osthole on Treatment of Hyperlipidemic and Alcoholic Fatty Liver in Animals

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AIM: To evaluate the effects of osthole on fatty liver, and investigate the possible mechanism. METHODS: Quail model with hyperlipidemic fatty liver and rat model with alcoholic fatty liver were set up by feeding high fat diet and alcohol, respectively. These experimental animals then were treated with osthole 5~20 mg/kg for 6 weeks, respectively. And then the lipid of serum, the lipid of hepatic tissue, and coefficient of hepatic weight were mensurated. RESULTS: After treatment the levels of serum TC, TG, LDL-C, coefficient of hepatic weight, and the hepatic tissue contents of TC and TG were significantly decreased, and the activity of SOD in liver was improved. In alcohol-induced fatty liver rats, level of MDA in liver was decreased. In high fat-induced fatty liver quails, GSH-PX in liver was significantly improved. The histological evaluation of liver specimens demonstrated osthole dramatically decreased lipid accumulation. CONCLUSION: Osthole possessed the theraputic effects on alcohol or high fat-induced fatty liver, the mechanism might be associated with its antioxidation.

P250005

Pharmacoinformatics Research on Drug Information and Drug Target Information

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Pharmacoinformatics is a new across science of several sciences, such as bioinformatics, chemical informatics, drug information and so on. Pharmacoinformatics research based on drug information and drug target information is very important for new drug discovery. Drug information research can reveal the features of drug functional groups from several drug structures, while drug target information research on the feature of active sites can give us more information on the features of its ligand structures, such as chemical space, electrostatics, hydrophobicity, and hydrogen bonds and so on. Therefore, novel drug discovery will benefit from pharmacoinformatics research on drug functional group information, drug target active site information and drug-like information.

Key words: Pharmacoinformatics, Drug functional group, Drug target active site

P250006

The Effect Of Bifid Triple Viable To Endotoxemia And Some Cytokines Of Liver Cirrhosis Patients

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Objective: The study is conducted to investigate the clinical significance of Bifid Triple Viable that was used to treat endotoxemia of liver cirrhosis on the base of general therapy, and to observe the change of endotoxin and some cytokines (IL-1α, IL-6, TNFα) in plasma during the treatment. Methods: 60 hospitalizing patients with liver cirrhosis in the uncompensated period were included in present study. The patients satisfied the conditions were separated into two groups at random. One is control group, another is BTV group. Results: The level of endotoxin, IL-1α, IL-6, TNFα in BTV group after therapy were lower than that before therapy, the differences were significant ($p < 0.05$); The level of endotoxin, IL-1α, IL-6 TNFα, in BTV group after therapy were lower than that in control group. The differences were significant ($p < 0.05$). Conclusions: Applied Bifid Triple Viable to treat endotoxemia of liver cirrhosis can sharply decrease the endotoxin level, and can down-regulate some cytokines (IL-1α, IL-6, TNFα) in plasma, and also can improve the liver function.

Key words: Bifid Triple Viable; Endotoxemia; Interleukin-1α;

P26. Immunopharmacology and Inflammation

P260001

Memantine Protects Hippocampal Neuronal Function in Murine HIV-1 Encephalitis

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Memantine, a low-to-moderate-affinity NMDA receptor antagonist, can be used to treat cognitive impairment associated with Alzheimer's disease. To examine its therapeutic potential for HIV-1 associated dementia, we studied the neuroprotective effects of memantine on hippocampal synaptic function in a severe combined immunodeficient (SCID) mouse model of HIV-1 encephalitis (HIVE). Human monocyte-derived macrophages (MDM) infected with HIV-1 were stereotactically injected into the basal ganglia of SCID mice, generating HIVE. Impaired synaptic transmission and long-term potentiation (LTP) were detected in the CA1 region of hippocampal brain slices of HIVE mice. Memantine-treated HIVE mice showed significant improvements in synaptic function during frequency facilitation tests and LTP induced by high frequency stimulation when compared to untreated animals. Immunocytochemical measures of neuronal antigens mirrored the neuronal physiological tests. These results demonstrate that memantine attenuates hippocampal synaptic impairment in murine HIVE and provides a rationale for its use in infected humans who experience cognitive decline. Supported by NIH grant R01 NS41862.

Key words: Memantine, LTP, AIDS

P260002

Uterine relaxant effect of subtype selective α-adrenergic receptor antagonists in vitro alters in inflammation-induced preterm birth in rats

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Catecholaminergic stimulation exerts potent myometrial contractions via the α-adrenergic receptors (α-ARs) in the late-pregnant rat uterus where the α_{1A}-AR subtype occurred to be the most abundant. In this study, the uterine relaxant effect of subtype selective α-AR antagonists was studied in vitro, in the uteri of

rats in inflammation-induced preterm labor.

Preterm labor was evoked by the administration of E. coli endotoxin on day 18-19-20 of pregnancy. AR subtype mRNA expressions were detected by RT-PCR. Rhythmic contractions of isolated uterine rings were elicited by electric field stimulation and relaxant effect of selective antagonists (WB4101 for α_{1A}; AH11110A for α_{1B}; BMY7378 for α_{1D}) were tested. Slight changes were detected in the expression of α_{1A}- and α_{1D}- AR mRNA, but a significant increase of α_{1B}- AR mRNA in case of tissue inflammation. The relaxant potency of WB4101 increased in inflammation, and surprisingly, AH11110A appeared to be very effective in relaxing the uterus in inflammatory preterm labor in contrast with its very limited relaxing effect in nontreated controls.

In conclusion, α_{1A}- and α_{1B}- AR antagonists are promising new tocolytics in inflammation-induced preterm birth.

P260003

Hepatic Ischemia Reperfusion Injury Increased LTC4 Synthesis by Up-regulation of mRNA Expression of LTC4S and Enhancement of LTC4 Synthesis Enzymes Activity in Rats

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To further explore the mechanisms of LTC4 generation during hepatic I/R. Using hepatic partial I/R injury rat model, we examined LTC4 content? the activities and mRNA expression of LTC4 synthesis enzymes including LTC4S, mGST2 and mGST3 with RT-PCR and RP-HPLC. Liver damages were assessed by serum ALT, AST measurement and histological observation. SOD, MDA and GSH were used to evaluate lipid peroxidation and cytotoxicity. Compared with those in control, the mRNA expression of mGST2 and mGST3 in I/R liver tissue were lower ($P < 0.05$), LTC4 content, LTC4 synthesis enzymes activities and the mRNA expression of LTC4S were significantly increased ($P < 0.05$), and this was accompanied by serum ALT and AST elevation ($P < 0.01$), liver tissue SOD and GSH decrease and MDA increase ($P < 0.05$), as well as histological damage. These results demonstrated that hepatic I/R down-regulated gene expression of mGST2 and mGST3 and enhanced the activities of LTC4 synthesis enzyme; these results also suggested that LTC4 enhancement after hepatic I/R was partly caused by LTC4S gene expression up-regulation and LTC4 synthesis enzymes activities augment, and maybe associated with liver damage.

P260004

CYCLOSPORIN INFLUENCES THE ACTIVITIES OF RENAL AMINOPEPTIDASES

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We test the hypothesis that the aminopeptidase (AP) participate within renal (Ki) effects induced by cyclosporin A (CsA). Ki soluble (S) and particulate (M) AP activity levels of CsA-treated and control mice were evaluated, as well as Ki caspase 3 activity, hematocrit, urinary protein and plasma osmolality, creatinine and uric acid. CsA increased caspase 3 (38%), hematocrit (15%) and osmolality (4%). CsA increased neutral (96%), basic (98%), cystyl (200%), prolyl imino (91%) and pyroglutamyl (64%) AP in S of Ki cortex. Acid (123%) and basic (19%) AP increased in the S of Ki medulla. Increased levels in the cortex were detected for acid (40%) and pyroglutamyl (69%) M AP. CsA increased cortical S (94%) while decreased medullar M (38%) prolyl dipeptidyl AP IV. With the exception of prolyl dipeptidyl AP IV, AP in M returned to levels similar to controls after 15 days of CsA withdrawal, and AP in S did not regress. These changes on Ki AP associated with mild Ki impairment caused by CsA should be considered into the elaboration of new potential strategies for preventing nephrotoxicity during the treatment with CsA.

Immunosuppression; peptidases. Supported by FAPESP and CNPq

P260005

METHOTREXATE AND CYCLOSPORIN INFLUENCE THE ACTIVITIES OF PROLYL DIPEPTIDYL AMINOPEPTIDASE IV AND PROLYL OLIGOPEPTIDASE OF MURINE MACROPHAGES

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This study was undertaken to evaluate the effects of methotrexate (MTX) and cyclosporin A (CsA) on macrophage (Mf) membrane-bound (M) and soluble (S) prolyl dipeptidyl aminopeptidase IV (DPPIV), which cleaves inflammation mediators such as interferon-gamma, and S prolyl oligopeptidase (POP), which cleaves the nociceptive mediators bradykinin and substance P. Mice were treated with MTX or CsA and a half of each group received intraperitoneal injection of thioglycollate (TGE). Resident (RE) and TGE Mfs were harvested by washing the peritoneal cavity. MTX increased DPPIV (S: 110%; M:99%) and POP (60%) while CsA inhibited POP (21%) in TGE Mfs. DPPIV and POP activities in RE Mfs were not affected by MTX and CsA. The effect of MTX on DPPIV activity of TGE Mfs and its absence on RE Mfs suggest that DPPIV is related to the immunossupressor action of MTX. The opposite actions of MTX and CsA observed on TGE Mf POP activity may influence the intensity of the analgesic action of these drugs. These data provide scope for additional studies on combined therapy with MTX and CsA.

Immunosuppression; peptidases. Supported by FAPESP and CNPq

P260006

Dendritic cells and regulatory cells in autoantigen induced murine immune tolerance model

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Aim: To investigate the preventive effect of autoantigen insulin given subcutaneously on IDDM murine model and the influence on the phenotype and function of dendritic cell (DC) and CD4+CD25+ regulatory T cells. Methods: The IDDM model was established by injection of multiple low dose of streptozotocin (STZ) 40 mg.kg-1 intraperitoneally for 5 days in Babl/c mice. The bovine insulin (100μg) in IFA was given subcutaneously weekly for 4 weeks. The blood glucose was examined weekly. Pancreas tissues were taken for histopathologic examination. DC precursors from bone marrow and lymphocytes from spleen were isolated. The phenotype of DC and $CD4^+$ $CD25^+$ regulatory T cells were analyzed by FACS. T cell stimulating activity by DC was determined by allo-MLR. Results: The blood glucose in mice given insulin was well controlled, the amount of DC with CD11c was increased, expression of CD86 and MHC-II was low and the capacity of stimulating T cell proliferation by DC was lower than those from the normal mice but higher than which from model mice, and the ratio of $CD4^+CD25^+$ T cells were significantly enhanced. Conclusion: Subcutaneous administration of insulin can confer protection to mice from IDDM. The immune protection may be associated with establishing immune tolerance by improving the function of abnormal DC and promoting the production of $CD4^+CD25^+$ T cells in vivo.

Key Words: Autoantigen; Dendritic cells; Regulatory cells

* Project supported by the National Natural Science Foundation of China (No 30200343).

P260007

Effect of statins on IL-18 production and adhesion molecule expression in human monocytes

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IL-18 production was detected in the medium of monocytes treated with HMG-CoA reductase inhibitors, pravastatin and fluvastatin, but not with the statin-derived LFA-1 inhibitor LFA703, which did not inhibit HMG-CoA reductase. Pravastatin and fluvastatin also induced the production of IL-18, TNF-α and IFN-γ in PBMC in contrast to LFA703. IL-18 production by PBMC is located upstream of the cytokine cascade activated by these statins. The IL-18-induced cytokine production was demonstrated to be dependent on adhesion molecule expression on monocytes. In the absence of IL-18, pravastatin and fluvastatin inhibited the expression of ICAM-1 and induced the expression of CD40, whereas LFA703 had no effect. In the presence of IL-18, pravastatin, fluvastatin and LFA703 similarly inhibited the expression of ICAM-1 and CD40 as well as the production of IL-12, TNF-α and IFN-γ. The effects of pravastatin and fluvastatin but not LFA-703 were abolished by the addition of mevalonate, indicating the involvement of HMG-CoA reductase in the action of pravastatin and flu-

vastatin. It was concluded that LFA703 has the inhibitory effect on IL-18-initiated immune response without any activation on monocytes.

P260008

Role of matrix metalloproteinases in the inflammatory response in human airway cell based assays and in a rat model of airways inflammation.

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Matrix metalloproteinases (MMP) are believed to be involved in the turnover/degradation of extracellular matrix, however, evidence suggests that they may also be involved in inflammation. We have previously measured an increase in MMP expression in our human cell assays and rat models of airway inflammation. The objective was to determine the role of MMPs in these models by using a broad spectrum MMP inhibitor (MMPI). In LPS stimulated THP-1 cells and primary human lung tissue macrophages the MMPI had no significant affect on the release of TNF, IL-8, IL-1, GRO, MIP-1 or IL-6. In the LPS-driven rat model of airway inflammation, the MMPI did not affect mediator release or cellular burden. The MMPI, however, did significantly reduce levels of MMP-9. In an airway disease model the MMPI did not reduce cellular inflammation but did significantly reduce elastase-induced emphysema. In summary, for the first time, this data shows that in these pre-clinical models MMPs do not play a role in the increase in inflammatory mediator release or cellular burden, but do in the breakdown of airway structure.

P260009

Comparison of anti-inflammatory and anti-leukocyte accumulation effects of statins

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Statins have been proven to possess anti-inflammatory activities unrelated to cholesterol lowering actions. Here we compared the anti-inflammatory and anti-leukocyte accumulation effects of atorvastatin, simvastatin and lovastatin in carrageenan-induced rat paw edema as an acute inflammatory model. Wistar rats were received 1, 5, and 10 mg/kg of drugs orally 20, 12, 6, and 1h prior to inflammation induction. We found that all three statins reduce both the maximal oedema response attained during 4h and neutrophils infiltration in inflammation zone.

Lovastatin had the lowest and atorvastatin had the greatest effects. The statins did not alter plasma cholesterol and triglycerides. Atorvastatin (10 mg/kg) caused the most potent and dose-related inhibition of the carrageenan induced inflammation (45% reduction; $p < 0.001$) and leukocyte accumulation (70% reduction; $p < 0.001$).

Atorvastatin was comparable to indomethacin in this model. The result of this study shows that the antiinflammatory potency of statins is according to their inhibitory potency on hydroxy-methyl-glutaryl CoA reductase but unrelated to lipid reduction.

P260010

Effect of phytoncide on human NK activity and intracellular perforin, granulysin and granzyme in NK cell

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In order to explore the effect of forest bathing on the human immune system, we investigated the effect of phytoncide on natural killer (NK) activity and the expression of perforin, granzyme A and granulysin in human NK. We used NK-92 MI, a human NK cell line. NK-92 MI expresses CD56, perforin, granzyme A and granulysin, and is highly cytotoxic to K562. Phytoncide significantly increase cytolytic activity of NK-92 MI in a dose-dependent manner and significantly increase the expression of perforin, granzyme A and granulysin.

Phytoncide also partially, but significantly, restore decreased NK activity and intracellular perforin, granzyme A and granulysin in NK-92 MI induced by dichlorvos, an organophosphorus pesticide.

Pretreatment with phytoncide partially prevents dichlorvos-induced inhibition of NK activity. Taken together, these data indicate that phytoncides significantly enhance human NK activity and this effect partially mediated by induction of intracellular perforin, granzyme A and granulysin. Keywords: Granulysin; Granzyme A; NK; Perforin; Phytoncide. This work was supported by a research project for utilizing advanced technologies in agriculture, forestry and fisheries.

P260011

Effects and mechanisms of Shaoqiduogan on immunological liver fibrosis

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Shaoqiduogan (SQDG) is a compound produced from Radix Paeoniae Rubra and Radix Astragali. This study was aimed to examine the effect of SQDG on human albumin induced immunological liver fibrosis in rats. The hyaluronic acid (HA) and procollagen III (PCIII) were assessed by radioimmunoassay. SQDG decreased HA, PCIII, hydroxyproline content and improved the histological appearance of the liver sections. SQDG reduced lipid peroxidation and restored activities of antioxidase. In vitro, SQDG raised the matrix metalloproteinase 13 (MMP-13) level and reduced the tissue inhibitors of metalloproteinase 1 (TIMP-1) level in HSC-T6 cell stimulated by transforming growth factor-beta1 (TGF-β1). The expression of $G\alpha i2$ and $G\alpha s$ on HSC-T6 cell membrane induced by TGF-β1 were detected by Western-blot analysis. SQDG inhibited expression of $G\alpha i2$ and elevated expression of $G\alpha s$. Moreover, SQDG promoted expression of MMP-13, inhibited the expression of TIMP-1 and collagen-I. These results indicated that SQDG may facilitate the collagen degradation of HSC-T6 induced by TGF-β1 via elevating the MMP-13/TIMP-1 ratio and controlling the expression of $G\alpha i2$ and $G\alpha s$.

Key words: shaoqiduogan; liver fibrosis; G protein

P260012

The role of endogenous hydrogen sulfide in regulating the severity of sepsis and associated organ injury

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Endogenous hydrogen sulfide (H_2S), a vasodilator and neurotransmitter, is naturally synthesized in a reaction catalyzed by cystathionine-γ-lyase (CSE) and/or cystathionine-β-synthase (CBS). However, little is known about its role in systemic inflammation. The aim was to investigate the potential role of endogenous H_2S in cecal ligation and puncture (CLP) induced sepsis. Swiss mice were subjected to CLP and treated with either saline (i.p.) or DL-propargylglycine (PAG, 50 mg/kg i.p., CSE inhibitor; $n = 12$ in each group). CLP induced sepsis significantly increased plasma H_2S concentration and liver H_2S synthesis as compared with sham operated animals. Induction of sepsis resulted in a significant up-regulation of CSE mRNA in liver. In contrast, prophylactic and therapeutic administration of PAG significantly reduced the level of cytokines and chemokines in lung, liver and plasma. PAG treatment also markedly decreased lung permeability and improved liver function and animal survival rate after CLP. Therefore, the effect of inhibition of H_2S formation suggests that H_2S plays a pro-inflammatory role in regulating the severity of sepsis and associated organ injury.

P260013

Study on immune function of polysaccharides from Asparagus officinalis

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To study the immune function of polysaccharides from Asparagus officinalis on S180 tumor mice.

After oral administration of polysaccharides solution (25, 50, 100 mg/kg) to S180 mice for a week. Thymus and spleen index, anti-sheep red blood cell (SRBC), number of antibody secreting cell (NASC) in spleen and phagocytic activity were detected, lymphocytic transformation rate (LTR) in spleen was de-

termined using MTT
methods. The results showed thymus and spleen index, LTR, anti-SRBC and NASC in spleen significantly increased after administration (3.53 ±0.80 vs 5.10 ±0.47 mg/g, P<0.05; 5.69 ±0.92 vs 7.49 ±1.18 mg/g, P<0.05; 1.047 ±0.012 vs 1.154 ±0.016, P<0.05; 6.46 ±0.12 vs 8.18 ±0.29, P<0.05; 0.403 ±0.008 vs 0.471 ±0.007, P<0.05). Phagocytic activity also increased significantly (phagocytic index: 0.53 ±0.017 vs 0.72 ±0.029, P<0.01); (phagocytic ratio: 32.30 ±1.098 vs 60.53 ±2.022, P<0.01). In conclusion, polysaccharides from Asparagus officinalis enhanced immune function of S180 mice.

P260014

IL-1β contributes to synoviocytes proliferation and G-protein alterations in fibroblast-like synoviocytes of rat with collegen-induced arthritis

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To study the alterations of guanine nucleotide regulatory proteins (G proteins) in fibroblast-like synoviocytes (FLS) of collegen-induced arthritis(CIA) under the stimulation of IL-1β, and to elucidate the possible pathogenesis. Primary cultures of CIA FLS were used. The proliferation of FLS was measured by MTT. The function of stimulatory G proteins(Gs) by cholera toxin(CT) −mediated [32P] ADP-ribosylation and inhibitory G proteins(Gi) by pertussis toxin(PT) −mediated [32P] ADP-ribosylation have been investigated in FLS. The proliferation of FLS was significantly increased by IL-1β. The labeling of Gs by CT was reduced, however, the labeling of Gi by PT was significantly increased under the stimulation of IL-1β. These showed that the augmentation of IL-1-induced FLS proliferation was associated with enhanced function of Gi and decreased function of Gs with CIA rat. The results indicate that the alterations of G proteins function play an important role in the proliferation of FLS, which may be used to explain the pathogenesis of CIA.

Key words: fibroblast-like synoviocytes; Gi proteins; Gs proteins.

Aknowledgement: Project supported by the National Science Foundation of China, No. 30572356

P260015

G protein-coupled signal transduction in synoviocytes of immune arthritis

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G proteins are partners of G-protein-coupled receptors (GPCRs). GPCRs catalyze guanine nucleotide exchange on Gαsubunits, enabling both activated Gα and Gβγsubunits to target downstream effector. Diverse extracellular signals regulate receptors to modulate cellular physiology. GPCRs signaling via heterotrimeric G proteins is attenuated rapidly by G protein-coupled receptor kinase (GRK). GPCRs phosphorylation is to promote the binding of arrestin proteins which block interactions of receptors and G-proteins. Regulators of Gprotein signaling are GTPase-activating proteins that attenuate signaling by G proteins. G proteins-AC-cAMP signal transduction play a crucial role in pathogenesis of immune arthritis. Gαt mRNA, protein express and function were decreased, and Gαt mRNA, protein express and function of were increased in synoviocytes of rats with immune arthritis. The "cross-talk" was found between MAPK signal transduction and G proteins associated signal transduction. Activation of MAPKs was regulated by Gαt and Gαt signal transduction pathway. G proteins transmembrane signal pathway became new target for treatment of arthritis arthritis.

Key words: G protein, MAPK signal transduction, arthritis

P260017

Substance P Plays a Key Role in Hydrogen Sulfide-Induced Lung Inflammation

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Hydrogen sulfide (H_2S) is a naturally occurring gas, which has been shown to be a potent vasodilator. Using different experimental models (such as caerulein-induced acute pancreatitis, carrageenan-induced hindpaw edema, and LPS-induced endotoxemia), we have earlier shown that H_2S acts as a mediator of inflammation. In this study, we have investigated the involvement of substance P in H_2S-induced lung inflammation.

Intraperitoneal administration of NaHS (10 mg/kg), an H_2S donor, to mice caused a significant increase in circulating levels of substance P (1.86 fold increase over control). H_2S alone could also cause lung inflammation, as evidenced by 1.58 fold increase over control in lung myeloperoxidase activity and histological evidence of lung injury. In substance P deficient mice, the preprotachykinin-A (PPT-A) knockout mice, H_2S did not cause any lung inflammation. Furthermore, pretreatment of mice with CP-96,345 (2.5 mg/kg, i.p.), an antagonist of the neurokinin-1 (NK-1) receptor, protected mice against lung inflammation caused by H_2S. These results demonstrate a key role of SP in H_2S-induced lung injury.

P260018

Anti-inflammatory and immunomodulatory effects of the glucosides of chaenomeles speciosa and its relative mechanism

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To observe the anti-inflammatory and immunomodulatory effects of glucosides of Chaenomeles speciosa(GCS) and relative mechanism in collagen-induced arthritis (CIA) rat. The effects of GCS were measured by histopathological assessment of synovium, IL-1, TNF and PGE_2 production in synoviocytes cAMP level and mRNA expression of Gi, Gs, and TNF in synoviocytes. There were significant secondary inflammatory reactions in CIA rats, companying the elevation of IL-1, TNF and PGE_2. GCS could significantly inhibit inflammatory swelling, IL-1, TNF and PGE_2 production, and reduced elevated spleens index, proliferation of T cell and B cell.

GCS increased cAMP level and mRNA expression of Gs, and inhibited mRNA expression of Gi, TNF, and reduced histopathological changes significantly. GCS has anti-inflammatory effects and immunomodulatory activities. The effects of GCS on rats CIA may be related to modulating G protein-AC-cAMP signal transduction of synoviocytes.

Key words: Chaenomeles speciosa; glucoside; immunomodulatory; collagen-induced arthritis

P260019

Antiinflammatory and analgesic effects of total glucosides of Cape Jasmine

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To research antiinflammatory and analgesic effects of total glucosides of Cape Jasmine (TGCJ). Carrageen were used to induce paw swelling in rats. Dye exudation induced by acetic acid and tampon granuloma induced in rats with tampon embedding method were studied to observe the antiinflammatory effects of TGCJ. Pain threshold of mice were determined with hot-plate test and the response of writhes induced by acetic acid was looked-in analgesic effects of TGCJ. TGCJ (80, 40 mg/kg) significantly inhibited carrageen-induced rat paw edema and tampon granuloma famation in rat. TGCJ(160, 80, 40 mg/kg) significantly inhibited the dye exudation, reduced the number of writhes induced by acetic acid, and increased pain threshold of mice. TGCJ has significant antiinflammatory and analgesic effects, which indicate that TGCJ is the effective part of Cape Jasmine.

Key words: TGCJ; therapeutical application; inflammation; pain

P260020

The modulation of G protein-coupled receptor kinases 2 on synoviocyte function and the effects of total glucosides of paeony

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We investigated the expression and cellular distribution of G protein-coupled receptor kinases 2(GRK2) in synovial tissue from rat with collagen-induced arthritis(CIA), and analyzed the modulation of GRK2 on synoviocytes and the

effects of total glucosides of paeony(TGP). Western blot results indicated that GRK2 expression in synovial tissue of CIA rats significantly increased at disease onset(d14) and the peak(d26), and returned to normal level on d46. Immunohistochemistry of joint sections revealed that GRK2 was expressed in synovial cells, superficial chondrocyte, and endothelial cells of blood vessels. GRK2 level in CIA synoviocytes was increased significantly, companying with the elevation of proliferation. Anti-GRK2 mAb induced a decrease in GRK2 level and a further increase of proliferation in CIA synoviocytes. TGP(12.5ug/ml) could increase GRK2 expression, and inhibit the proliferation of CIA synoviocytes induced by anti-GRK2 mAb. We demonstrated,for the first time, that GRK2 was expressed in synovial tissues and could modulate synoviocytes function. The therapeutic effects of TGP could be associated with its ability to ameliorate the hyperfunction of synoviocytes via improving GRK2 level.

P260021

Suppression of (5R)-5-hydroxytriptdide (LLDT-8) on Allograft Rejection in Full MHC-Mismatched Mouse Cardiac Transplantation

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(5R)-5-hydroxytriptolide (LLDT-8) is a new compound derived from triptolide, which is the major immunosuppressive fraction of Tripterygium wilfordii Hook. F. Here we tested LLDT-8 in major histocompatibility complex (MHC)-mismatched cardiac transplantation and investigated the underlying mechanisms. LLDT-8 administered orally induced the survival prolongation of allogeneic cardiac graft.

Histological results showed that LLDT-8 well preserved myocardium and significantly reduced infiltration of the graft with inflammatory cells. LLDT-8 decreased IL-2 production in recipient splenocytes stimulated by concanavalin A (ConA) ex vivo. LLDT-8 significantly inhibited the immunoreactivity of recipient to specific donor alloantigens, but preserved immunity to third-party alloantigens and mitogen. While the flow cytometry analysis showed LDT had a normalizing effect on the splenic lymphocytes population (CD4+, CD8+ T cell). LLDT-8 decreased CCR5 and their ligands MIP-1α and MIP-1β mRNA expressions in allografts. The results outline the great potential of LLDT-8 as a therapeutic tool in transplant rejection.

Key words: LLDT-8; Transplantation; Chemokine; Immunosuppression
Acknowledgment: Grant: No. KSCX2-SW-202

P260022

(5R)-5-Hydroxytriptolide Inhibits iNOS Expression in IFN-gamma- and LPS-Stimulated Macrophages

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(5R)-5-hydroxytriptolide (LLDT-8) is a novel analog of triptolide that has antiarthritic effect. Here, we investigated the effect of LLDT-8 on nitric oxide (NO) production and iNOS expression in macrophage.

Peritoneal macrophages and macrophage cell line Raw264.7 cells were stimulated with IFN-gamma or LPS followed by analysis with Griess method, flow cytometry, RT-PCR, Westernblot, EMSA. LLDT-8 significantly reduced NO generation by inhibiting iNOS expression at mRNA and protein level, rather than by interfering its enzymatic activity. In IFN-gamma-stimulated cells, LLDT-8 suppressed the transcription of STAT1alpha and IRF-1 but displayed no effect on IFN-gamma receptor level. After LPS challenge,LLDT-8 abrogated the expression of LPS receptor complex, including CD14, TLR4 and MD-2; decreased the phosphorylation of SAPK/JNK, Erk1/2 and p38 MAP kinase; retarded the degradation of IkappaBalpha; and ameliorated the DNA binding activity of NF-kappaB. These results suggest that LLDT-8 reduces NO production and iNOS expression by inhibiting IFN-gamma-triggered IRF-1 expression and LPS-triggered MAPK phosphorylation and NFkappaB activation.

Key words: iNOS; IFN-gamma; LPS
Grant: No. KSCX2-SW-202

P260023

Inhibition of S-Adenosyl-L-Homocysteine Hydrolase by DZ2002 Induces Immunosuppression in vitro and invivo

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AIM: A potent reversible type III inhibitor of S-adenosyl-L-homocysteine hydrolase (SAHH), methyl 4-(adenin-9-yl)-2-hydroxybutanoate (DZ2002) was determined its immunologic effects. METHODS: In vitro, the immunosuppressive effect of DZ2002 on T cell and macrophage were examined. Invivo, DZ2002 was evaluated for its immunosuppressive efficacy in delayed type hypersensitivity reaction (DTH), ovalbumin (OVA) immunized mice. RESULTS: DZ2002 reduced both a mixed lymphocyte reaction and IL-12 production from in vitro stimulated splenocytes. In addition, levels of CD80 and CD86 on human monocytic THP-1 cells were decreased in the presence of 0.1-10 mM DZ2002 and, decreases were also seen in IL-12 and TNF-a production from both thioglycollate-stimulated peritoneal macrophages and THP-1 cells. In vivo, DZ2002 suppressed DTH, OVA specific lymphocyte proliferation and anti-OVA IgG production. IL-2 and IFN-g productions as well as anti-OVA IgG2a and IgG3 were markedly decreased in mice treated with DZ2002. Conclusion: DZ2002's immunosuppressive effects are likely attributed to not only T cell inhibition, but also the obstruction of macrophage.

Key words: DZ2002; OVA
Acknowledgement: Grant: KSCX2-SW-202

P260024

Anti-inflammatory effect and mechanism of osthol in rats

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Aim: To investigate the anti-inflammatory effect and mechanism of osthole (Ost) Methods: Carrageenan-induced hind paw edema in rats were prepared. The nitric oxide synthase (NOS) activity was measured by NADPH-diaphoras stain assay, nitric oxide (NO) content by Griess diazotization assay, malondialdehyde (MDA) content by Thibabituric acid mehtods. And PG content assayed by Uv-vis spectrophotometry with 278nm after 0.5 mol·L^{-1} KOH methanol reagent dissimilating. catalyzing the isomerization at 50 °C. Results: The increase in NO2- observed 4h after carrageenan administration was inhibited by Ost in a dose-dependent manner. In the presence of Ost 100 mg/kg, NOS activities remained at near blank control levels. Mean while results showed reduced MDA production in the presence of Ost,. Ost markedly suppressed the generation of PG in inflamed paws. Conclusion: The effects of Ost antiinflammatory activities may be associated to a suppression of content of PG,, NO, MDA, and cNOS activity by inhibition of calcium entry and elevating cGMP levels way.

Key words: osthole; MDA; NOS; NO
Acknowledgment: This study was supported by the Natural Science Foundation of Jiangxi Province No. 95502

P260025

Beta2-agonists and glucocorticoids repress eotaxin gene transcription in human airway smooth muscle cells: selective inhibition of histone H4 acetylation

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Eotaxin is an eosinophil chemoattractant implicated in asthmatic airway inflammation. We have shown before that beta2-agonists and glucocorticoids (GCs) additively inhibit its production in human airways mooth muscle cells, but the mechanisms are unclear. In this study we showed that eotaxin gene transcription by TNF was mediated mainly by the transcription factor NF-kappaB (p65/p50) as analyzed by reporter gene assay and electrophoretic mobility shift assay. Chromatin immunoprecipitation assay demonstrated that TNF also induced histone H4 acetylation on lysines 5 and 12 and p65 binding to the eotaxin promoter, resulting in gene transcription. Beta2-agonists and GCs inhibited eotaxin gene transcription not by altering NF-kappaB nuclear translocation or promoter binding capability, but by selectively inhibiting histone H4 acetylation and p65 in vivo promoter binding. Additive inhibition was achieved when the drugs were combined. Our findings reveal a novel mechanism by which beta2-agonists, like GCs, regulate NF-kappaB-mediated inflammatory gene expression through inhibition of histone acetylation, and provide an explanation for the benefits that result when these agents are combined to treat asthma.

P260026

Sulfated Polymannuroguluronate, a Novel Anti-AIDS Drug Candidate, Inhibits T Cell Apoptosis by Combating Oxidative Damage of Mitochondria

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Sulfated polymannuroguluronate (SPMG) has entered the Phase II clinical trial as the first anti-AIDS drug candidate in China. Hereinn, SPMG was effective at protecting T lymphocytes against apoptosis. Further studies indicated that SPMG significantly elevated mitochondrial membrane potential of T cells, inhibited mitochondrial release of cytochrome c, enhanced the activities of mitochondrial enzyme complex I, III and V, and subsequently increased ATP level and ATP/ADP ratio. In addition, SPMG potently suppressed reactive oxygen species (ROS) generation in mitochondria and scavenged free radicals. The molecular mechanism underlying the ATP-involved and ROS-dependent anti-apoptosis of SPMG is characterized to be due to its engagement with mitochondrial import receptor and ADP/ATP carrier in T-cell mitochondrial membrane. All these might shed new light on the understanding of anti-AIDS functions of SPMG by protecting T cells of persons infected with HIV.

Abbreviations: SPMG, sulfated polymannuroguluronate; MMP, mitochondrial membrane potential; FITC, fluorescein-5-isothiocyanate; PMSF, phenyl methyl sulphonyl fluoride; ESI-MS, electrospray ionization-mass spectrum; ROS, reactive oxygen species.

P260027

The selective inhibition of Th1-related immune response by obaculactone

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The present study aims at elucidating a new mechanism underlying the immunosuppressive properties of the small compound obaculactone via influencing Th1 cells and its specific transcription factor, T-bet in comparison with cyclosporin A. As the result, obaculactone significantly inhibited the ConA-induced liver damage with a notable reduction in aminotransferases and a marked improvement of histological damage. Furthermore, this compound notably reduced IFN-γ, TNF-α, IL-2 and T-bet. At the same time, cyclosporin A also strongly inhibited the liver damage and the inflammatory process. However, it has been shown that the expression of only Th1-related molecules such as T-bet and IFN-γ, but not Th2-related molecules such as GATA-3 and IL-4 was inhibited by obaculactone in polarized Th1 or Th2 cells. On the other hand, cyclosporin A potently inhibited both Th1 and Th2 cytokines. In summary, obaculactone, which is different from immunosuppressants so far as cyclosporin A, has been found to show a selective effect on Th1 cells.

Key words: obaculactone, Th1 cells, inflammatory liver injury

Acknowledgement: This study was supported by National Natural Science Foundation of China (No. 30230390 and 30500617).

P260028

Selective inhibition of T cell-mediated immune response by natural products

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Most immunosuppressants have toxic effects due to non-specific efficacy. Our study is focused on the selective regulation on T cell immunity by natural products.

For T cell activation, extracts from Si-Ni-San and Artemisia vestita, were found dominantly to inhibit the induction phase of DTH and improve related diseases such as contact sensitivity. In addition, fusaruside, acerebroside, showed a novel immunosuppression with a selective regulation on STAT1 signaling pathway in Tcell activation.

For activated T cell function, we found that astilbin, a flavanone, improved various DTH-related diseases. The effect was confirmed mainly to be a selective targeting to activated T lymphocytes with an induction of apoptosis through mitochondria pathway. In addition, some other compounds, such as obaculactone, also showed aselective inhibition on activated T lymphocytes.

In summary, we have found that some natural products may selectively regulate the T cell immunity. Such selective regulation targeting to special disease stage or cell population may pave a new approach to immunosuppressive therapy.

Key words: T cells, immunosuppression, natural products.

Supported by NSFC (No. 30230390, 30472174).

P260029

Cydosporin A, an immunosuppressive drug, significantly inhibits the expression of B and T lymphocyte attenuator in T cells

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OBJECTIVE: To examine the effects of cyclosporin A (CsA) on the expression of B and T lymphocyte attenuator (BTLA)-a recently identified immune inhibitory receptor. METHODS: Spleen cells were isolated from C57 mice and stimulated with concanavalin A (ConA) in the presence or absence of CsA for 24 hours.

Cells were immunostained and analyzed by flow cytometry for surface expression of BTLA and CD25. T cells proliferation was measured using [3H]-Thymidine incorporation assay. RESULTS: CsA significantly reduced ConA-induced BTLA protein expression in CD4+ and CD8+ T cells. This suppression was not dependent on the inhibitory effect of CsA on T cell proliferation, because low dose of CsA had no effect on T cell proliferation but can reduce the expression of BTLA proteins. CD25 which was under the control of NF-Kappa B only showed modest reduction in the presence of CsA. CONCLUSIONS: Our data suggested that calcineurin/NFAT-dependent pathway may play an important regulatory role on BTLA production.

Key words: BTLA; CsA; calcium signal.

Acknowledgements: This work was supported by grants from C03020504, 2003-85, 2005-546 and 973 Program, 2003CB515501 (Y.Z.).

P260030

Effect of montelukast and heparin on acute phase symptoms of antigen-induced rhinitis in guinea pigs

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The aim of the study was to test the effect of heparin (HP) and a specific cysteinyl leukotriene LTD4 receptor antagonist, montelukast (MT) on the sneezing, nose rubbing, nasal airway resistance (NAR) and cellular infiltration (CI) induced by nasal antigen challenge in sensitized guinea pigs. Male Dunkin Hartley guinea pigs were sensitized to, and challenged with, ovalbumin. Initially sneezing and nose rubbing were evaluated. Three days later animals were anaesthetized with pentobarbital, and the trachea was cannulated caudally toward the nasal cavity for measurement of NAR using a ventilator/flow method. Drugs were administered iv prior to ovalbumin challenge. NAR was measured for 30 minutes post challenge while CI was assessed from nasal lavage solutions collected 60 minutes post challenge. Both MT and HP significantly inhibited NAR and CI. However, they failed to inhibit sneezing and nose rubbing. In conclusion, MT (presumably by antagonism of LTD4 receptors) inhibits NAR and CI. In addition, HP inhibits CI and this may account for its role in inhibition of NAR.

Key words: montelukast, sensitization, heparin, ovalbumin.

Funded by Rhinopharma Ltd, Canada.

P260031

Involvement of nitric oxide in nasal congestion during the acute phase of antigen-induced rhinitis in guinea pigs

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The main aim of the study was to examine the role of nitric oxide (NO) in nasal airway resistance (NAR) and cellular infiltration (CI) during the acute phase of allergic rhinitis. The effect of dexamethasone was also studied.

Male Dunkin Hartley guinea pigs were sensitized to, and challenged with, ovalbumin. Animals were anaesthetized by ip pentobarbital and the trachea cannulated caudally, toward the nasal cavity, for measurement of NAR using a ventilator/flow method. Drugs were administered intravenously prior to ovalbumin chal-

lenge.

NAR was measured for 30 minutes post challenge while CI was assessed from nasal lavage solutions collected 60 minutes post challenge. NG-nitro-L-arginine-methyl ester (L-NAME), for non-selective nitric oxide inhibition, significantly inhibited NAR whereas dexamethasone did not. Additionally, both drugs failed to inhibit CI. In conclusion, nitric oxide is involved in nasal congestion in the acute phase of allergic rhinitis presumably throughits potent vasodilatory effects.

Key words: Nitric oxide, sensitization, L-NAME, ovalbumin.

Funded by Rhinopharma Ltd, Canada.

P260032

Ethanol extract from Artemisia vestita, a traditional Tibetan medicine, exerts anti-sepsis action through downregulating MAPKs and NF-kappaB pathways

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Artemisia vestita Wall. (AV), a traditional Tibetan medcine, has wide clinical applications on inflammatory diseases. However, its molecular mechanism of anti-inflammatory effect is little known. In this study, we examined the anti-sepsis action and mechanism of the ethanol extract from AV (AV-ext). AV-ext significantly improved the survival of mice with sepsis and remarkably reduced the expressions of TNF-alpha, interleukin-1beta and cyclooxygenase-2 in vivo and in vitro. Moreover, AV-ext dose-dependently suppressed the activation of mitogen-activated protein kinases (MAPKs) such as p38, extracellular signal-regulated kinase and c-Jun NH2-terminal kinase in endotoxin-evoked RAW 264.7. Furthermore, AV-ext inhibited the activation of nuclear factor-kappaB (NF-kappaB), as well as the degradation and phosphorylation of inhibitory kappaB. Taken together, these results reveal that AV-ext inhibits TNF-alpha release from macrophages by suppressing MAPKs and NF-kappaB pathways and suggest that AV-ext may be beneficial for the treatment of endotoxin shock or sepsis.

Key words: sepsis, Artemisia vestita, MAPKs, NF-kappaB

This work was supported by National Natural Science Foundation of China (No. 30230390).

P260033

Inhibition of Arginase I Activity by RNA Interference Attenuates Interleukin-13 Induced Airways Hyperresponsiveness

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Study Objective: Arginase I is activated in hepatic helminths infection, moreover it is also employed by Th2 cells & STAT6 pathway and is associated with allergic disorders. How arginase I contributes to, and is regulated by, allergic inflammation remains unknown.

Methods: We employed a murine model of ovalbumin (OVA) induced airways disease or instillation of IL-13 into mouse lung to investigate the correlation between expression of arginase I and development of AHR. We also determined the role of arginase I by inducing loss of function specifically in the lung by employing RNA interference.

Results: OVA induced AHR correlated directly with increased expression of arginase I. Expression of arginase I, but not eosinophilia or mucus-secretion, temporally correlated with the development, persistence and resolution of IL-13 induced AHR. Moreover, inducing loss of function of arginase abrogated the development of IL-13-induced AHR.

Conclusion: Our data suggest an important role for arginase I in the modulation of IL-13 induced AHR, and identify a potential pathway distal to cytokine receptor interactions for the control of IL-13 mediated bronchoconstriction in asthma.

P260034

Effect of aspirin, paracetamol and their nitric oxide donating derivatives on exudates cytokine and PGE_2 production in zymosan-induced air pouch inflammation in rats

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Effects of different doses of aspirin, compared with equimolar doses of nitric oxide (NO)-donating aspirin (NCX4016), and of a single dose of paracetamol and compared with an equimolar dose of NO-donating paracetamol (NCX 701) were investigated in acute zymosan-induced air pouch inflammation in rats. Aspirin, at 10, 30 and 100 mg/kg, increased IL-1b levels in exudates reaching significant difference vs. the control group at the maximal dose only, however, a significant increase has been seen in TNF-a level for all of the doses tested. NCX4016 did not cause changes in both exudate IL-1b and TNF-a levels. Although paracetamol increased significantly exudates TNF-a level compared to the control group and NCX701 group, both treatments did not change significantly the levels of exudates IL-1b.

The results of this study indicate that, although both drugs inhibited the synthesis of PGE_2 in a similar way, aspirin and NCX4016 possess different effects on cytokine production or release.

Key words: Inflammation, Cytokines, Aspirin, NO-aspirin

Acknowledgements: This study was supported by a grant from TUBITAK (SBAG-2671).

P260035

Therapeutic Efficacy of Pioglitazone in Acute Gouty Arthritis Rats

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Thiazolidinedione (TZD), drugs in the clinical therapy of type 2 diabetes mellitus, were proven to exert antiinflammation effects in rheumatoid arthritis and other chronic inflammation, but their effects on acute gouty arthritis have not been reported so far. In this experiment, a monosodium urate (MSU)-induced rat model of acute gouty arthritis (GA) was used to investigate the anti-arthritis effects of pioglitazon, an agonist of peroxisome proliferators-activated receptor (PPAR) gamma. The clinical symptom of GA rats was inspected and the mRNA expression of PPAR gamma and related inflammatory factors in arthritic rat synovium was dynamic detected by RT-PCR. It was showed that the therapeutic effects of pioglitazone were not obvious at 24 hrs, but significant at 48hrs after MSU injection in ameliorating the inflammation, swelling, disability and histopathologic changes and regulating the mRNA expression of PPAR gamma and some inflammatory factors of rats. Our results firstly proved that pioglitazone has its anti-inflammatory properties in acute gouty arthritis.

Key words: pioglitazone; gouy arthritis; PPAR gamma

Acknowledgment: Supported by a grant (YC0216) from AMMS

P260036

BURN-INDUCED HEPATIC AND RENAL INJURY IS PREVENTED BY ROSIGLITAZONE IN WISTAR ALBINO RATS

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In the present study we investigated the putative protective effect of rosiglitazone, a PPARgamma agonist, against hepatic and renal injury induced by thermal trauma. Under anesthesia, rats were exposed to a 90 °C bath for 10 s to induce thermal trauma. Rosiglitazone (4 mg/kg ip) or saline was administered immediately after and at the 12th h of the injury. Rats were decapitated at 24th h and tissue samples were taken for determination of malondialdehyde (MDA), and GSH levels, and myeloperoxidase (MPO) activity. Serum AST, ALT levels, and creatinine, and BUN were determined. TNF-α, IL-1β and lactate dehidrogenase (LDH) were also assayed in serum samples. Skin scald injury caused significant decrease in tissue GSH, and significant increases in MDA levels and MPO activity. Serum ALT, AST, creatinine and BUN levels, as well as LDH, IL-1β and TNF-α, were elevated significantly in the burn group. Rosiglitazone treatment reversed the biochemical indices induced by thermal trauma, suggesting that it possesses an anti-inflammatory effect on burn-induced damage in remote or-

gans and protects against oxidative damage by a neutrophil - dependent mechanism.

Key words: Burn, rosiglitazone, myeloperoxidase, glutathione

P260037

CD63 and CD203c used as markers of in vitro basophil activation in patients with penicillins allergy

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The sensitivity and specificity of flow cytometric basophil activation test by detection of CD63 and CD203c expression was assessed, as well as its relationship with specific IgE and IgG in patients with penicillins allergy.

Fourty - three patients with clinical allergy to penicillins and 15 healthy control were included for examinating the expression of CD63 and CD203c by flow assay stimulation test (FAST). Radioallergosorbent test (RAST) and enzyme - linked immunosorbent assay (ELISA) were used for investigating the levels of specific IgE and IgG. Of the 43 patients, 28 (65.12%) were positive to FAST- CD63, 25 (58.14%) to RAST, 14 (32.56%) to ELISA and 39(90.70%) to either one or the others. Furthermore, the coincident rates of FAST- CD63 with allergic history, skin test, specific IgE and IgG were 65.12%, 44.19%, 55.81%, 46.15%, respectively. However, there was no marked expression of CD203c after contact with penidlins haptens. Flow cytometric quantitation of CD63, not CD203c, may be a useful approach to determine the allergic state in patients with penicillins allergy. If combined with RAST and ELISA, the sensitivity will be largely improved.

Key words: CD63, CD203c, basophil, penicillins allergy

P260038

Inhibitory effects of 2, 3, 5, 4'- tetrahydroxystilbene - 2 - O - beta - D - glucoside on experimental inflammation and cyclooxygenase 2 activity

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The effects of 2, 3, 5, 4'- tetrahydroxystilbene - 2 - O - beta - D - glucoside (THSG), extracted from the roots of Polygonum multiflorum Thunb, on experimental inflammation and cyclooxygenase - 2 (COX- 2) activity were investigated. The carrageenin (CGN) - induced rat paw edema model and dimethylbenzene - induced mouse ear edema model were prepared; MTT assay, semi - quantitative RT - PCR, Western blot, ELISA were adopted in lipopolysaccharide (LPS) - induced mouse RAW264.7 macrophage cells. THSG 23, 46 and 92 mg $\cdot kg^{-1}$ per oral (po) could dose - dependently inhibit mouse ear edema with the inhibitory rate of 87% at 92 mg $\cdot kg^{-1}$. THSG 32, 64 and 128 mg $\cdot kg^{-1}$, po, also dose - dependently inhibited rat paw edema with the inhibitory rate of 56% at 128 mg $\cdot kg^{-1}$, at 6 hour. Indometacin (Indo) 13 mg $\cdot kg^{-1}$, po, showed 90% inhibition in the former model and 9 mg $\cdot kg^{-1}$, po, did 57% inhibition in latter model. In RAW264.7 cells, LPS 1 $\mu g \cdot ml^{-1}$ significantly up - regulated prostaglandin E_2 (PGE_2) production, (generated from exogenous arachidonic acid (AA) through the catalyze of inducing COX- 2) by 35%, which could be antagonized by THSG 1, 10, 100 $\mu mol\ l^{-1}$, in concentration - dependent matter and the percentage of inhibition of THSG 10 $\mu mol\ l^{-1}$ was 40%. NS - 398 10 $\mu mol\ l^{-1}$ decreased PGE_2 production by 42%. Moreover, THSG 1, 10, 100 $\mu mol\ l^{-1}$ markedly inhibited the LPS - induced expression of COX - 2 protein and mRNA ($P > 0.05$), but did not obviously influence COX - 1 protein expression and mRNA ($P > 0.05$).

P260039

Effect of angelica A3 active component on isolated rat uteruses cyclooxygenase - 2 expression

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Objective To study the effect of Angelica A3 active component (A3) on lipopolysaccharides (LPS) induced rat uteruses cyclooxygenase - 2 (Cox - 2) gene expression up - regulation. Methods RT- PCR and Western blot were used to analyze the uteruses cyclooxygenase - 2 mRNA and protein expression level. Results LPS 1 µg/mL could significantly increase the level of Cox - 2 mRNA and protein expression from normal control group 0.159 ±0.021 and 122.2 ±19.7 to 0.381 ±0.141 and 183.6 ±16.7 (n = 8) respectively. A3 10, 20, 40, 80, 160, 320 mg/L could concentration - dependently inhibit increased Cox - 2 mRNA and protein expression stimulated by LPS respectively from tween - 80 control group 0.462 ±0.164 and 187.8 ±13.5 to 0.408 ±0.136 and 162.6 ±16.3, 0.368 ±0.126 and 155.0 ±17.0, 0.306 ±0.065 and 148.4 ±14.3, 0.250 ±0.084 and 133.6 ±13.3, 0.138 ±0.016 and 125 ±15.4, 0.008 ±0.003 and 119.4 ±14.4 (n = 8). Conclusion The mechanism of the effects of A3 on antiinflammatory, analgesic and anti - dysmenorrhea may be related with the inhibition of the Cox - 2 gene expression.

Key words: Angelica, A3, cyclooxygenase - 2

P260040

Blockade of neurokinin - 1 receptor attenuates CC and CXC chemokine production in acute pancreatitis and associated lung injury

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The neuropeptide substance P (SP) and its receptor neurokinin - 1 receptor (NK - 1R) play a key role in the pathogenesis of acute pancreatitis (AP). The present study aimed to investigate the involvement of CC and CXC chemokines in SP - related pathogenesis of this condition. In a mouse model of caerulein - induced acute pancreatitis, a selective NK- 1R antagonist CP- 96,345 was used to block the interaction of SP and NK - 1R. The temporal and dose - related effects of caerulein hyperstimulation and CP- 96,345 treatment on various chemokine expressions were examined. The results showed that MCP - 1, MIP - 1α and MIP - 2 were early mediators upregulated in both the pancreas and lungs after AP induction, whereas RANTES was a later mediator induced only in the pancreas. Treatment of CP- 96,345 significantly suppressed caerulein - induced increase in chemokine mRNA and protein levels. Additionally, in the pancreas chemokines were immunohistochemically localized to acinar cells and the infiltrating leukocytes, while in the lungs they were expressed by alveolar macrophages, epithelial and endothelial cells. We thus identified chemokines as important mediators in SP - related pathway in the pathogenesis of AP.

P260041

AUROTHIOMALATE INHIBITS COX - 2 EXPRESSION IN ACTIVATED CHONDROCYTES AND IN OSTEOARTHRITIC CARTILAGE.

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Aurothiomalate is used in the treatment of arthritis. Cyclooxygenase - 2 (COX - 2) is expressed in osteoarthritic cartilage and produces proinflammatory prostanoids in the joint. In the present study, we investigated the effects of aurothiomalate on interleukin - 1β (IL - 1β) - induced COX - 2 expression and prostaglandin E_2 (PGE_2) production in immortalized murine H4 chondrocytes and in human osteoarthritic (OA) cartilage. Aurothiomalate inhibited IL - 1β- induced COX - 2 expression at mRNA and protein level, and subsequent PGE_2 production in chondrocytes in a dose - dependent manner. In the further mechanistic studies, the effect of aurothiomalate on the degradation of COX - 2 mRNA was tested by actinomycin assay. The half - life of COX - 2 mRNA following IL - 1β treatment was 3 h and aurothiomalate reduced it to about 1.5 h. To study the clinical relevance of the finding we investigated the effects of aurothiomalate on COX- 2 expression in human OA cartilage. Aurothiomalate reduced COX - 2 expression in OA cartilage at drug concentrations which have been measured in synovial fluid following treatment with aurothiomalate. The results suggest an additional anti - inflammatory mechanism for aurothiomalate.

Key words: COX - 2, chondrocyte, aurothiomalate

P260042

Mast cell mediated histamine release and pro - inflammatory cytokine production are attenuated by gallic acid

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The discovery of drugs for the treatment of inflammatory allergic diseases such as, asthma and allergic rhinitis is an important subject in human health. Gallic acid is a polyphenyl natural product from gallnut and green tea.

The aim of the present study was to elucidate whether gallic acid modulates the inflammatory allergic reaction and to study its possible mechanisms of action. Gallic acid attenuated IgE-induced histamine release from mast cells by the modulation of cAMP and calcium. Gallic acid decreased the phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated pro-inflammatory cytokine gene expression in human mast cells. The inhibitory effect of gallic acid on the pro-inflammatory cytokine was nuclear factor-κB and p38 mitogen activated protein kinase dependent. In addition, gallic acid inhibited compound 48/80 or IgE-induced systemic allergic reaction. Our findings provide evidence that inhibitory effect of gallic acid on the mast cell-derived inflammatory allergic reactions, and suggest the mechanisms of action. Furthermore, in vivo and in vitro antiallergic effect of gallic acid suggests a possible therapeutic application of this agent in inflammatory allergic diseases.

P260043

Effects of Jingjie Volatile Oil on carrageenan-induced acute inflammation in the rats

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Previous studies have demonstrated that the volatile oil of Jingjie (Sto), major effective components of Chinese herb Schizonepeta tenuifolia Briq. (Jingjie), is able to inhibit different acute inflammation induced by carrageenan, fresh egg white, xylene or acetic acid in mice or rats. This study investigated the anti-inflammatory potential of Sto treatment in one model of acute inflammation (carrageenan-induced air vesicle synovitis) where eicosanoids, proinflammatory cytokine and oxyradical play a crucial role in the inflammation processes. Sto (0.2, 0.1 ml/kg) attenuated the inflammation parameters: total leukocytes, the number of polymorphonuclear and the protein concentration in the exudate, as well as significantly reduced the activity and levels of phospholipase2, malondialdehyde (MDA), prostaglandins (PGE) and TNF in the exudate of air vesicle synovitis model. Sto were also able to inhibit IL-1 release in abdominal macrophage and regulate IL-2 release in spleen cells in model rats. The results suggest that Sto exerts potent anti-inflammation effects that could be, in part, related to suppress arachidonic acid metabolism, antioxidation, and regulatory action on the release of proinflammatory cytokines.

Key words: Jingjie Volatile Oil (Sto); arachidonic acid metabolism; MDA; cytokines

Acknowledgements

Research supported by grants from technology project of the Department of Education of Sichuan province (2000yy)

P260044

Effect of treatment with an neurokinin-1 receptor antagonist on the expression of adhesion molecules in acute pancreatitis

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The effect of treatment with CP96,345, a neurokinin-1 receptor antagonist, on the regulation of ICAM-1, VCAM-1, E- and P-selectin expression during acute pancreatitis (AP) was studied. AP was induced in male balb/C mice by 10 consecutive hourly intraperitoneal (i.p.) injections of caerulein. In the treatment groups, CP96,345 was administered at 2.5 mg/kg i.p. either 30 min before or 1 hour after the first caerulein injection. The animals were sacrificed and the lungs and pancreas were isolated for RNA extraction and RT-PCR, or immunohistochemical (IHC) staining. The mRNA expression of the four adhesion molecules was upregulated in the pancreas during AP. Treatment with CP96,345 effectively reduced the expression of E- and P-selectin, but not ICAM-1 and VCAM-1. In the lungs, ICAM-1, E- and P-selectin mRNA expression increased during AP, which could be suppressed by the antagonist. Pulmonary VCAM-1 expression was not affected during AP. Similar expression pattern was seen in the IHC stainings. These results provide important information of the regulation of adhesion molecule expression during AP.

P260045

A new model of FCA induced monoarthritic pain for pharmacological studies in rabbits

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The present study aimed at adapting the Freund's complete adjuvant (FCA) induced monoarthritic model for rabbits. Male rabbits were injected with FCA into the right wrist joint. The incapacitance in forelimbs was measured using an "Incapacitance tester" originally aimed for rats. The pain after injection of FCA showed two phases: first peaking with 60% incapacitance at 6-24 hours and gradually decreasing to 17% by Day 5. This was followed by a second increase with a sustained 40-50% incapacitance from Days 8-21. Indomethacin (p.o.) and Valdecoxib (p.o.) dose-dependently reversed the incapacitance both in the acute (24 h) phase with ID50 values of < 0.3 mg/kg and in the chronic phase (14 days) with ID50 values of 7.4 and 1.8 mg/kg, respectively.

Whereas the COX inhibitors completely reversed the acute phase, they provided only partial relief in the chronic phase. In contrast, the B1 receptor antagonist Lys-DALBK (100 nmol/kg, i.v.) produced complete reversal in the chronic but partial reversal in the acute phase. In conclusion, (1) the test proved to be suitable for pharmacological studies in rabbits; (2) B1 antagonists may be superior over COX inhibitors in the treatment of chronic pain conditions.

P260047

Anti-oxidative activity of Paeonol contributes to amelioration of atherosclerosis in cholesterol-fed rabbits

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Paeonol is a compound isolated from the root cortex of Paeonia suffruticosa Andrews, that has been used in oriental medicine as both an analgesic and anti-inflammatory agent. In this study, we examined the anti-atherogenic potential of Paeonol using cholesterol-fed rabbits. The rabbits were divided into a normal group ($n = 6$), and cholesterol-fed group (1% cholesterol-diet for 12 weeks, $n = 18$). After then, the cholesterol-fed rabbits were divided into two groups treated with Paeonol (75 mg/kg, 150 mg/kg per day, $n = 6$) and a vehicle group ($n = 6$) for 6 weeks. After 6 weeks' treatment, the atherosclerotic lesions were significantly reduced in the Paeonol group. Paeonol increased the SOD activity and reduced the content of MDA in serum and aortic tissue, and suppressed the over-expression of NF-κB in aortic wall of cholesterol-fed rabbits. In vitro study, Paeonol also significantly inhibited the copper ion-mediated oxidation of LDL. The results of present study suggest that the anti-atherogenic effect of Paeonol is probably in close relation to its anti-oxidative property in addition to its plasma lipid lowering activity resulting in an amelioration of lesion development in cholesterol-fed rabbits.

Key words: Paeonol; atherosclerosis; anti-oxidative activity

Acknowledgement: This work was supported by grants from Excellent Youth of Anhui Province (No. 4043047).

P260048

Mechanism of action of Disintegrin, Rhodostomin, in suppression of endotoxin-induced activation of monocyte

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Endotoxin injection has been widely used to study the acute inflammatory response. In the present study, we found that rhodostomin, a snake venom disintegrin from Calloselasma rhodostoma, significantly decreased the production of tumor necrosis factor-α (TNF-α) in septic mice induced by lipopolysaccharide (LPS). To understand the mechanism of this obvious inhibition, we evaluated the in vitro effects of rhodostomin on LPS treated human monocyte cell line, THP-1. Flowcytometric analysis revealed that FITC-conjugated rhodostomin concentration-dependently bound to LPS-activated THP-1. In the presence of rhodostomin, both LPS-induced THP-1 adhesion to fibronectin-coated well, and migration through were inhibited. Moreover, we also found that rhodostomin blocked the expression of tissue factor on THP-1 cells stimulated by LPS. Taken together, these results suggest that rhodostomin interacted with monocytes to in-

terfere with the activation and function of monocytes trigged by LPS. Whether rhodostomin exhibits marked anti-sepsis activity is worth to be further investigated.

Key words: Sepsis, Monocyte, LPS, Disintegrin.

P260049

Exogenous catecholamines interaction with β-endorphin levels in patients with haemorrhagic shock

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Study objective: The aim of this study was to determine β-endorphin plasma levels in correlation with exogenous catecholamines infusion, in patients with haemorrhagic shock. Methods: 44 patients with acute haemorrhage were studied, divided into two groups: Group1 (n = 22) included patients who received exogenous catecholamines the first 24 hours after admission, and Group2 (n = 22) patients who didn't. 12 patients with minor trauma without haemorrhage served as controls. Blood samples were collected at 0, 2, 4 and 24 hours after admission and analyzed for β-endorphin with specific ELISA method. Results: Both groups had elevated β-endorphin levels at 0 and 2 hours after admission. No significant difference was observed between the groups, but Group 1 showed greater values of β-endorphin at 0 and 2 hours time points. The same group had lower systemic blood pressure and greater trauma severity. Conclusion: A significant elevation of β-endorphin levels was observed for both groups immediately after injury. Exogenous catecholamines can influence β-endorphin release.

Key words: β-endorphin, haemorrhage, catecholamines

P260050

Specific IgG and IgE antibodies in sera in patients with penicillins allergy

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To elucidate the relationship between IgG antibodies and penicillins allergy, between IgG and IgE antibodies in the allergic patients. Radioallergosorbent test and enzyme-linked immunosorbent assay were used to examine 8 kinds of specific IgE and IgG antibodies in the sera of 249 patients with penicillins allergy. Except BPA-IgG, 7 kinds of antigenic determinants IgG antibodies levels were significantly higher than that of control group ($P < 0.05$). The positive rate of IgG antibodies to major antigenic determinants (42.17%) was significantly higher than that of minor antigenic determinants (8.84%) ($P < 0.01$). The positive rate of IgG antibodies of patients with allergic history was significantly higher than that of patients with positive skin test ($P < 0.05$). Positive rates of specific IgG and IgE were 46.99% and 57.83%, while positive rates of detection of IgE and IgG simultaneity were 77.91%. Not only IgE but also IgG were involved in the development of penicillins allergy. IgG antibodies to major antigenic determinants probably play an important role in the process of penicillins allergic reaction.

Key words: penicillins, allergy, IgG, IgE

P260051

Therapeutic effects and mechanisms of total flavonoids of saciente on adjuvant arthritis in rats

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The study was to investigate the therapeutic effects and mechanisms of total flavonoids of saciente (TFS) on adjuvant arthritis in rats. The model of adjuvant arthritis (AA) was induced by injection of Freund's Complete Adjuvant (FCA). Secondary paw swelling of AA rats was measured with volume meter and polyarthritis index was scored. The splenocyte lymphocytes proliferation, IL-1 and IL-2 production were assayed by cell proliferation assay. Prostaglandin E_2 production was determined by radioimmunoassay. AA rats with treatment of TFS (80, 160, 320 mg·kg^{-1}, ig) significantly inhibited secondary inflammatory reaction (secondary swelling、multiple arthritis、pathologic change of ankle arthritis) in AA rats. The low response of splenocytes to ConA and LPS and the decreased IL-2 synthesis were reversed by TFS treatment (160, 320 mg·kg^{-1}, ig), while the elevated IL-1 and PGE_2 released from PM_Φ were also reduced. These results suggest that TFS has significant a therapeutic effect on AA rats, which may be related to its immunoregulatory actions.

Key words: saciente; inflammation; immunoregulation; experimental arthritis

P260052

Eukaryotic expression of hTM and Preparation of Its Monoclonal Antibody

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To prepare monoclonal antibody (McAb) specific to human thrombomodulin (hTM), The recombinant plasmid pThr402 was transfected into CHO cells by lipofectamine 2000 reagent. The CHO cells, expressing hTM on membranes, were obtained after selecting by G418. That was confirmed by flow cytometry and Western blot. Then the McAb anti-hTM was prepared with classical hybridoma technique, and 1 hybridoma cell lines (NH-1) was obtained. The Ig subclasses of the McAb was IgG1 and the titers of ascitic McAb was 1×10^{-6}. Flow cytometry, CELISA and Western blot assays demonstrated that NH-1 could specifically recognize hTM expressed on CHO-TM5 and HUVEC. Mean while, The better tissue specificity of antigen recognized by NH-1 was identified by immunochemical staining. hTM was expressed mainly on vascular endothelial cells, NH-1 can specifically recognize the natural hTM, which will be of greater value to us in our research on the functions and clinic values of hTM.

P260053

AUTOCRINE ACTIONS OF NADPH OXIDASE-DERIVED O2.- IN CORONARY ARTERIAL MYOCYTES

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The present study tested the hypothesis that extracellular O2.? derived from NADPH oxidase (NOX) serves as an autocrine to activate physiological response in coronary arterial myocytes (CAMs). By simultaneously monitoring intracellular ($[O_2.^-]_i$) and extracellular $O_2.^-$ ($[O_2.^-]_O$ levels, M1-receptor agonist oxotremorine (OXO) was found to stimulate a more rapid increase in $[O_2.^-]_O$ than $[O_2.^-]_i$ in CAMs. Addition of SOD outside cells blocked OXO-induced increases in both $[O_2.^-]_i$ and $[O_2.^-]_O$. Silencing NOX subunit, Nox 1 by siRNA blocked increases in both $[O_2.^-]_i$ and $[O_2.^-]_O$, but silencing Nox 4 only attenuated increase in $[O_2.^-]_i$. By ESR spectrometry, OXO was found to increase $[O_2.^-]_O$ by 35%, which was blocked by Nox 1 siRNA (by 74%). This M1-activation of NOX stimulated SOD-blockable Ca^{2+} release in CAMs ($\triangle[Ca^{2+}]_i$ = 821 ± 67 nM). NOX was also shown to be activated by NO donor, spermine NONOate (by 67%). These results suggest that NOX-derived $[O_2.^-]_O$ exerts an autocrine action to stimulate intracellular Ca^{2+} release and that NOX can be activated by NO, which may counteract the action of excessive NO around CAMs.

Key Words: NADPH oxidase, autocrine, redox signaling, coronary artery
(Supported by NIH grants HL057244-10 and HL075316-01).

P260054

BLT1 and BLT2 Both Mediate Leukotrine B4-Induced Effects in vitro

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Leukotriene B4 (LTB4) is a potent chemoattractant and considered to be an inflammatory mediator. Two G protein-coupled receptors for LTB4, namely BLT1 and BLT2, have been cloned and shown to be high- and low affinity LTB4 receptors, respectively. In the experiment, we investigate whether BLT1 or

BLT2 or both mediate the effects of LTB4 on the proliferation of HIB64, a rat synovial cell line, the production of IFN-γ by rat CD4+ lymphocytes and the chemotaxis to rat leukocytes. It was shown that LTB4 accelerated the incorporation of [3H] thymidine in HIB64 cells, IFN-γ production by CD4+ lymphocytes and exerted chemotactic activity to leukocytes, however, these effects could be inhibited by U-75302 or LY255283, antagonists for BLT1 or BLT2. These findings suggested that both of BLT1 and BLT2 can mediate the roles of LTB4 in vitro.

P260055

Anti-inflammatory activity of S-diclofenac, a novel H_2S-releasing diclofenac derivative

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The objective of this study was to evaluate the potential anti-inflammatory effect of S-diclofenac (2-[(2,6-dichlorophenyl) amino] benzeneacetic acid 4-(3H-1,2,dithicle-3-thione-5-yl)-phenyl ester). H_2S is slowly released from S-diclofenac (100 uM) on exposure to rat plasma or liver homogenate in vitro. S-diclofenac pretreatment (47.2 umol/kg, i.p.) of rats inhibited lipopolysaccharide (LPS, 10 mg/kg)-induced inflammation asassessed by reduced tissue myeloperoxidase (MPO) activity. The enhanced (c.f. diclofenac) anti-inflammatory effect of S-diclofenac was associated with downregulation of enzymes which synthesise nitric oxide, prostanoids and H_2S plus reduced plasma IL-1β, TNF-a and elevated plasma IL-10 concentrations. Reduced NF-kB p65, cfos and enhanced SP-1 DNA-binding activity were observed in livers from S-diclofenac-pretreated, LPS-injected animals. We propose that H_2S release in vivo inhibits a number of molecular targets resulting in augmented anti-inflammatory activity. We further suggest that a strategy centering on slow-releasing H_2S compounds may prove of value in the treatment of inflammation.

P260056

Analysis of spinal COX-1 and COX-2 mRNA expression in the model of monoiodoacetate-induced osteoarthritis

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Objective: To establish the role of spinal cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) in the model of experimental induced osteoarthritis. Methods: Wistar rats received an intrarticular injection of either monoiodoacetate or saline into right knee joint. Animals were killed 1, 5, 14 and 31 days after monoiodoacetate injection The levels of spinal COX-1 and COX-2 mRNA were analysed by real-time PCR. The production of COX-2 protein was also tested by ELISA. Results: The first day after monoiodoacetate injection, real-time PCR revealed balanced, but significantly raised levels of COX-1 and COX-2 mRNA with respect to control. At day 5 and 14, levels of COX-2 mRNA were significantly increased in comparison to levels of COX-1 mRNA and to control. At day 31, the expression of both genes was almost equable, but still significantly increased in comparison to control. Conclusions: The present findings indicate that expression of spinal COX-2 mRNA is in the model of experimental induced osteoarthritis dominant, but the role of spinal COX-1 mRNA is upregulated with the time.

Key words: osteoarthiris, cyclooxygenase, spinal cord, real-time PCR.
Acknowledgements: VZ MSM0021620816 and GAUK 74/2005/C/3.LF.

P260057

Immunologic specificities of the dendritic cells-based immunotherapy for cancer patients

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Dendritic Cells(DCs) may be suited for immunotherapy for its capability to stimulate naive T cell. DCs were established from the peripheral blood leukocytes of cancer patients by culturing in the presence of Flt-3 ligand, GM-CSF, IL-4, and TNF-αfor 14 days. At day 15, DCs were incubated with autologous T cells and lysate of the cancer tissues. At day 18, intact cancer tissues were incubated with autologous activated T cells for 4 days and examined the morphology of the cancer tissue and T cells by scanning electrone microscopes. The differentiated cells showed typical morphology of DCs including multiple processes and profuse cytoplasm. The cells stained positively with CD1a, CD83 and CD86. Activated cytotoxic T lymphocytes and veiled cells adhered and destroyed the cancer tissues. However, normal tissues were not attacked by T cells. This study indcated that DCs with enhanced antigen presenting activity can be generated from leukocytes, and that they may be used as potential vaccines in the immunotherapy or strategy for minimal residual disease of cancer.

Key Words: Dendritic cells, Cancer, Cytotoxic T lymphocytes, Immunotherapy.

P260058

Anti-Inflammatory Effects of Glycogen Synthase Kinase-3β Inhibitor in a Mouse Asthma Model

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Glycogen synthase kinase 3β (GSK-3β) is known to regulate various cellular functions including inflammatory responses. We hypothesized that inhibition of GSK-3β may have anti-inflammatory effects in a mouse asthma model. BALB/c mice were sensitized with ovalbumin (OVA) and challenged with aerosolized OVA. TDZD-8, a non-ATP competitive GSK-3β inhibitor, was administrated by i.v. injection one hour before OVA challenge.

TDZD-8 significantly reduced the OVA-induced eosinophilia in a dose-dependent manner and inhibited the levels of IL-5 in bronchoalveolar lavage (BAL) fluid. TDZD-8 also suppressed the mRNA levels of IL-13 and gob-5. Histological studies revealed that TDZD-8 substantially reduced the inflammatory cell infiltration and mucus secretion in the lung tissue. TDZD-8 did not alter IgE and OVA-specific IgE serum level. On the other hand, OVA-induced increase in airway resistance and reduction in dynamic compliance were inhibited by TDZD-8. Our findings reveal for the first time that inhibition of GSK-3β may have therapeutic potential for the treatment of allergic airway inflammation.

P260059

The immunotherapeutic effects of ginsenoside Ro in mice with diabetes mellitus

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Aim To study the immunotherapeutic effects of ginsenoside Ro in multiple low dose streptozotocin (MLD-STZ) induced diabetic mice. Methods The diabetic mice were administered either ginsenoside Ro (25, 50, 150 mg/kg/day) or saline per os and sacrificed after 10 or 20 days of treatment. Blood glucose level and the number of pancreas islet beta cells were measured, the nonspecific proliferation and specific proliferation ability of splenocytes to ConA and Insulin respectively were tested using [3H] thymidine incorporation assay, the level of cytokine IFNγ and IL-4 secreted by splenocytes were determined by ELISA method, and the expression of peroxisome proliferator-activated receptor gamma (PPARγ) gene was characterized using semi-quantitative RT-PCR. Results In day 20 of treatment, in experimental groups (50, 150 mg/kg/day), the level of blood glucose and IFNγ, and specific proliferation ability of splenocytes to Insulin, decreased significantly; The number of islet beta cells, the level of IL-4 and PPARγ mRNA, however, increased significantly. Conclusion Ginsenoside Ro showed immunomodulatory and antihyperglycemic effects by revising the imbalance of Th1 and Th2 in MLDSTZ induced diabetic mice.

P260060

Laboratory study of chronic eczema treated by Polysaccharide Nucleic Acid Fraction of Bacillus Calmette Guerin (BCG-PSN)

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Objectives: To investigate the mechanism of immuneoregulation of BCG-PSN on balb/c mice of chronic eczema caused by 2,4-Dinitrofluobenzene (DNFB). Methods: 40 balb/c mice of chronic eczema were divided into model group, the group of BCG-PSN (0.015 mg/kg), BCG-PSN (0.030 mg/kg) and BCG-PSN (0.060 mg/kg). drugs were given through muscle every other day. on the weekend of third, detected the percentage of CD4+T、CD8+T lymphocytes of

peripheral blood with flow cytometry and calculated the ratio of CD4+T and CD8+T; measured serum levels of IL-2、IL-4 and γ-IFN with Double-antibody sandwich ELISA method. Results: After treated by BCG-PSN, the serum levels of IL-2、γ-IFN was increased significantly ($P<0.05$), while the serum levels of IL-4 was decreased significantly ($P<0.05$); the ratio of CD4+T and CD8+T was increased significantly ($P<0.05$).

Conclusions: The mechanism of BCG-PSN may be related to the regulation and modulation the imbalance of T lymphocyte subgroup and cytokines production so as to enhance the cellular immunity in balb/c mice of chronic eczema.

Key words: Chronic eczema BCG-PSN DNFB

Acknowledgement: I thank members of department of pharmacology for their helpful instructions and comments.

P260061

Effects of an inducible nitric oxide synthase inhibitor on the formation of pro-inflammatory hydrogen sulphide in lipopolysaccharide-treated rat.

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This study examined the effects of an iNOS inhibitor N-(3-(aminomethyl) benzyl) acetamidine (1400W) on H_2S metabolism in LPS-injected rats. Administration of LPS (10 mg/kg, i.p.; 6h) resulted in an increase in plasma TNFa, IL-1B and NOx concentrations, H_2S biosynthesis from added cysteine, CSE mRNA, and iNOS in liver and kidney. Pre-treatment with the non-selective NOS inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) (100, 50, 25 mg/kg, i.p.) did not significantly decrease plasma TNFa and IL-1B concentration, H_2S biosynthesis or CSE mRNA expression in LPS-injected rats. In contrast, 1400W (10, 5, &1 mg/kg, i.p.) administration resulted in a dose-dependent inhibition of the LPS-mediated rise in plasma TNFa and IL-1B concentration, H_2S biosynthesis from cysteine and CSE mRNA expression. These results show for the first time that 1400W downregulates the biosynthesis of pro-inflammatory H_2S suggesting that constitutive NOS isoforms play a protective role in endotoxic shock and that 'cross talk' could possibly exist between NO and H_2S.

Hydrogen sulphide, nitric oxide, 1400W, L-NAME

We thank A*STAR for FA's Graduate Scholarship and Office of Life Science (NUS) for financial support R-184-000-074-712.

P260062

Anti-hapatofibrotic effects of total glucosides of paeony via G protein-coupled signal on hepatic stellate cells

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Total glucosides of paeony (TGP) is an active compound extracted from roots of paeonia lactiflora Pall. Our previous study showed that TGP has anti-hepatic injury and anti-hepatofibrotic effects. In the present study, the effects of TGP on the changes of the expressions of G-protein and G-protein-AC-cAMP signal transduction pathways stimulated by platelet-derived growth factor BB (PDGF-BB) in hepatic stellate cells (HSC) were investigated. The changes of the expression of G-protein on HSC-T6 cell membrane induced by PDGFBB ($10\ \mu g \cdot L^{-1}$) were detected by Western-blot analysis. The results showed that PDGF-BB remarkably increased the expression of Gαi2, but had no effect on expression of Gαi1、Gαi3 and Gαs. PDGF-BB decreased the level of cAMP with concentration-dependently. Furthermore, the tendency of cAMP was closely related with the proliferation of HSC-T6. The expression of Gαi2 was remarkably inhibited by TGP, which also increased the level of cAMP, and inhibited the proliferation of HSC-T6. The results indicate that TGP may inhibit the proliferation of HSC-T6 induced by PDGF-BB via regulating G-protein-AC-cAMP pathway.

Key words: TGP, HSC, PDGF, G-protein

P260063

Immunomodulating effect of a tellurium compound AS101 on Interleukin-10 and the involvement activation of MAPK signaling pathway in Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease. Th2 T-cells are thought to play a key pathogenic role in AD. IL10 is overexpressed in AD suggesting a general bias toward IL10 production. Predisposition toward IL10 gene overexpression may be a key element in the pathogenesis of AD. Recently it was reported that IL10 is overexpressed also in skin lesions of AD patients and believed to be an important factor in the pathogenesis of the disease. Thus the regulation of IL10 production is a potential solution for immunotherapeutic intervention in AD. Reduction in IFN-γ secretion in AD individuals cannot be ruled out, as it has been implicated in the pathogenesis of the disease. The study shows that IL10 level was higher in AD patients compared to healthy donors and IL-2 and IFN-γ levels were low. The addition of the tellurium compound ammonium AS101 inhibits the production of IL10, while increasing the production of IL2 and IFN-γ. These changes correlate with the inhibition of p38. This effect of AS101, together with its excellent clinical safety profile in humans, suggests that it has potential as a therapeutic agent for AD.

Key words: Atopic dermatitis, AS101, IL10, p38

P260064

Artrofoon, an oral anti-TNF alpha therapeutic, is effective in both rheumatoid arthritis and osteoarthritis: results of clinical trials

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Recent trials have shown superior efficacy of Artrofoon (anti-TNF alpha antibodies, ultra-low doses for oral use - AF) over diclofenac (DF) in rheumatoid arthritis (RA). About 57% of patients treated with AF (8 tablets/day) met ACR20 criteria after 6-month therapy (20% of patients treated with 100 mg of DF daily). The aim of this study was to test clinical efficacy and safety of AF in osteoarthritis (OA). In a pilot open-label, comparative trial patients with OA were randomized to receive either AF (8 tablets/day, n=30) or DF (100 mg/day, n=22). At month 6, total WOMAC (primary endpoint) decreased by 36.1 and 29.5 points in AF and DF arms respectively. Scores of WOMAC subscales: pain, stiffness and physical function (secondary endpoints) in AF group improved by 8.4, 2.8, and 25.1 points respectively. Although Artrofoon's onset of action was slower at month 1, by month 3 the both drugs equalled in efficacy, and at month 6 AF was more effective than the comparator. Unlike DF, AF did not cause drug-related adverse events.

Artrofoon is a novel oral anti-TNF-alpha antibody therapeutic that holds great promise both in RA and OA.

P260065

INHIBITION OF iNOS EXPRESSION AND NO PRODUCTION BY ANTI-INFLAMMATORY STEROIDS. ROLE OF HISTONE DEACETYLATION

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Tampere University Hospital, Finland In inflammation, nitric oxide (NO) is produced by inducible nitric oxide synthase (iNOS) induced by bacterial products and cytokines, and NO acts as a proinflammatory and cytotoxic mediator. The aim of the present study was to investigate the mechanisms how glucocorticoids inhibit iNOS expression in activated macrophages.

Dexamethasone and a dissociated glucocorticoid RU24858 inhibited NO production, and iNOS protein and mRNA expression in murine J774 macrophages exposed to bacterial lipopolysaccharide (LPS). In the presence of a glucocorticoid receptor (GR) antagonist mifepristone, dexamethasone and RU24858 had no effect on NO production. The role of histone deacetylation in the glucocorticoid effect was studied by using three inhibitors of histone deacetylases (HDACs); non-selective trichostatin A and apicidin, and HDAC1 selective MC1293. HDAC inhibitors reversed the effects of dexamethasone and RU24858 on iNOS expression

or NO production.

These results suggest that glucocorticoids inhibit iNOS expression and NO production in activated macrophages by a GR-mediated and GRE-independent manner possibly through histone deacetylation and transcriptional silencing.

Key words: inflammation, nitric oxide, histone deacetylation, glucocorticoids

P260066

A macrophage-based nanopartide system for drug delivery: Pharmacokinetic and anti-viral activities in a murine model of HIV-1 infection

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Nanotechnology-derived cell based systems, nanoparticle indinavir (NP-IDV) were packaged into bone marrow-derived-macrophages (BMM) as drug carriers for anti-retroviral delivery. Drug trafficking and disease outcomes were assessed over time in HIV-1 infected humanized mice treated with NP-IDV packaged in BMM. Cell trafficking was evaluated by SPECT, MRI and histologic tests. BMM distribution showed the spleen to contain 3-5-fold greater than liver on day 7. Tissue and sera were >50 nM/ml at two weeks when administrated NP-IDV packaged in BMM. A single administration of NP-IDV-BMM significantly reduced infected cells in virus-challenged NOD/SCID mice reconstituted with human peripheral blood lymphocytes. CD4+ T cells were restored after NP-IDV-BMM administration. These results provide, for the first time, proof of concept towards the use of NP delivery system in anti-retroviral therapy.

P260067

Effect of amodiaquine on P. acnes/LPS-Induced hepatitis in mice through the elevation of endogenous histamine.

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Histamine is a well known mediator of allergic inflammation. In addition, histamine has been demonstrated to be involved in the regulation of innate and aquired immune responses through H2-receptors. In the previous study, we observed that inducible histamine protect mice from lethal hepatitis by reducing the excessive cytokine response in the liver. In the present study, we examined the effect of amodiaquine, a specific inhibitor of histamine N-methyltransferase, on the hepatitis in mice. Heat-killed P. acnes (1 mg, i.v) followed by challenge with a low dose of lipopolysaccharide (LPS, 1 μg) induced acute and massive liver injury. Amodiaquine at 2 and 5 mg/kg dose-dependently increased histamine levels in the liver associated with the decrease in telemethylhistamine levels. At same doses, amodiaquine inhibited the hepatitis and reduced the lethality of mice. Amodiaquine decreased the plasma levels of TNF-α as well as the expression of TNF-α mRNA in the liver. These results suggested that amodiaquine inhibited hepatitis and lethality by reducing TNF-α production through the elevation of histamine in the liver.

P260068

Substance P and Caerulein Induce Chemokine Synthesis in Pancreatic Acinar Cells.

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Chemokines and substance P (SP) play a key role in acute pancreatitis. Pancreatic acinar cells produce MCP-1 in response to caerulein hyperstimulation. In mice with pancreatitis, SP levels and expression of NK-1 receptors in pancreatic acinar cells are increased. In this study, we investigated the effect of caerulein and SP on pancreatic acinar cells. Acinar cells secreted CC chemokine MCP-1, MIP-1alpha and CXC chemokine MIP-2 when treated with caerulein or SP alone. Combined treatment of caerulein and SP caused a further increase in the levels of MCP-1, MIP-1alpha, and MIP-2, which was accompanied by a significant increase in NFκB activation compared to the treatment with caerulein or SP alone. These results suggest that both SP and caerulein are acting through NFκB pathway to induce chemokine synthesis. To further confirm this, acinar cells were treated with NEMO-binding domain (NBD), a selective inhibitor of NFκB activation. Treatment with NBD significantly attenuated the stimulation in chemokine synthesis caused by treatment with both caerulein and SP. This study shows that caerulein and substance P induce chemokine synthesis through NFκB pathway.

Key words: Chemokine, Substance P, NFκB

P260069

CHANGES IN BLADDER MYELOPEROXIDASE ACTIVITY INDUCED BY CYCLOPHOSPHAMIDE AND ACROLEIN. ROLE OF iCOX

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Aims: The role of myeloperoxidase (MPO) on the genesis of cyclophosphamide (CYP) and acrolein (ACR)-induced cystitis was investigated. Methods: Rats received: a) saline; b) ACR: 5 mg/Kg or CYP: 75 mg/Kg, i.p; c) ACR or CYP + Rofecoxib (ROF), 20 or 15 mg/kg, p.o.; d) Meloxicam (MEL), 25 or 15 mg/Kg, i.p e) Ketoprofen (KET), 20 mg/kg, i.p. After 6 h (for CYP) or 24 h (for ACR) bladders were taken and MPO activity measured. Results as MPO: Abs./mg protein/40 min/room temp.

CONTROL CYP + SALINE CYP + ROF CYP + MEL CYP + KET
0.42 ±0.19 2.16 ±0.13** 0.80 ±0.12** 0.70 ±0.28* 0.54 ±0.10***
CONTROL ACR + SALINE ACR + ROF ACR + MEL ACR + KET
0.53 ±0.11 2.32 ±0.31** 0.75 ±0.15** 1.06 ±0.17** Not done

Different from control at *p < 0.05; **p < 0.01; ***p < 0.001. Conclusions: ACR and CYP induced an increase of MPO activity. Protection by KET, non-selective COX inhibitor (i-COX) indicates that COX-1 could be involved in this effect. These results suggest that CYP possibly through ACR provokes neutrophils incoming to the bladder, with PGs production.

Key words: cyclophosphamide, myeloperoxidase. (Supported by grant CDCH.-065221.2005 to ABA)

P260070

Effect of Celecoxib on inflammatory mediators in rats exposed to gamma irradiation

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Rats were irradiated using a Gamma cell 40 with a ^{137}Cs source either acutely as single irradiation doses of 2 and 7.5 Gy or chronically by fractionated exposure to 7.5 Gy delivered as 0.5 Gy twice weekly for 7.5 weeks. Rats were then exposed to 2 models of acute inflammation: carrageenan paw oedema and 6-day air pouch and one for chronic inflammation: adjuvant-induced arthritis. Celecoxib (Pfizer) was injected 1 h before carrageenan in the acute models and on day 14 for 7 days in the chronic model.

Irradiated rats showed a greater inflammatory response than controls associated with higher levels of prostaglandins, TNFalpha, IL-1beta, IL-6, LTB4 and COX-2 activity in plasma and exudates as well as higher levels of malondialdehyde and lower levels of superoxide dismutase. Celecoxib markedly reduced the extent of leukocytic infiltration and prevented the changes induced by irradiation in the tested parameters. In many respects it was superior to diclofenac (a reference non-selective COX inhibitor used for comparison) as a protective agent against gamma-irradiation induced damage.

Keywords: celecoxib, gamma-irradiation, inflammation.

P260071

TLR2 mediates bleomycin-stimulated maturation of dendritic cells and activation of T cells: Implication of pulmonary injury

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Anti-tumor drug bleomycin induces pneumonitis and fibrosis that limit its therapeutic application. We hypothesis that bleomycin activates dendritic cells (DCs) via Toll-like receptors (TLRs) that direct polarization of T cells and participate in pulmonary injury. Bleomycin-induced DCs maturation and expression of cy-

tokines were analyzed by flow cytometry. The expression and activity of TLRs were determined by PCR or western blot. The polarizing capacity of bleomycin-treated DCs was examined by allogenic-mixed lymphocyte reaction. We found that bleomycin enhanced expression of TLR2 and activated the TLR2 signal pathway. Bleomycin-induced maturation of DCs and alteration of cytokine production in DCs were completely blocked by anti-TLR2 but not by TLR4 antibody. Bleomycin-activated DCs promoted polarization of Th1- and Th2-cells via activation of TLR2. Moreover, inhibition of TLR2 significantly attenuated bleomycin-induced pulmonary injury. Our results suggest that bleomycin is a specific ligand of TLR2; bleomycin activation of TLR2 and its signaling pathway contribute into pathogenesis of bleomycin-induced pneumonitis and pulmonary fibrosis.

Key Words: bleomycin, TLRs, DCs, Th1/Th2 response

P260072

Adjuvant application of Th1-inducing TLR agonists and routing application of Th2-inducing TLR agonists inhibit tumor growth and metastasis

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TLR-mediated inflammation involves in tumor growth and metastasis. TLR agonists may induce pro- or antitumor activity, depending on their triggering Th1/Th2 immune response. We wonder if adjuvant application of TLR agonists or routing application of TLR agonists produces different effects on tumor growth and metastasis.

Animals were pretreated or treated with PG-LPS, EC-LPS, CpG, or EC-LPS plus CpG. Tumor growth and metastasis were determined as described previously. Biochemical and immunological changes were investigated using flow cytometry, RT-PCR, or ELISA. Pretreatment of animals with EC-LPS or CpG but not PG-LPS slightly attenuated tumor growth and metastasis. However, pretreatment of animal with EC-LPS plus CpG significantly inhibited tumor growth and metastasis. Routing application of EC-LPS plus CpG promoted tumor metastasis but PG-LPS significantly inhibited tumor growth and metastasis. Our results suggest that tumor growth and metastasis are prevented by Th1-inducing TLR agonists or attenuated by Th2-inducing TLR agonists, indicating that inflammation plays an important role in the process of tumor growth and metastasis.

Key words: tumor growth, metastasis, TLR agonist, tumor immunity

P260073

ORAZIPONE DECREASES INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION AND NITRIC OXIDE PRODUCTION IN ACTIVATED MACROPHAGES

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Inflammation, inducible nitric oxide synthase (iNOS) produces nitric oxide (NO), which has proinflammatory and destructive effects. Compounds that inhibit expression or activity of iNOS have a promise as anti-inflammatory drugs in diseases like arthritis and asthma. Orazipone is a novel sulfhydryl modulating compound with anti-inflammatory properties. We investigated the effects of Orazipone on iNOS expression and NO production in J774 murine macrophages exposed to lipopolysaccharide (LPS).

Orazipone, but not its non thiol modulating analogue inhibited iNOS protein expression and NO production in a dose-dependent manner. In addition, iNOS mRNA levels were significantly decreased by Orazipone when measured by quantitative PCR 3 h after the exposure to LPS. Orazipone prevented the activation of nuclear factor kappa B (NFkappaB), which is a critical transcription factor for iNOS.

In conclusion, Orazipone decreased iNOS expression and NO production along with its inhibitory effect on NFkappaB in activated macrophages. The effect is implicated in the anti-inflammatory action of Orazipone.

Key words: inflammation, inducible nitric oxide synthase, Orazipone.

P260074

Poor inhibition of calcineurin activity is associated with the onset of acute rejection after lung transplantation

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Forty patients, who have received conventional immunosuppression, were enrolled during a 22-month period. Calcineurin activity was measured once weekly during the first month after transplant and then once monthly for ar least six months. Calcineurin activity was determined in mononuclear cells isolated from whole blood by quantifying by HPLC the dephosphorylation of phospho-RII peptide, a substrate of calcineurin. The results of the first 25 enrolled patients have been analysed so far and show that the activity of calcineurin was increased in patients developing acute rejection. These results suggest that the immunosuppressant treatment should be enhanced in patients exhibiting high levels of calcineurin activity in order to reduce the onset of acute rejection.

P260075

STAT1 contributes to TLR3 ligand inhibition of liver regeneration and inversely correlates with hepatocyte proliferation in HCV patients

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Previously, we have demonstrated that the TLR3 ligand poly I:C, a synthetic double-stranded RNA which is generated during viral infection, suppresses liver regeneration in the partial hepatectomy (PHx) model, and IFNgamma partly contributes to such inhibition. Here we examined the role of IFN-gamma-activated downstream signals and genes (STAT1, IRF-1, p21cip1) in poly I:C/IFN-gamma suppression of liver regeneration and hepatocyte proliferation. Disruption of the STAT1 gene enhanced liver regeneration and abolished poly I:C suppression of liver regeneration, the inhibitory effect of poly I:C on liver regeneration was also diminished in IRF-1$^{-/-}$ and p21cip1$^{-/-}$ mice. In vitro treatment with IFN-gamma-inhibited cell proliferation of wild-type mouse hepatocytes but not STAT1$^{-/-}$ mouse hepatocytes. The inhibitory effect of IFN-gamma on hepatocyte proliferation was also partially diminished in IRF-1$^{-/-}$ and p21cip1$^{-/-}$ mouse hepatocytes, but was enhanced in SOCS$^{-/-}$ mouse hepatocytes. Finally, activation of STAT1 was detected in the livers of patients with chronic hepatitis C infection, and correlated inversely with hepatocyte proliferation in these patients.

P260076

Tumor Necrosis Factor-alpha Plays an Important Role in Mediating the Neurotoxicity Caused Indirectly by Human Immunodeficiency Virus Type-1 Tat

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HIV-1 infection causes, with increasing prevalence, neurological disorders characterized in part by neuronal cell death. The HIV-1 protein Tat has been shown to cause mitochondrial dysfunction and increase levels of intracellular calcium, proinflammatory cytokines and neuronal cell death. Here, we tested the hypothesis that a non-neurotoxic epitope of Tat can, through actions on inflammatory cells, increase neuronal cell death. Tat1-72 and a mutant Tat1-72 lacking amino acids 31-61 (mTat) concentration-dependently and markedly increased TNF-alpha production in human U937 monocytic cells differentiated with PMA. Supernatants from these cells treated with either Tat1-72 or mTat were neurotoxic and their immunoneutralization with an anti-TNF-alpha antibody decreased Tat1-72- and mTat-induced neurotoxicity. These results demonstrate that the neurotoxic epitope of Tat1-72 is different from the epitope that is indirectly neurotoxic following production of TNF-alpha from inflammatory cells, and suggest that therapeutic interventions against TNF-alpha might be beneficial against

HIV-1 associated neurological disorders. (Supported by NCRR grant P20 RR17699-01)

P260077

Gab2 Antisense Oligonucleotide Blocks Mast Cell Function

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Aggregation of high-affinity IgE receptor triggers signaling events vital for mast cell degranulation and activation. Recent progress revealed a critical role of an adapter molecule Grb2-associated binder-like protein 2 (Gab2) in mast cell functions. The purpose of this study was to develop an antisense oligonuclectide (ASO) targeted at Gab2 and to examine its immunomodulatory effects in rat basophilic leukemic (RBL)-2H3 cells. A phosphorothioate-modified ASO targeted at the predicted carboxyl-terminal of Gab2 mRNA was able to selectively knock down Gab2 mRNA and protein in RBL-2H3 cells. The ASO blocked IgE-mediated mast cell release of preformed mediators beta-hexosaminidase and histamine. Gab2 ASO inhibited IgE-induced phosphorylation of Akt, p38 mitogen-activated protein kinase and protein kinase C delta in mast cells. Increases in cytokine mRNA levels (e.g. IL-4, 6, 9 and 13, and TNF-alpha) induced by IgE were suppressed by the ASO. Gab2 ASO prevented RBL-2H3 cell adhesion to fibronectin and random migration in cell culture chambers. Gab2 ASO may have therapeutic potential for mast cell-dependent disorders such as allergic asthma. (Supported by a grant BMRC 01/2/21/17/046)

P260078

Up-regulation of Interleukin-10 and Interleukin-6 Production In Macrophages by Adrenomedullin: Role of the Protein Tyrosine Kinase and Mitogen-Activated Protein Kinases Wong

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Adrenomedullin (ADM) is a potent vasorelaxant peptide that has important regulatory roles in cardiovascular system. Transgenic mice over-expressing ADM are resistant to septicaemic shock. Lipopolysaccharide (LPS) induces activation of a variety of proteins involved in inflammatory response, including mitogen-activated protein kinases (MAPKs) and protein tyrosine kinases (PTKs). In this study, we used a rat alveolar macrophage cell line, NR8383, to study the effects of ADM on LPS-induced production of interleukin (IL)-6 and IL-10. We demonstrated that both IL-6 and IL-10 productions were increased by ADM and TNF-alpha. Inhibition of p42/44 and p38 MAPKs partially reduced IL-6 and IL-10 productions; the inhibition was reversed by ADM but not by TNF-alpha. Inhibition of PTKs by genistein markedly reduced LPS-induced production of IL-6 and IL-10 by over 90%; but the inhibition on IL-6 production was significantly reversed by ADM. In conclusion, our results indicate that ADM might play important roles in regulation of cytokine production in inflammatory response via PTK-dependent and PTK-independent signal pathways.

Acknowledgement: This work was supported by the University of Hong Kong CRCG Grant

P260079

Effective osteosarcoma cytolysis using cytokine-induced killer cells co-incubated with tumor RNA-pulsed dendritic cells

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Osteosarcoma at late stage has posed a challenge for neo-adjuvant treatments. The application of cytokine induced killer (CIK) cells to osteosarcoma constitutes a promisingly strategy. In this study, completely autologous CIK cells, DCs and tumor cells were used while $CD3^+CD56^+$ cells were isolated from heterogeneous CIK cells. We showed superior cytotoxicity of the CIK cells post co-cultured with tumor RNA-pulsed DCs than with non-pulsed DCs. The advantage of the co-culture with RNA-pulsed DC was lost when high CIK cell density was employed for tumor cytotoxic assay, but was maintained in purified $CD3^+CD56^+$ cells isolated from the CIK cells. This phenomenon could explain by the effect of suppressive factors in heterogeneous CIK cells, so experiments using the purified $CD3^+CD56^+$ cells exhibited no density-dependent suppression of anti-tumor cytolysis as observed in those using CIK cells.

Keywords: CIK; Osteosarcoma; Dendritic cell; Immunotherapy

P260080

Dendritic cells pulsed with total tumor RNA enhanced cytokine-induced killer (CIK) cells-induced glioblastoma multiforme cytolysis

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Dendritic cells (DCs) play a critical role in cell-mediated immunity as potent antigen presenting cells. DCs could induce strong anti-tumor responses both in vitro and in vivo. DCs were shown to enhance the cytotoxicity of NK cells. We generated CIK cells, a novel type of effector cells differentiated from normal lymphocyte. This study aimed to elucidate the effects of CIK cells after co-culturing with DCs against glioblastoma multiforme cells. The results revealed that tumor-derived RNA-pulsed DCs can enhance the immune responses of CIK cells against glioblastoma multiforme cell line but these effector cells did not appear to have the cytotoxic effect against normal cells (human umbilical vein endothelial cells (HUVEC) and fibroblasts) in vitro. This study may be beneficial for the development of adoptive immunotherapy using immunologic effector cells against glioblastoma multiforme in the future.

P260081

Effect of Nitric Oxide Donors on Human Mast Cells

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Both nitric oxide (NO) and mast cells participate in inflammation and their interactions have been studied in rodents. However, rodent mast cells are heterogeneous to human mast cells and it is our aim to investigate the effects of NO donors on anti-IgE-induced mediator release from human mast cells. Human mast cells cultured from progenitor cells in human buffy coat were incubated with NO donors alone or together with 5 mM N-acetylcysteine (NAC) for 30 min before activation with anti-IgE. The levels of histamine, prostaglandin D2 and cysteinyl-leukotrienes released were measured. NAC and the NO donors, sodium nitroprusside, NOR-3 Diethylamine NONOate and S-Nitroso-N-acetylpenicillamine alone all failed to modulate anti-IgE induced mediator release. However, dose dependent inhibitions of mediator release were observed with all three NO donors in the presence of NAC. These results suggest that NO released from NO donors may not be stable enough to interfere with mast cell activation but the bioavailability of the released NO may be increased by the free radical scavenger, NAC.

Key words: Nitric oxide, mast cells, N-acetylcysteine.
Acknowledgement: This work is supported by RGC Grant CUHK4337/03M

P260082

Effects of Estrogen Agonists on Mast Cells Histamine Release

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The mast cells have been implicated to mediate some of the non-reproduction related actions of estrogens. However, direct effects of estrogens on mast cells have not been extensively studied. It is hence the aim of our study to investigate effects of the natural estrogen, estradiol and selective estrogen receptor modulators

(SERMs), tamoxifen and raloxifene on rat peritoneal mast cells (RPMC). Purified RPMC obtained from ovalbumin sensitized rats were challenged with anti-rat-IgE antibodies subsequent to incubation with the estrogen agonists for 30 minutes and histamine release was assayed. Estradiol dose dependently inhibited anti-IgE induced histamine release with maximum inhibition of around 25% attained at 0.05uM. However, the inhibitory potency decreased with further increase in estradiol concentration and was totally diminished at 50uM. In contrast, the SERMs did not inhibit but enhanced anti-IgE induced histamine release above 5uM. These results suggest that estradiol does not modulate mast cell activation through the conventional genomic pathway since the mast cellaction appeared within 30 min and was not mimicked by the SERMs.

Key words: estrogen, mast cell, histamine

P260083

Switching of Th1/Th2 expression profiles on CD4$^+$ T cells after the incubation with dendritic cells pulsed with mite extract allergens

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Mite extract allergens have been used in the induction of immune tolerance in patients allergic to house dust mite. The injection of the mite extract allergens would be taken up by regional dendritic cells (DCs) followed by the processing of the allergens to T helper cells type 2 (Th2). The effect of crude mite extract/Der p 1 treatment of DCs on the Th1/Th2 expression profiles of the co-cultured CD4$^+$ T cells of asthmatic patients allergic to Derp 1 were examined. The extract was prepared from soluble portion of homogenized house dust mite. DCs were pulsed with mite extract followed by the co-incubation with autogeneic CD4$^+$ T cells. We observed the shift toward Th2 expression patterns on CD4$^+$ cells, while the Th1 profiles remained unchanged. The in-house crude mite extract preparation carried similar functional effects to that of commercially purified Der p 1.

Key words: dendritic cells, mite, allergen

P260084

Influence of Percutaneous Absorption of Recombinant Human Interferon-alpha 2b Cream to Interleukin-18 And Interferon-alpha in BLAB/c

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BACKGROUND: IL-18 (Interleukin-18) and IFN-α (Interferon-alpha) both are important immunoregulatory factors. RHIFN-α2b (Recombinant Human IFN-α2b) cream is one of popular topical drugs for antivirus and immunoregulatory therapy. OBJECTIVE: To find in BLAB/c skin model rubbed with RHIFN-α2b cream whether the drug influence Mouse IL-18 and IFN-α in plasma and skin. METHODS: In vivo, BLAB/c abdominal bare skin topically treated with RHIFN-α2b cream by rubbing once a day for 10 days which part to 24h and 10d groups. In vitro, BLAB/c abdominal bare skin slice treated with RHIFN-α2b cream in culture inserts, cultured by medium in pore plate in 12h, and then samples of plasma and medium detected concentration of Human IFN-α, Mouse IL-18 and IFN-α by ELISA Assay. RESULTS: In plasma, Increase of Human IFN-α depress Mouse IFN-α notably, but not to Mouse IL-18. Adversely in skin, Increase of Human IFN-α depress Mouse IL-18 notably, but not to Mouse IFN-α. CONCLUSION: The study suggests that RH IFN-α2b cream influence the Mouse IL-18 and IFN-α adversely between plasma and skin by per cute m.

Key words: IL-18, IFN-α, IFN-α2b, Percutaneous Absorption ACKNOWLEDGEMENT: NNSFC (No: 30572269)

P260085

Modulation of eosinophil migration by Mangifera indica L. extract (Vimang)

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The effects of Vimang, an aqueous extract of the stem bark of Mangifera indica L. (Anacardiaceae), on cell migration in an experimental model of asthma was investigated. In vivo treatment of T. canis-infected BALB/c mice for 18 days with 50 mg/kg Vimang reduced eosinophil migration into the bronchoalveolar space and peritoneal cavity. Also, eosinophil generation in bone marrow and blood eosinophilia were inhibited in infected mice treated with Vimang. This reduction was associated with inhibition of IL-5 production. In all these cases the effects of Vimang were more selective than those observed with dexamethasone. Moreover, Vimang treatment is not toxic for the animals, as demonstrated by the normal body weight increase during infection. These data confirm the potent anti-inflammatory effects of Vimang and support its potential use as an alternative therapeutic drug to the treatment of eosinophilic disorders including those caused by nematodes and allergic diseases.

P260086

The potential side effects of cydosporine A: its inhibition on CD4+CD25+Treg cells in mice

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CD4$^+$CD25$^+$ regulatory T (Treg) cells are essential for the maintenance of immunologic self-tolerance as well as transplant tolerance. As an immunosuppressive agent, Cyclosporin A (CsA) is widely used by transplanted patients. Here, the effects of CsA on CD4$^+$CD25$^+$ Treg cells in mice were determined. Methods: Balb/c mice were injected with CsA or control solution for 1 month. The levels, phenotype and function of CD4$^+$CD25$^+$ Treg cells in these mice were then detected. Results: The percentages and total cell numbers as well as the immunosuppressive function of CD4$^+$CD25$^+$ Treg cells in the periphery blood and spleens were significantly reduced after the treatment with CsA. The total numbers of CD4$^+$CD25$^+$ Treg cells in the thymus of CsA-treated mice were markedly reduced than control mice. The phenotype of CD4$^+$CD25$^+$ Treg cells became activated and memory in CsA-treated mice. In addition, CsA decreased the levels of Foxp3 in CD4$^+$CD25$^+$ Treg cells. Conclusions: CsA significantly impaired the development, homeostasis and function of CD4$^+$CD25$^+$ Treg cells. This study might be of significance to guide the clinical usage of CsA.

Key words: CD4$^+$CD25$^+$ Treg cells, CsA, Foxp3

P260087

The Immunosuppressive Effects of Novel Artemisinin Derivatives in vitro and in vivo

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A series of novel artemisinin derivatives with nonsteroidal anti-inflammatory drug structure, were synthesized and evaluated on their immunosuppressive activity. MTT assay and [^{3}H]-thymidine incorporation were used to evaluate the cytotoxicity and splenocyte proliferation. Cytokines were detected with the enzyme-linked immunosorbent assay. Dinitrofluorobenzene (DNFB) induced-delayed-type-hypersensitivity (DTH), quantitative hemolysis of sheep red blood cells (SRBC) and collagen-induced-arthritis (CIA) were used to evaluate immune responses in vivo. Among them, SM735, SM934 and SM905 exhibited lower cytotoxicity and higher inhibition activity on splenocyte proliferation, and dose-dependently inhibited proinflammatory cytokine production [interleukins (IL)-12, interferon (IFN)-γ and IL-6]. In vivo, the compounds suppressed DTH, QHS and CIA responses. The results demonstrated a strong immunosuppressive activity of SM735, SM934 and SM905 both in vitro and in vivo, and outlined a great potential of artemisinin derivatives as immunosuppressive agents.

Key words: artemisinin; non-steroidal anti-inflammatory agents; immunosuppressive activity

P260088

The protective effect of Baicalin on Concavalin A-induced liver injury and the related mechanism

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To examine the protect effect of Baicalin (BA) in Concavalin A (Con A) –induced hepatitis and explore the possible mechanisms, forty Balb/c mice subjected to Con A to induce acute liver injury. Mice were pretreated with BA three times before Con A injection to determine the prophylatic effect. Liver injury was assessed by quantification of plasma transaminase activities and histological analysis. Apoptosis was detected by TUNEL method and tissue caspase activity assay. Cytokine concentrations in plasma and medium supernatant collected from BA-treated primary splenocyte and mouse macrophage line were determined by enzyme-linked immunosorbent assay (ELISA). The protective effect of BA on hepatocyte was detected. Results showed that BA inhibited Con A-induced liver injury by modulating the inflammatory mediators and alleviating the apoptosis in mice. In vitro, BA inhibited the immune cell activation and cytokine production. BA also alleviated TNF-α/Act D induced hepatocyte injury. In conclusion, BA suppressed Con A-induced liver injury as an immune-response modifier, might be a valuable drug in protecting T-cell mediated liver injury.

Key Words: Baicalin; liver injury; inflammatory mediators

P260089

A Combined COX and LOX Inhibitor Regimen Fails to Mimic the Action of Dexamethasone Against Cisplatin-Induced Acute and Delayed Emesis in the Ferret

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The anti-emetic action of glucocorticoids in the clinic may involve an ability to reduce the synthesis of eicosanoids. In the present studies, therefore, we investigated the potential of the non-selective COX inhibitor, indomethacin, and the LOX inhibitor, MK-886 (3-1-(p-chlorobenzyl)-5-(isopropyl)-3-tert-butylthioindol-2-yl)-2,2-dimethylpropanoic acid), to reduce cisplatin (5 mg/kg, i.p.)-induced acute and delayed emesis in the ferret. Indomethacin (3-30 mg/kg/8 h, i.p.) potentiated significantly cisplatin-induced retching + vomiting ($P < 0.05$) and was also emetic when used alone. Conversely, MK-886 (1-10 mg/kg/8 h, i.p.) was inactive to modify cisplatin-induced emesis ($P > 0.05$). The combination treatment of indomethacin (10 mg/kg/8 h, i.p.) with MK-886 (10 mg/kg/8 h, i.p.) did not affect significantly ($P < 0.05$) cisplatin induced retching + vomiting, but had a different profile ($P < 0.05$) from dexamethasone (1 mg/kg/8 h, i.p.), which produced a trend for a reduction. Further studies are required to fully elucidate the mechanism of anti-emetic action of glucocorticoids.

Key words: Emesis, cyclooxygenase, lipoxygenase, dexamethasone.

The research was supported by the RGC of Hong Kong (CUHK 4049/98 M).

P260090

Humoral and Cellular Immunomodulation Induced by Propoxure in C57-bl/6 Mice

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Propoxure (PPX), a well-known carbamate insecticide, has been used in agriculture and public health programs for decades. We examined the effects of PPX on humoral (PFC & HA) and cellular (DTH) responses. Female C57b1/6 mice were administered PPX (0.2, 2, 10 mg/kg/day i.p. for 28 days). On the day 28, mice were examined for DTH, PFC and HA responses to SRBC. Spleen CD4/CD8 percentage and absolute numbers also were measured. Furthermore In vitro lymphocyte proliferation response to PHA was measured. PPX at 10 mg/kg/day could suppress DTH and increase $CD4^-/CD8^+$ T-cell percentage. On the other hand, PPX at 2 mg/kg could increase PFC and HA responses against SRBC. Subchronic PPX at low dose (0.2 mg/kg/day) could not show any significant effects on humoral or cellular responses. In conclusion, subchronic PPX at high dose (10 mg/kg), has cellular immunosuppressive effects. However, PPX at 2 mg/kg does not change cellular responses but may stimulate humoral responses. It seems that PPX has no adverse effects on mice immune system at low doses as 0.2 mg/kg, which is 10 fold greater than PPX Allowed Daily Intake limit.

Key words: Immunomodulation; C57b1/6 mice; Propoxure

P260091

Nitric oxide production in endothelial cell culture is inhibited by melatonin

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Considering that melatonin modulates endothelial function (vascular tonus and permeability), and that bradykinin activates endothelial nitric oxide synthase (NOS), our aim was to investigate if melatonin alters endothelial NO production in vitro. Endothelial cells were incubated with a fluorescent dye selective either for NO (DAF-FM, 5 uM) or Ca^{2+} (Fluo3, 5 uM) and fluorescence was determined. Bradykinin (1-100 nM) increased both cytosolic Ca^{2+} ($pD2 = 7.86 \pm 0.06$; $n = 3$) and NO production ($pD2 = 8.38 \pm 0.07$; $n = 4$), but only the last effect was abolished by melatonin (0.1^{-1} nM) and N-acetylserotonin (0.01^{-1} nM), while the selective agonist for MT3 receptors (5 MCA-NAT 1 nM) had no effect. In addition, despite the presence of MT receptors as revealed by RT-PCR assay, nonselective (luzindole, 10 uM) or MT2 selective (4P-PDOT, 100 nM) antagonists did not prevent melatonin effect, suggesting that this effect is mediated by calmodulin antagonism, as observed for nNOS. Thus, melatonin modulation of bradykinin effect could be the basis for a putative diurnal variation of endothelial function.

Financial support: FAPESP, CAPES, CNPq.

Key words: melatonin, nitric oxide, endothelium

P260092

Hydrolysis of extracellular nucleotides by CD39/ENTPD family members: prominent effects on thrombosis, vascular inflammation and immune reactions.

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Ecto-nucleotidases of the CD39/E-NTPDase family are expressed in the vasculature and immune systems. These ecto-enzymes hydrolyze extracellular nucleotides, ultimately to the respective nucleosides, to regulate P2-receptor signaling. Spatial and temporal expression of CD39/NTPDase1 by vascular and immune cells could regulate thrombotic and immune reactions in vivo.

CD39 has the potential to modulate thrombotic reactions viz. platelet activation after ischemia reperfusion in vivo. Increases of NTPDase1 biochemical activity within microparticles associated with evolving arteriolar thrombi also seems to impede further ADP-mediated platelet activation. CD39 is also a surface marker of Tregulatory cells (Treg). Co-ordinated expression of CD39 on Treg and the adenosine A2A receptor on activated effector T cells (Teff) generates an immunosuppressive loop. Adoptive transfer of Cd39 null Treg fails to inhibit allograft rejection in vivo and null mice also develop autoimmune manifestations and exhibit vascular thrombophilia. Pharmacologic modalities to modulate or boost NTPDase1 expression may suppress deleterious vascular or immune reactions, as seen in autoimmune disease and transplant graft rejection.

P260093

Characterization of Urate excretion in isolated perfused kidney of streptozotocin-induced diabetic rats

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AIM Many studies have demonstrated that patients with diabetes show low plasma levels of uric acid. In the present study we compared the renal handling of uric acid (UA) by isolated perfused kidney (IPK) in streptozotocin-induced diabetic rats and response to probenecid with intact rats. Methods. The left kidneys of male wistar rats were isolated and perfused in recirculating mode with Krebs-Henseleit buffer containing amino acids and BSA ($n = 7$). Inulin was added to the perfusate to permit estimation of glomerular filtration rate (GFR). An uricosuric, probenecid, was administered to the IPK perfusate to investigate its role. During the 90-min experimental period, urine was collected in 10-min intervals and perfusate was collected at the midpoint of these intervals. inulin clearance (Cl-In), clearance of uric acid (Cl-UA), fractional excretion of uric acid

(FE - UA) , and 90 - min urinary UA excretion (UA - U) were determined. Results . IPK in diabetic rats had significantly higher GFR, urine flow rate , FE- UA, Cl - UA and UA - U compared to intact group , but renal handling of UA in diabetic rats had not been improved by probenecid . Conclusions . UA renal clearance increase in type 1 diabetes , which leads to hypouricemia , may not be relevant to UA tansporter function in the proximal tubule but to higher GFR, the role responsible for UA uptake by UA tansporter remains to be determined.

Key Words : isolated perfused kidney , UA excretion , probenecid

P260094

Saponin Fraction from Cleditsia sinensis Inhibits Collagen - induced Arthritis in DBA/1 Mice

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In the present study , we investigated the therapeutic potential and underlying mechanisms of saponin fraction from anomalous fruits of Cleditsia sinensis (SFGS) on collagen II (CII) - induced arthritis (CIA) in DBA/1 mice . SFGS (50 , 100 and 200 mg/ kg) , orally administered from the day of immunization , dose - dependently alleviated disease severity , postponed the onset and reduced the incidence rate of CIA. Histological analysis revealed that joints of CIA mice treated with SFGS showed scarce inflammatory cell infiltration and slight synovium hyperplasia and focal bone erosion . Furthermore , SFGS treatments lowered the serum anti - CII autoantibody levels , and suppressed the delayed type hypersensitivity against CII in ears of CIA mice . The findings indcated that SFGS ameliorated inflammation and joint destruction in CIA mice , which may be consequence of suppression on CII - specific humoral and cellular immunity . SFGS should be a candidate of novel therapeutic agents for rheumatoid arthritis .

P260095

Effect of sodium azulene sulfonate on capsaicin - induced pharyngitis in rats

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Sodium azulene sulfonate is clinically used as a therapeutic agent of pharyngitis . There has been no documentation on the effect of sodium azulene sulfonate on pharyngitis in laboratory models , probably because of no availability of such models . We recently established a pharyngitis model using capsaicin application on pharyngeal mucosa in rats . The present study investigated the anti - pharyngitis activity of intrabuccal sodium azulene sulfonate comparing with those of ruthenium red (RR, vanilloid receptor antagonist) , ascorbic acid (AA, anti - oxidative compound) , povidone iodine (PI , gargle as disinfectant , oxidative compound) and diclofenac sodium(DS , cyclooxygenase/ COX inhibitor) . As an anti - pharyngeal effect , the capsaicin - induced plasma exudation in the pharyngeal mucosa of the rat was evaluated . The capsaicin - induced plasma exudation in the pharyngeal mucosa was inhibited by sodium azulene sulfonate as well as RR and AA, but not by PI and DS ; PI rather promoted the plasma exudation . In conclusion , the anti - pharyngitis effect of sodium azulene sulfonate was demonstrated for the first time in a laboratory model .

Key words : sodium asulene sulfonate , pharyngitis , capsaicin , anti - pharyngitis effect

P260096

Sinomenine improves trinitrobenzene sulfonic acid - induced murine colitis by balance of Th1 and Th2 cytokines

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Sinomenine is a pure alkaloid extracted from the Chinese medical plant Sinomenium acutum. The therapeutic efficacy of sinomenine was confirmed in patients with rheumatoid arthritis . The aim of the present study was to evaluate therapeutic effects of sinomenine on T- helper cell type 1 - mediated experimental colitis , 2 , 4 , 6 - trinitrobenzene sulfonic acid (TNBS) induced colitis in mice . Two hour following colonic instillation of TNBS , sinomenine with several doses was given by gastric gavages once daily for 7 days . Comparing with the ethanol control group and the 30 mg/ kg dose group , the 100 mg/ kg and 200 mg/ kg dose groups of sinomenine were shown improvements of weight loss , macroscopic and histologic scores , myeloperoxidase activity . Th1 cytokine , tumour necrosis factor - alpha and interferon gamma expression in protein and mRNA levels was decreased , and Th2 cytokine interleukin - 10 was increased in mucosa after 7 days treatment . However , sinomenine has no effects in interleukin - 12 expression in both protein and mRNA level in mucosa . Our findings suggest that sinomenine improves TNBS - induced colitis in mice and the therapeutic mechanism might be related to Th1 and Th2 balance in mucosa .

P260097

Protective effects of BX471 , a non - peptide CC chemokine receptor - 1 antagonist , on acute respiratory distress syndrome

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Chemokines have been shown to play a critical role in the pathogenesis of acute respiratory distress syndrome (ARDS) . BX471 is a potent non - peptide CC chemokine receptor - 1 (CCR1) antagonist in both human and mouse . The aim of the present study was to evaluate the effect of prophylactic and therapeutic treatment with BX471 on ARDS that was caused by acute pancreatitis (non - infective) or sepsis (infective) in the mouse and to investigate the underlying mechanisms . In acute pancreatitis induced by caerulein hyperstimulation and in sepsis induced by cecal ligation and puncture , treatment with BX471 significantly protected mice against lung injury by attenuating myeloperoxidase activity , an indicator of neutrophil sequestration , in lungs and attenuating lung morphological changes in histological sections . In both models blocking CCR1 by BX471 led to a downregulation of intercellular adhesion molecule - 1 , P - selectin and E - selectin expression in lungs compared with vehicle - treated controls . These findings suggest that interfering with neutrophil migration and activation by targeting CCR1 may represent a novel method to prevent disease progression in ARDS .

P260099

Anti - inflammatory effects of somatostatin receptor subtype 4 selective agonist J - 2156 in rodents

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The aim of the present study was to investigate a , sst4 selective synthetic agonist , J - 2156 , on sensory neuropeptide release and acute inflammatory processes . Electrically - induced release of substance P, calcitonin gene - related peptide and somatostatin from isolated rat tracheae was measured with radioimmunoassay . Mustard oil - induced neurogenic inflammation in the rat hindpaw skin was determined by Evans blue accumulation and in the mouse ear with a micrometer . Dextran - , carrageenan - induced non - neurogenic inflammation was measured by plethysmometry . Granulocyte accumulation evoked by IL - 1 beta or zymosan in the murine back skin was determined with myeloperoxidase assay . J - 2156 (10 - 2000 nM) concentration - dependently diminished neuropeptide release . It also inhibited neurogenic and non - neurogenic acute inflammatory processes but did not influence IL - 1 beta or zymosan - induced leukocyte accumulation . These results suggest that J - 2156 acting on sst4 inhibits neuropeptide release and vascular components of inflammation therefore opens new way in antiinflammatory treatment .

Keywords : somatostatin; sst4 receptor ; neuropeptides ; inflammation ; .
Grants : OTKA F - 046635 , T - 046729 , RET - 008/ 2005

P260100

Effect of monoamine uptake blocker antidepressants on the inflammatory response in noradrenalin transporter knock - out (NETKO) and B6 (WT) mice

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Object : Study whether inhibitors of the monoamine uptake system might modulate the LPS - induced inflammatory responses and this contributes to their antidepressant effect .

Methods : NETKO and WT mice were treated ip . with 10 mg/ kg of drugs , 30 minutes before LPS induction . Proand anti - inflammatory cytokine production was measured by ELISA technique . Results : Nisoxetine , desipramine , GBR12909 , and citalopram were effective on LPS - induced cytokine production in acute experiments not only in WT but also in NETKO mice . Combination of NET inhibitors with SSRIs or DAT inhibitors , resulted in an additive effect only in the NETKO animals . These effects could be reversed by propranalol demonstrating the role of NE via b - adrenoceptors , although in this case , the source of NE can not be noradrenergic varicosity . The antiinflammatory effect was more significant in acute experiments ,to achieve the antidepressant effect chronic administration was necessary . Conclusions : Our results show that SSRIs , despite their selectivity on the uptake system, can also enhance the noradrenergic neurotransmission by blocking the reuptake of NE by the serotoninergic terminals .

This work was supported by OTKA T - 046896 grant

P260101

Immune Cell Distribution in Mice Infected with Friend Leukemia Virus Treated with Modified GMDP

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The aim of present study , to analyze the immunophenotype changes induced by modfied muramyl glycopeptide (mGMDP) , arises from our findings concerning NK cell activation in the course of Friend virus (FV) infection . The effect of mGMDP followed in two mouse strains DBA/ 2 (sensitive , NK1 .1 $^{-}$) , and C57/ BL6 (resistant , NK1 .1 $^{+}$) allowed us to map the involvement of NK1 .1 antigen in viral pathogenesis and tumor development . The preventive treatment was followed on days 7 , 14 , and 21 after FV inoculation . The initial stimulation induced by FV (day 7) was replaced by progressive loss of T , B and NK cell numbers with contemporary proliferation of TER119 + leukemic cells in DBA2 (day 14) , but not in BL6 mice . On day 21 mGMDP partially recovered the number of monocytes , cytotoxic (NK, CTL) , and NKT cells in DBA2 , and enhances those in BL6 mice . Taken together , mGMDP activates the natural immunity in DBA2 , while increases the expression of NK1 .1 in BL6 mice . The CD11b and NK1 .1 receptors were detected as target structures of mGMDP therapy impact .

Key words : Friend virus , NK1 .1 , modified GMDP

Supported by grants GAUK 92/ 2004 , A500200620 , MZ - QF 3115 and Inst . Res . Concept Z40550506 , AV0Z50200510

P260102

Friend Virus Infection Modulated by a Modified GMDP

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The effect of modified muramyl glycopeptide a synthetic immunomodulator (IM) with adjuvant activity on Friend virus (FV) infection was studied . Two mouse strains , sensitive DBA/ 2 and resistant C57/ BL6 , were treated with 2 doses of IM prior to the virus inoculation . Splenomegaly , survival , splenocyte proliferative response to mitogens , and NK cell function were monitored . The preventive application of IM (day 10 and 3 prior to FV) significantly increased the survival rate of DBA/ 2 mice on day 45 post FV infection , even if did not influence the tumor development . IM temporarily restored the splenocytes proliferative response to LPS and NK activity . In C57 BL/ 6 mice , FV didn't induce malignant transformation while the immune responses were partially inhibited similarly to those in DBA/ 2 mice . IM restored the splenocytes response to both LPS , and T - cell mitogens . Our results indicate that preventive application of modified GMDP delay the FV induced disease progress as well as immunosuppression , and have a potential to improve antiviral therapy .

Key words : Friend virus , modified GMDP, NK cells , proliferation

Supported by grants GAUK 92/ 2004 , MZ - QF 3115 , and Inst . Res . Concept Z? 40550506 , AV0Z50200510

P260103

Glycoconjugates Induce NK Cell Differentiation and Functional Activation

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This study focuses to the effect of N - acetyl - β- D - glucosamine glycoconjugates (GCs) on cytotoxic cells (NK, NKT, CTL) morphology , distribution in different immune compartments , activation , and functional endpoints . FACS analysis following the morphology (FSC/ SSC) , phenotype (CD49b/ CD8) and activation markers (NK1 .1 , CD69) , as well as cytotoxic activity of purified spleen CTLs and NK cells using B16F10 melanoma model were performed . GCs induced cytotoxic cells (NK, CTL) transformation to monocyte and granulocyte morphology , without changes in NK1 .1 or CD69 , while NKT cells strongly down - modulate NK1 .1 expression in blood and spleen . In contrast , NK and NKT cells infiltrating the tumor up - regulate CD69 antigen . Both NK and CTL lytic activity increased against B16F10 target cells , but only NK cells - mediated cytotoxicity enhanced against IC - 21 targets . Summarizing these results , NKT cells can be considered as primary targets for GCs , initiating a cascade of events , leading to the NK cell differentiation , migration into tumor microenvironment and subsequent functional activation .

NK cell , GlcNAc - glycoconjugates

Supported by grants A5020403 , A400200503 , A500200620 , 524/ 05/ 0267 , AV0Z50200510

P260104

GLYCOGEN SYNTHASE KINASE - 3b INHIBITOR, TDZD - 8, ATTENUATES THE LIVER INJURY CAUSED BY ISCHEMIA - REPERFUSION IN THE RAT

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Glycogen synthase kinase 3b (GSK - 3b) is a serine/ threonine protein kinase involved in the modulation of the inflammatory response . GSK - 3b may play a pivotal role in the regulation of the activation of NF - kB and , deregulation of the enzyme has been implicated in the pathogenesis of several diseases . Knowing that the liver is particularly susceptible to ischemia/ reperfusion injury which is evident after conditions such as shock , trauma ,transplantation , and surgical hepatectomy , here we investigate the effects of a GSK - 3b inhibitor , TDZD - 8 (1 mg/ kg , i . v ., administered 30 min before ischemia) , on the liver injury caused by ischemia - reperfusion injury of the organ . In male Wistar rats , blood supply was interrupted to 3/ 4 of the liver during 30 minutes , followed by 2 hours of reperfusion . Ischemia - reperfusion resulted in hepatic injury , as assessed by the significant rise in the serum levels of ALT, AST, and LDH compared to sham - operated animals ; this injury was significantly reduced($p < 0.05$) by the pre - treatment with TDZD - 8 . Thus , inhibition of GSK - 3b may represent a novel approach for the therapy of liver injury caused by ischemia - reperfusion .

Key Words : GSK - 3b , Reperfusion injury , Liver , Rat

P260105

Effect of agmatine on carrageenan - induced acute lung inflammation in rats .

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The purpose of this study was to investigate the effect of agmatine onset of carrageenan - induced lung inflammation . When compared with carrageenan - treated rats exhibited a preponderance of pleural exudation and polymorphonuclear cell infiltration . Lung myeloperoxidase activity, an index of neutrophil infiltration and activation, was significantly increased in rats . Consistent with the biochemical markers of inflammation ,increased lung damage , as assessed by nitrosative stress and lipid peroxidation, was observed in carrageenantreated rats . In the lung exudate obtained from agmatine treated rats , a significant reduction in TNF - alpha was observed . The increases in polymorphonuclear cell infiltration , luminol and lucigenin chemiluminescence values were also reduced with agmatine treatment in

comparison with saline group. These results demonstrate that agmatine presents remarkable anti-inflammatory activity.

Key words: Agmatine, carrageenan, inflammation

P260106

PAR2-mediated protective mechanism against cerulein-induced pancreatitis

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Protease-activated receptor 2 (PAR2) is widely expressed in many tissues including pancreas. We previously reported that intra-pancreatic PAR2 activation protects pancreatic cell damages induced by various noxious stimuli. The aim of this study is to find out the molecular mechanism of protective effects by PAR2 activation in cerulein-induced pancreatitis. In this study, it was found that cerulein stimulation evoked a hyperphosphorylation of extracellular signal regulated kinase (ERK) in rat pancreas. Interestingly, PAR2 activation decreased the hyper-phosphorylation of ERK and the treatment of ERK inhibitors prior to cerulein injections significantly decreased pancreatic damages. PAR2 activation also strongly increased mRNA expression levels of pancreatitis-associated protein (PAP) I and PAP II in rat pancreas. Recent observations suggested that PAP I may have a protective effect against inflammatory damages in pancreas. The above results imply that the protective mechanisms by intra-pacreatic PAR2 activation may involve the dephosphorylation of ERK and induction of PAP I expression.

P260107

Anti-inflammatory activity of saponin fraction from Ilex pubescens

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Ilex pubescens (Mao-Dong-Qing, MDQ) is a commonly used Chinese herbal medicine for heart and inflammatory diseases. In this study, the anti-inflammatory fractions of MDQ were isolated and their pharmacological activity and chemical constituents were investigated. Fractions were obtained by solvent extractions and column separations and their anti-inflammatory activities were compared in two animal models, i.e. the paw edema of rats induced by carrageeman and histamine. Eight fractions were screened and fraction 8 was identified as the most potent faction in terms of anti-inflammatory action. Further fingerprinting and pharmacological studies revealed that the main chemical components in fraction 8 were saponins and fraction 8 showed significantly and dose-dependently suppression on the paw edema when given intraperitoneally in a range of dosage from 12.5-100 mg/kg. These findings have provided scientific data for our understanding on MDQ's effect and the way of search for the compounds with anti-inflammatory activity from this plant.

Key words: Ilex pubescens anti-inflammatory saponin

P260108

The protection of extract of Cyrtomii Rhizoma against lung impairment is related to the inhibition of complement components

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Purpose: To test the effect of Cyrtomii Rhizoma extract on the complement and its protection against lung impairment. Methods: The effect of Cyrtomii Rhizoma was carried out by the method of classical pathway of complement activation. By using complement-depleted serum, it is easy to identify which component was inhibited by the drug. Animal's lung impairment was induced by lowing the blood pressure and injection of lipopolysaccharide directly into trachea. The blood was taken to test the carbon dioxide and the complement activity. Results: Cyrtomii Rhizoma extract can inhibit the activation of complement system. And it probably affects the C3 or C4 but not C9. Animals with lung impairment were treated with 10 mg/kg drug. Compared with the control group, the rising level of carbon dioxide was decreased. The complement activity was also degraded. The coefficient correlation was -0.9318. Conclusion: Cyrtomii Rhizoma extract shows protecting role against lung impairment by inhibiting the complement components.

Key words complement inhibition; Cyrtomii Rhizoma; lung impairment
Acknowledgement: The project is granted by science and technology commission of Shanghai municipality (ID034319233).

P260109

Construction of cell lines expressing the human somatostatin receptors SSTR1 and SSTR4

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The peptide hormone somatostatin has been shown to inhibit the release of inflammatory neuropeptides from the nerve endings of capsaicin sensitive neurons and thus prevent inflammation. Previous studies indicated that somatostatin exerts its anti-inflammatory effects through the SSTR4 and possibly the SSTR1 receptor. To facilitate large scale screening for pharmacologically active compounds acting on these targets, two new cell lines were created which express the human SSTR1 and SSTR4 receptors. CHO-K1 cells were transfected by a lentiviral system containing the human SSTR1 and SSTR4 cDNAs. In the constructs the SSTR cDNA is followed by an internal ribosome entry site allowing separate expression of the enhanced green fluorescent protein from the same mRNA. Stable and uniform expression of the SSTR receptors were demonstrated by RT-PCR, flow cytometry and immunohistochemistry. Binding of somatostatin-14, somatostatin analogue peptid TT-232, the selective SSTR4 ligand KD-5621 and the selective SSTR1 ligand KD-7825 were demonstrated by a radioactive binding assay.

Keywords: cell line, somatostatin, SSTR1, SSTR4
Supp. by grant: RET 008/2005

P260110

Resveratrol prevents CsA inhibition of proliferation and osteoblastic differentiation of mouse bone marrow-derived mesenchymal stem cells through an ER/NO/cGMP pathway

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The purpose of this study was to investigate the effects of resveratrol (RSVL) and cyclosporin A (CsA) on proliferation and osteoblastic differentiation of mouse BMSC cultures. Application of RSVL (10^{-8}-10^{-6} mol/l) resulted in a dose-dependent increase in [^{3}H]-thymidine incorporation, ALP activity and calcium deposition of BMSCs cultures, which was accompanied with the increase of NO production and cGMP content. Concurrent treatment with the estrogen receptor antagonist ICI182,780 (10^{-7} mol/l) or the NO synthase inhibitor, L-NAME ($6x10^{-3}$ mol/l) abolished the RSVL (10^{-6} mol/l)-induced increase in NO production and cGMP content and eliminated the RSVL-induced increase in proliferation and osteoblastic differentiation of BMSCs. In contrast, CsA (10^{-6}-10^{-5} mol/l) dose-dependently decreased [^{3}H]-thymidine incorporation, ALP activity and calcium deposition, which was accompanied with the reduction of NO production. Concurrent treatment with RSVL (10^{-6} mol/l) significantly reversed the CsA ($3x10^{-6}$ mol/l)-mediated decrease in NO production and restored the proliferation and differentiation potential. Our data suggest that RSVL may act through an ER/NO/cGMP pathway to reverse the inhibitory effect of CsA on BMSC cultures.

P260111

Selective Kallikrein Inhibitors Attenuate Hemorrhagic Lesions Caused by Kinin Antagonists in Experimental Acute Pancreatitis

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Kinin B2 antagonists prevent edema in acute pancreatitis but also cause hemorrhagic lesions. We investigated whether this is due to reduced influx of endogenous protease inhibitors and increased tissue kallikrein activity.

Pancreatitis was induced in anesthetized rats by i.v. infusion of cerulein. Rats were pretreated with the B2 antagonist icatibant and/or selective inhibitors of tissue kallikrein (TKI) and plasma kallikrein (PKI) [Evans et al., 1996]. The pancreatic tissue was analyzed for hemoglobin. Icatibant inhibited edema formation but caused a pronounced increase in tissue hemoglobin. Although TKI also in-

hibited edema, vascular damage was absent.

Hemorrhage caused by icatibant was largely attenuated by combined TKI and PKI. Influx of endogenous protease inhibitors was significantly reduced by icatibant and TKI. Tissue kallikrein activity was increased 10- 100 fold by icatibant, but was inhibited by TKI. We conclude that increased levels of active kallikrein in the pancreas cause hemorrhagic lesions when edema is absent. Inhibition of kallikreins thus could be a promising strategy for the prevention of hemorrhagic lesions in acute pancreatitis.

P260112

ROLE OF TRPV1 RECEPTORS IN BLEOMYCIN- INDUCED SCLERODERMA IN MICE

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Neuropeptides released from the activated capsaicin- sensitive, TRPV1 receptor- expressing sensory nerves modulate inflammatory processes. This study examines the role of TRPV1 and calcitonin- gene related peptide(CGRP) in bleomycin- induced scleroderma using transgenic mice.

Cutaneous sclerosis of TRPV1 receptor and CGRP gene- deficient mice (TRPV1$^{-/-}$ and CGRP$^{-/-}$) and their wild- type (WT) counterparts was induced by daily s.c. bleomycin injection during 30 days. Composite histological sclerosis score was calculated on the basis of thickening, leukocyte infiltration and amount of collagen boundles. The collagen- specific amino- acid, hydroxyproline, in the skin was measured with spectrophotometry. Quantitative real- time RT- PCR was used to determine type I collagen- alpha mRNA.

Bleomycin induced a marked skin thickening and fibrosis. Both sclerosis score and hydroxyproline content of the skin were significantly increased in TRPV1$^{-/-}$ and CGRP$^{-/-}$ mice compared to WT animals. Type I collagen alpha mRNA was significantly higher in bleomycin- treated TRPV1$^{-/-}$ mice.

These data suggest that CGRP released by TRPV1 activation exerts a protective action against fibrosis.

Grants: ETT- 598/2003, RET- 008/2005

P260113

Protective mechanism of bicyclol on immune- mediated liver injury in mice

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OBJECTIVE: Bicyclol, a new antihepatitis drug, has been found to protect against liver injuries induced by certain hepatotoxins. The present study was to investigate the mechanism of its protective effect on concanavalin A (ConA)- induced liver damage in mice. METHODS: Mice were pretreated with bicyclol (200 mg/kg/day) for four days before injection with ConA (25 mg/kg). The serum levels of cytokines were determined by ELISA method, the expressions of iNOS, IκB and ICAM- 1 in liver were measured by western blotting analysis. Hepatic cytokines expression was determined by quantitative RT- PCR. RESULTS: The increase of serum aminotransferases, IL- 6, IFNγ, liver IL- 6 and IFNγ production induced by ConA were markedly reduced in mice pretreated with bicyclol. The induced expression of iNOS protein, ICAM- 1, and the degradation of IκB protein caused by ConA were also inhibited by bicyclol treatment. CONCLUSION: Bicyclol protect mice against ConA by its inhibition of NFκB activation and iNOS expression, reduction of inflammatory cytokines and ICAM- 1.

Key Words: Bicyclol, ConA, IκB, iNOS, ICAM- 1

P260114

Inhibitory effect of Bulleyaconitine A(BLA) on some immune function in Balb/c Mice

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Objective: The aim of this study was to determine whether tulleyaconitine A (BLA), extracted from Acnoitum lontounense T. L. Ming as a beneficial analgetic and anti- inflammatory drug in southwest China, had inhibitory effect on some immune function. Methods: BLA 0.32 mg/kg or 0.16 mg/kg or 0.08 mg/kg were given intramuscularly from d0 to d7. After mice were sacrificed on d8, spleen- and thymus- index were recorded, splenocyctes proliferation were stimulated with or without concanavalin A or lipopolysaccharide. Phocysosis function of peritoneal macrophages (MΦ) was tested with neutral- red phocysosis assay. Interleukin- 2(IL- 2) in Supernatarts of splenocyctes and interleukin- 1(IL- 1) and nitric oxide (NO) in supernatants of macrophages were detected. The level of total IgG in serum was measured by ELISA method. Results: BLA 0.32 mg/kg inhibited splenocyctes proliferations, reduced the levels of IL- 1, IL- 2, and NO in supernatants. Treatment with BLA 0.32 mg/kg and 0.16 mg/kg lowered the thymus- index with the reduction of total IgG in serum. BLA suppressed phocysosis function of Mφ. Conclusion: BLA had the suppressive effect on some immune function of Balb/c mice.

Key Word: Bulleyaconitine A; total IgG; cytokine; lymphocytes proliferation

P260115

Contrd of Nocturnal Melatonin (MEL) Surge by TNFalpha (TNF) in rodents and humans- A "feed- back" of immune response on circadian timing

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Although MEL and analogs have been shown to interfere in immune response, the converse was not evaluated yet. Here we explored TNF effect on the transcription of the rate limiting enzyme in melatonin synthesis and tested if a similar modulation is seen in humans, expecting a common response for nocturnal and diurnal animals. Aa- nat mRNA levels from TNF (30ng/ml, 30min)- treated rat pineals stimulated with noradrenaline (100nM, 5h, 66.5 ±21.06% over basal, n=3) were significantly reduced (9.9 ±3.3, n=3, $p<0.05$), as determined by real- time RT- PCR. N- acetylserotonin levels followed gene transcription (36.8 ±3.9 vs 8.3 ±2.9 ng/well).

The evaluation of MEL and TNF in colostrum of 18 puerperae (11 healthy, 7 with mastitis) showed nocturnal MEL surge in healthy mothers (day; 4.1 ±0.4; night; 39 ±3 pg/ml, $p<0.01$), which have TNF values bellow the detection limit of the method (2.3 pg/ml), but not in mothers with mastitis (TNF: 34 to 547 pg/ml). Taking into account that melatonin inhibits neutrophil translocation, this effect of TNF on pineal gland is essential for mounting an inflammatory response.

Support: FAPESP, CNPq, CAPES

P260116

Modulation of Liuwei Dihuang decoction, a traditional Chinese medicinal prescription, on the immune responses in Campylobacter Jejuni (CJ)- primed mice

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Liuwei Dihuang decoction (LW) is a classical famous prescription for "nourishing kidney- yin" in traditional Chinese medicine. In this study its therapeutic effect and mechanism on autoimmune disease were explored.

Campylobacter jejuni (CJ)- primed mice were used as the animal model with autoimmune disease. The methods such as enzyme- linked immunoassay, plaque- forming cells (PFC) assay, FACS, RT- PCR and electrophoretic mobility shift assay were used. It was found that LW(5 and 10g/kg, i.g.) for 15d alleviated the liver chronic inflammation, decreased serum titers of anti- dsDNA and anti- nuclear antibodies, PFC production response and splenocyte proliferation of CJ- primed mice. The elevated percentage of Th cells, decreased Ts cells and ratio of IFN- g/IL- 10 mRNA and intensified IL- 10 NFAT expression in splenocyte of CJ- primed mice were all reversed. These results demonstrated that LW modulate the disordered immune responses in CJ- primed mice. This effect may be related with its restoration on the balances of Th/Ts and Th1/Th2 cells.

Key words: Liuwei Dihuang decoction, immunomodulation, Th/Ts, Th1/Th2

Acknowledgement: This study was supported by National Basic Research Program of China (G1999054401)

P260117

Therapeutic effect of a new immunomodulator H1521 on the progression of experimental lupus nephritis

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Transferring DBA/2 spleen cells into (C57BL/6 ×DBA/2) F1 (BDF1) mice induces a chronic graft-versus-host disease (GVHD) that resembles systemic lupus erythematosus in human. This study examined the effect of a newly developed immunomodulator H1521 on mice undergoing chronic GVHD and the possible mechanism.

BDF1 mice injected with DBA/2 spleen cells were treated orally with H1521 at 32 and 64 mg/kg for 6 weeks.

Beneficial effect was seen at 32 mg/kg and this treatment significantly suppressed the development of glomerulonephritis. The highly altered pattern of thymic subpopulations in the non-treated animals was normalized after H1521 treatment. IFN-gamma levels were significantly higher in the H1521-treated group in supernatants from cultured splenocytes, while IL-4 levels were unchanged, resulting in a shift from Th2 to Th1 cytokine dominance. Supernatants from cultured peritoneal macrophage cells taken from H1521-treated mice contained lower levels of TNF-alpha in comparison with those from untreated mice. Results suggested that H1521 administration might be of therapeutic benefit in experimental lupus.

Key words: lupus; glomerulonephritis; immunomodulation; Th1/Th2 cytokine

P260118

PfS-C, the aqueous extract from Periploca forrestii Schltr, is a potential anti-inflammatory immunosuppressant for treatment of Rheumatoid Arthritis

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Periploca forrestii Schltr (PfS) is a folk medicine usually used for prevention of Rheumatoid Arthritis (RA). In order to evaluate the anti-inflammatory immunosuppressive effect of the aqueous extract of PfS (PfS-C), the effect of PfS-C on acute inflammation, chronic inflammation, cellular immunoreaction and pain were studied with the croton oil induced ear edema, cotton pellet induced granuloma, 2,4-Dinitrochlorobenzene induced delayed-type hypersensitivity and acetic acid induced writhing response, respectively. The complete Freund's adjuvant (CFA) induced adjuvant arthritis (AA) rat was also used as the animal model to evaluate its treatment of RA. It was found that PfS-C (i.g.) inhibited acute inflammation, chronic inflammation and cellular immunoreaction. It alleviated the pain induced by acetic acid. Moreover PfS-C obviously inhibited paw edema, ankle girth, lymphocyte proliferation and IgG production, and increased the percentage of $CD4^+CD25^+$ regulatory T cells in peripheral blood of CFA induced AA rat. The results suggested that PfS-C might be a potential anti-inflammatory immunosuppressant for treatment of RA.

Key words: Periploca forrestii Schltr, anti-inflammatory, immunosuppressant

P260119

Differential Effects of IL-1α and IL-1β in Malignant Processes

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The family of the pro-inflammatory cytokine interleukin-1 (IL-1) consists of two agonistic proteins, IL-1α and IL-1β, and an antagonistic protein, the IL-1 receptor antagonist (IL-1Ra). In their recombinant form, IL-1α and IL-1β exert the same biological activities and bind to the same receptors. We have assessed the role of the IL-1/IL-1Ra molecules in the control of malignant processes. To distinguish between tumor cell and host-derived IL-1, we used knockout (KO) mice that lack functional genes of members of the IL-1 family, i.e. IL-1α, IL-1β, IL-1α and IL-1β (double KO) and IL-1Ra KO mice as well as 3-methylcholanthrene (3-MCA)-induced tumors in control and IL-1 KO mice. Microenvironment-derived IL-1β, rather than IL-1α, is essential for invasiveness of transplantable tumors and for chemical-induced carcinogenesis. IL-1β of both the malignant cell- and the host-origin were shown synergize in controlling invasiveness and metastasis of the tumor, while IL-1α was less important. Altogether, these results point to the therapeutic feasibility of the IL-1Ra, which neutralizes soluble IL-1 (mainly IL-1β), in tumor therapy, apart from its use in treatment of autoimmune diseases, such as RA

P260120

Influence of Eotaxin on the Chemotaxis Response of Primed and Non-Primed Human Eosinophils

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Eosinophils (EOS) isolated from healthy (HS) and allergic rhinitis subjects (ARS) were incubated with eotaxin or IL-5 before chemotaxis assay towards eotaxin or IL-5. Eosinophils were isolated using a magnetic cell sorting system. Chemotaxis of eosinophils from ARS towards IL-5 was 78% higher than that of healthy subjects.

Incubation of eosinophils with eotaxin did not change the interleukin-5-induced chemotaxis in HS, but it reversed the enhanced chemotaxis seen in eosinophils from ARS. Chemotaxis of eosinophils from ARS towards eotaxin was 65% higher than that of HS. Incubation of eosinophils with IL-5 significantly increased the eotaxin-induced chemotaxis in both subject groups, but such increases were markedly higher in cells from allergic patients. Our finding that eotaxin inhibits the enhanced eosinophil chemotaxis towards interleukin-5 in primed cells suggests that this chemokine may downregulate eosinophil accumulation into the nasal mucosa of allergic patients.

Key words: Allergic rhinitis; Eosinophil chemotaxis; Interleukin-5; Eotaxin.
Acknowledgment: Fapesp

P260121

AZITROMYCIN PRTOTECT AGAINST ETHANOL-INDUCED GASTRIC MUCOSAL DAMAGE IN RATS

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Neutrophil accumulation in the gastric mucosa has also been shown to induce microcirculatory abnormalities.

Excessive discharge of histamine could increase tissue blood flow as a consequence of vasodilatation is one of the characteristic of acute inflammation. Our study showed that ethanol given an oral dose (1 ml absolute) by gastric intubation induced massive submucosal and intramucosal hemorrhagie (UI = 14.85 mm) and increased accumulation of polimorphonuclear cells surrounding the hemorragic site, mononuclears in submucosa and mast cells. Administration of the azitromycin (given orally once daily 250 mg/BM, 4 days and last dose 2,5 h before stress) completly protected against stress bleeding and serious hyperaemia. Microscopic gastric mucose showed the cellular integrity and thickness of the mucus-secreting layer from the surface of the epithelium of the glandular mucosa, with low hyperaemia, focal accumulation of mononuclear cells (lymphocyts and monocyts) and perivascular mast cells in submucosis.

A role for gastric acute inflammation in gastric ulcer seems intuitively probable.

Key words: stress-ulcer, histology, azitromycin, rat

P260122

Desferrioxamine Inhibits NADPH Oxidase-Mediated Oxidative Stress and Adhesion Molecule Expression in a Murine Model of Inflammation

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Excess iron has been suggested to induce oxidative stress and accelerate the development of atherosclerosis. The goal of the present work was to determine whether the iron chelator desferrioxamine (DFO) could ameliorate oxidative stress and adhesion molecule expression in a topical in vivo model of inflammation. Dorsal air pouches were created in C57BL/6J mice by subcutaneous injection of air. DFO (100 mg/kg body weight) was directly injected into the air pouch once per day for 2 days, followed by lipopolysaccharide (LPS; 2.5 mg/kg body weight). The animals were sacrificed 24 hr later for analysis of oxidative stress markers and adhesion molecules in air pouch tissue.

Results showed that LPS up-regulated $p22^{phox}$, a catalytic subunit of NADPH oxidase. In parallel, LPS increased NADPH oxidase activity, superoxide levels, NFκB nuclear translocation and adhesion molecule expression. All of these effects

were strongly inhibited by DFO but not iron-loaded DFO, which was used as a control. These data suggest that metal chelation by DFO may exert anti-oxidant, anti-inflammatory and antiatherogenic effects in vivo by inhibiting upregulation of p22phox and limiting NADPH oxidase activity.

Key Words Adhesion molecule. DFO. LPS. $p22^{phox}$

This work was supported by NIH grant "Center of Excellence for Research on Complementary and Alternative Medicine Antioxidant Therapies" (CERCAT, NIH P01AT-002034).

P260123

Comparitive effects of dehydroepiandrosterone and analog on pro-oxidant damage

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Dehydroepiandrosterone (DHEA) is an steroid synthesis in adrenal cortex. DHEA has been found that the steroid induces serious deleterious side effects, such as inhibits cell growth and induces apoptosis. In this paper, compare pro-oxidant effects between DHEA and analog butane acid-(5-androsten-17-one-3β-ol)-diester (A1998). After separately administrated rats with DHEA 500 mg/(kg.d) or A1998 167, 500, 1500 mg/(kg.d) for a1, 2, 3 wk, change of liver and body weights were observed, and changes of lipid per-oxidation in liver mitochondrial and microsomal were measured by thiobarbituric acid-reactive substances (TBARS).

Administrating DHEA (500 mg/kg.d) decreased weight of rats and liver index with significant difference, compared with control group. Meanwhile, DHEA (500 mg/kg.d) and A1998 (1500 mg/kg) groups led the lipid peroxidation of liver mitochondrial and microsomal proteins to significant increase compared with the control group. In contrast, groups of A1998 (500 mg/kg.d) and A1998 (167 mg/kg.d) are normal. Compared with DHEA, A1998 can be developed to replace DHEA as an over-the-counter health food product.

Key words: Dehydroepiandrosterone; butane acid-(5-androsten-17-one-3β-ol)-diester; pro-oxidant damage

P260124

The effect of QingGan extract on the level of cytokines of experimental liver injury models

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Objective: To study the effect of QingGan extract on the level of cytokines of experimental liver injury models.

Method: The acute liver injury model was established by using D-Galactosamine (D-GalN). The immunological liver injury model was established by using Bacillus Calmette-Guerin (BCG) and lipopolysaccharide (LPS). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) activity and cytokines were determined.

Results: QingGan extract can decrease the transaminase level of both experimental liver injury models. It can also decrease TNF-α, IL-1β, IL-8 level of the D-GalN induced liver injury model in rats and TNF-α, IL-18 level of the immunological liver injury model induced by BCG and LPS in mice. Conclusion: QingGan extract has certain protective effect on experimental liver injury models. The mechanism perhaps related to the reduction of cytokines and need further research.

Key words: D-Galactosamine, lipopolysaccharide, liver injury.

P260125

Inhibition of VEGF by recombinant human endostatin contributes to improvement of rat adjuvant arthritis

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The formation of new blood vessels permits a supply of nutrients and oxygen to the proliferating synovial cells and augmented inflammatory cell mass in rheumatoid arthritis (RA). Angiogenesis inhibition is not dependent on a dysregulated immune system. Therefore, angiogenesis is an attractive target in treating RA. To investigate the mechanism by which recombinant human endostatin (RHE) inhibits angiogenesis, the number of new blood vessels, VIII factor related antigen and VEGF expression in synovial tissue were determined. It was found that RHE inhibited secondary rat paw swelling induced by CFA in a dose-dependent manner. Meanwhile, the number of new blood vessels in synovial tissue stained by HE was reduced after treatment with RHE. Additionally, RHE decreased the expressions of VIII factor related antigen and VEGF in both synovial tissue and primary cultured synoviocytes. These suggest that RHE inhibiting VEGF contributes to improvement of rat adjuvant arthritis.

Key words: recombinant human endostatin; adjuvant arthritis; angiogenesis; VEGF

P260126

Tilte: Changes in the expressions of NOS isoforms in pressure ulcer tissue of rats

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Pressure ulcer is considered to be a chronic wound accompanied by ischemia-reperfusion disorder. Generally, NO production in wound is augmented by an increased expression of nitric oxide synthases (particularly i-NOS). However, the changes in production and functional roles of NO in pressure ulcer are still unclear. The present study was performed to investigate the inflammation and changes in NOS expression in a new pressure ulcer model of rats. The animals were loaded a constant pressure (0.5 N/cm^2) for 5 days (2 hr/day) on their sacral area. In ulcer area, infiltration of numerous inflammatory cells was observed. As for NOS expression in ulcer area determined by western blot analysis, any NOS isoform (e-, n-, i-NOS) wasn't changed just after the last pressure-loading. Though, on the 3rd day after the last pressure-loading, expression of all NOS isoforms were increased. In particular, the level of i-NOS expression was markedly increased; it was about 5 times higher than that in control (unwounded) skin. These results suggest the possibility that inflammation induced by repeated pressure-loading involves an augmentation of NOS expression in ulcer area.

P260127

Extraction and Purification of Polysaccharides from Lappula Echinata Gilib and Observation on their Immunocompetence

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Objective: This research is to extract and purify polysaccharides from Lappula echinate Gilib (LEG), and study their immunological competence. Methods: The polysaccharides from LEG were purified by ion-exchange and gel chromatography on DEAE-Sephrose Fast Flow and Sephacryl S-200 column. The immunological function of the purified polysaccharide was studied in vitro with MTT method to observe the direct reaction and synergistic reaction with ConA on proliferation of murine lymphocytes. Result: The purified polysaccharide was a heteropolysaccharide that contented 57% glucose. It had obvious direct reaction on proliferation of lymphocytes at the concentrations of 0.01～0.2 mg/ml and this effect was dependent on dosage. Conclusion: The polysaccharide from LEG had the immunological competence.

Keyword: Lappula echinata Gilib (LEG), polysaccharides, immunocompetence

Acknowledgement: This study was supported by a project from Tianjin Medical University (No:2004XK35).

P260128

PPARα AGONISTS INHIBIT NO PRODUCTION BY ENHANCING iNOS DEGRADATION IN ACTIVATED MACROPHAGES

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Nitric oxide (NO) production through the inducible nitric oxide synthase (iNOS) pathway is increased in inflammatory and tissue cells in response to proinflammatory cytokines and bacterial products. In inflammation, NO has proinflammatory and destructive effects. Peroxisome proliferator-activated receptors (PPARs) are known to regulate the inflammatory processes. We examined the role of PPARα on the regulation of LPS-induced NO production and iNOS expression in murine J774 macrophages. LPS induced iNOS expression and NO production in J774 cells. PPARα agonists GW7647 and WY14643 inhibited LPS-induced NO production in a dose-dependent manner, but they had no effect on iNOS mRNA expression measured by quantitative RT-PCR. PPARα agonists reduced iNOS protein expression significantly when measured 12-24 h after addition of LPS but had only a minor effect at 8 h time point. Treatment with a proteasome inhibitor lactacystin reversed the effects of PPARα agonists. The results suggest that PPARα agonists reduce LPS-induced iNOS expression and NO production in J774 macrophages by enhancing iNOS protein degradation through proteasome pathway.

P260129

Imrecoxib inhibits interleukin-8 production through NF-kappaB activation signal pathway in HEK293 cells

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Objective: To investigate the mechanism of anti-inflammatory action related to nuclear factor-kappaB (NFkappaB) inhibition of imrecoxib, a novel and moderate selective cyclooxygenase-2 inhibitor. METHODS: Human embryonal kidney (HEK293) cells were treated with compounds or in combination with TNF-alpha. Cell viability and cytotoxicity were detected by MTT method and LDH assay, respectively. NF-kappaB activation was determined by luciferase reporter gene assay. Interleukine-8 (IL-8) content in medium was measured by ELISA. RESULTS: Imrecoxib was found to inhibit both constitutive and TNF-alpha-inducible NFkappaB activation obviously. Imrecoxib also suppressed IL-8 production and this suppression is significant for TNF-alpha-stimulated IL-8 production. No significant cytotoxicity and influence to cell growth from imrecoxib were observed. CONCLUSION: Imrecoxib inhibits NF-kappaB activation and therefore suppresses inflammatory cytokine IL-8 production.

Key words: imrecoxib; NF-kappaB; TNF-alpha; IL-8

Acknowledgement: This work was supported by "863" Project (No. 2001AA234021) and "973" Project of China (No.2004CB518906).

P260130

Heparin selectively inhibits both TNFa induced NF-kB and AP-1 activation in cerebral endothelial cells

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Heparin is well known as an anticoagulant. However, many recent studies have indicated that heparin can also show anti-inflammatory effects by inhibiting many inflammatory cytokines and adhesion molecules. The underlying mechanism of these has not been uncovered yet. In this study, we examined the effects of heparin on the proinflammatory transcription factors including NF-kB and AP-1 induced by TNFa in cerebral endothelial cells. We used bEnd.3 cells, a cell line originated from murine cerebral endothelial cells. We measured the activities of NF-kB and AP-1 using EMSA and Western blot for nuclear extracts. In this study, we found that heparin selectively inhibited the DNA-binding activity of NF-kB and AP-1 induced by TNFa. In the mechanism, however, we found that heparin did not affect the degradation of IkBa and the translocation of NF-kB induced by TNFa. The exact mechanism how heparin inhibits the DNA binding of NF-kB, not affecting the translocation of it, is under investigation. We also found that heparin inhibited the TNFa induced phosphorylation of c-jun. We believe that our finding provides the new insight into the mechanism of heparin as an anti-inflammatory drug beyond an anticoagulant.

P260131

Laboratory study of chronic eczema treated by Polysaccharide Nucleic Acid Fraction of Bacillus Calmette Guerin (BCG-PSN)

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Objectives: To investigate the mechanism of immuneoregulation of BCG-PSN on balb/c mice of chronic eczema caused by 2,4-Dinitrofluobenzene (DNFB). Methods: 40 balb/c mice of chronic eczema were divided into model group, the group of BCG-PSN(0.015 mg/kg), BCG-PSN(0.030 mg/kg) and BCG-PSN(0.060 mg/kg). drugs were given through muscle every other day. on the weekend of third, detected the percentage of $CD4^+$T、$CD8^+$T lymphocytes of peripheral blood with flow cytometry and calculated the ratio of $CD4^+$T and $CD8^+$T; measured serum levels of IL-2、IL-4 and γ-INF with Double-antibody sandwich ELISA method. Results: After treated by BCG-PSN, the serum levels of IL-2、γ-INF was increased significantly ($P<0.05$), while the serum levels of IL-4 was decreased significantly ($P<0.05$); the ratio of $CD4^+$T and $CD8^+$T was increased significantly ($P<0.05$). Conclusions: The mechanism of BCG-PSN may be related to the regulation and modulation the imbalance of T lymphocyte subgroup and cytokines production so as to enhance the cellular immunity in balb/c mice of chronic eczema.

Key words: Chronic eczema BCG-PSN DNFB

Acknowledgement: I thank members of department of pharmacology for their helpful instructions and comments.

P260132

Establishment of infectious tolerance in IDDM murine model and its mechanism investigation*

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To investigate the method and mechanism of establishing infectious tolerance in IDDM model by adoptive transfer of DC. We examined the properties of DC in IDDM induced by injection of multiple low dose of STZ in mice which treated with insulin subcutaneously. Infectious immune tolerance was established by DC injection cotransferred with diabetogenic spleen cells and lower dose of STZ in secondary recipients. Diabetic incidence together with $CD4^+$ $CD25^+$ T cells differentiation were observed and analyzed. DC abnormalities were found in diabetic mice with decreased expression of CD11c and lower MLR stimulation followed with insulitis. We showed that insulin administration once a week over 4 weeks resotred the functional and phenotype normality of DC. These dendritic cells with a normal surface marker and function adoptively transferred immune tolerogenic effects in recipients, which was associated with significant higher level of Treg cells compared with the control recipients received diabetic DC. Our findings suggest that DC generated by insulin subcutaneously treated mice can generate infectious immune tolerance to diabetes in a secondary STZ induced model which associates with Tcell regulatory pathway.

P260133

EFFECTS OF iNOS INHIBITOR 1400W ON INFLAMMATORY MEDIATORS IN OA CARTILAGE DETECTED BY ANTIBODY MICROARRAY

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The balance between anabolic and catabolic mediators is critical in the pathogenesis of osteoarthritis (OA). Interleukin-1 (IL-1) plays a central role in OA and its destructive effects are partly mediated by nitric oxide (NO) produced by iNOS pathway. In the present study we investigated secretion of 40 mediators regulating cartilage metabolism (e.g. cytokines and destructive enzymes) by human OA cartilage samples with an antibody microarray (RayBiotech). The role of NO in the production of these mediators was investigated by using a selective iNOS inhibitor 1400W.

Results: OA cartilage secreted spontaneously 28 out of the 40 measured mediators. IL-1β enhanced production of 26 inflammatory mediators along with increased NO production. Inhibition of NO production with a selective iNOS in-

hibitor 1400 W enhanced IL-10 production, and reduced the levels of MMP-10. Conclusions: OA cartilage produces many of the mediators involved in the pathogenesis of OA. The ability of 1400 W to enhance levels of protective IL-10 and to reduce production of destructive MMP-10 points to the anti-inflammatory and anti-erosive effects that iNOS inhibitors may have in the treatment of OA.

Key words: Osteoarthritis, Nitric oxide, IL-10, MMP-10

P260134

Development of a new pressure ulcer model of rats and its histopathdogical study

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Pressure ulcers continue to be a major health care problem because of much expense and time for treatment. To understand the complete etiology of pressure ulcer, new animal models which reflect the clinical conditions have been needed. The present study was carried out to develop a new pressure ulcer model of rat. To induce pressure ulcers, anesthetized male Wistar rats were loaded a constant pressure (0.5 N/cm^2) for 2 h/day during 5 days on sacral area by using a manchette for blood pressure measurement. Before loading pressure, the hairs of sacral area were removed, and the rats were soaked for 15 min in a warm water (37 °C) to humidify their skin surfaces. By hematoxilin-eosin (HE) stain for histopathological study, necrosis of skin organization where pressure was loaded was observed from epidermis to muscle layer. The lesion of muscle fibers was observed in not only the subjacent area where pressure was loaded but also its circumferences. Furthermore, rubefaction in epidermis, scabs and infiltration of numerous inflammatory cells were also seen. These results suggest that ulcers in this model might correspond to the stage II to III of clinical pressure ulcer.

P260135

A new acetaminophen (APAP) antipyretic and analgesic treatment strategy in children: using an initial loading dose

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A new antipyretic and analgesic APAP dosing schedule has been evaluated after revisiting APAP pharmacokinetics and pharmacokinetic-pharmacodynamic relationship. A lag-time to APAP maximal effect, ranging 7 to 20h, related to the time to obtain steady-state plasma concentrations and to a 1 to 2h lag-time in the time course to maximal antipyretic effect compared to time to maximal plasma concentration. To decrease this lag-time, the use of an initial APAP 30 mg/kg loading dose (twice a usual dose), followed by the usual 15 mg/kg/6h maintenance dose schedule has been suggested. Three controlled clinical trials in children were conducted:

- In febrile children a single 30 mg/kg (loading dose) demonstrated superiority to a 15 mg/kg single dose in time to 38.5 °C (-30 min), time below this temperature (+1h).
- Results of a repeated-dose trial confirmed these findings.
- Post-operative analgesic efficacy, clinical and biological safety were evaluated for 24 hours. A preventive post-operative nalbuphine-sparing effect that improved postoperative analgesia was observed in 1/3 more of the patients in the loading dose group. Excellent clinical and biological (liver enzymes) safety was recorded in both groups.

P260136

The effects of KLG-01 on bone protection

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AIM: To investigate the inhibitory effect of KLG-01 on bone destruction in mouse parietal bones and in collagen induced arthritis (CIA) rats and the suppressive effect of KLG-01 on osteoclastic bone destruction with cultured osteoclasts. METHODS: A coculture system constituted with MC3T3-E1 cells and bone marrow cells for osteoclasts formation was established in vitro. Stimulant IL-1β and different concentration of KLG-01 were added into the medium and the pits formed in the bone slice were measured. The calcium concentrations in rat parietal bones were quantitated. The effects of KLG-01 on bone protection in CIA rats were detected by X-ray assay. RESULTS: KLG-01 could significantly decreased bone lacuna and decreased Ca^{2+} releasing from rat calvarium induced by IL-1β. Furthermore, KLG-01 significantly ameliorated joint destruction in CIA rats. CONCLUSION: KLG-01 has significant inhibitory effect on bone destruction induced by IL-1β both in vivo and in vitro.

P260137

Effects of Pannax notoginseng total saponin on inflammatory immune factors in Atherosclerosis

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To investigate the effect of Pannax Notoginsed (PNS) on the content of interteukin-6 (IL-6), C-reactive protein (CRP) and circulation immune complex (CIC) in serum duing atheosclerogenesis in rabbits induced by high cholesterol food. Rabbits were divided into three groups: control group, atheros clerosis group and PNS group.

The level of IL-6, CRP and CIC were estimated at the end of 4, 6, 8 weeks. The extent of aortic atherosclerosis was measured with planimetry for the painted area. At the end of 4, 6, 8 weeks, serum level of IL-6, CRP and CIC in AS group were increased compared with control group, and there was significant difference in IL-6, CRP and CIC between AS group and PNS group ($P < 0.05$). The area and severity of aortic atherosclerosis in PNS group were decrease ($P < 0.05$). It was suggested that occurrence and development of AS had relation with inflammation and immune response. PNS could slow down the formation of AS by anti-inflammation and immune modulation. (This study was supported by NCF of China №30470465, 30371768)

Key words: atherosclerosis; Pannax Notoginsed; interteukin-6; C-reactive protein; circulation immune complex

P260138

Mechanism of Panax Notoginseng total saponin on stability of atherosclerotic plaques in rabbits

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Objective: To investigate the Mechanism of Panax Notoginseng total spaponin's effect on the stability of atherosclerotic plaques in rabbits. Methods: Atherosclerotic models in rabbits were made by hypercholesternia diet. Rabbits were divided into four groups, i.e. atherosclerosis model group and three therapeutic groups which were adminstrated ig PNS 15, 45, 120 mg/(kg d). TNF-α and IL-6 level in plaques and serum were determined with immunohistochemical and ELISA method, respectively. Results: 15, 45, 120 mg/(kg d) PNS decreased the expression of TNF-α and IL-6 in atherosclerotic plaques significantly after 8w therapy in rabbits ($p < 0.05$ or 0.01). The effects of PNS on serum TNF-α and IL-6 concentration were similar to that expression in plaques and correlated to PNS dose and therapeutic term. Conclusion: PNS could enhance stabilization of atherosclerotic plaques through anti-inflammation pathway.

Key Words: Panax Notoginseng total saponin; athero sclerosis; tumor necrosis factor-α; interleukin-6

(This study was supported by NCF of China №30470465, 30371768)

P260139

The effect of PAF recetor antagonist KWS-06 on acetic acid-induced gastric ulcer in rat and in vitro cultured gastric epithelia stimulated by TNF-α

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AIM: To investigate the protective effects and possible mechanisms of PAF recetor antagonist KWS-06 on gastric mucosa. METHODS: Wistar rats were randomly divided into five groups of 10 each, normal group, model group, and groups with 100 mg/kg, 30 mg/kg or 10 mg/kg of KWS-06. After 14 days of modeling the stomach tissues were collected to determine the ulcer size, histopathology, and gene expression. Human gastric epithelia GES-1 were stimulated with TNF-α, and the protein secretion and the gene expression were assessed. RESULTS: The

ulcer size were reduced and the bleeding and edema around the ulcer margin were alleviated by KWS-06. The TGF-αmRNA expression were augmented and the CINC-1 and iNOS mRNA expression were reduced by KWS-06 on the ulcer. The expressions of IL-8 and PAF receptor mRNA were augmented in GES-1 stimulated by TNF-α. KWS-06 could alleviate it. CONCLUSION: KWS-06 can alleviate the gastric ulcer, which maybe relate to increasing the expression of TGF-αand decreasing the expression of CINC-1 and iNOS. Moreover, KWS-06 can not only bind with PAF receptor competitively but also downregulate the expression of the PAF receptor.

Key words: PAF receptor antagonist

P260140

Effects of repeated antigen exposure to sensitized rats on agonist-induced NO production and its downstream signaling in nasal mucosal veins

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In allergic rhinitis, nasal obstruction is considered to be induced by both a dilatation of plexus caverosum and an increase in vascular permeability in nasal mucosa. Nitric oxide (NO), a powerful vasodilator, is suggested to be involved in allergic inflammation. In the present study, the effect of repeated antigen exposure on leukotriene D_4 (LTD_4)-induced NO production in nasal mucosa was investigated. The changes in mRNA expression of NO synthase (NOS) isoforms in nasal mucosae of the antigen-induced nasal hyperresponsive rats were also determined by immunoblottings. The mRNA level of iNOS, but not eNOS and nNOS, was significantly increased in nasal mucosae of repeatedly antigen challenged rats. In addition, the LTD_4-induced NO production in nasal mucosae of nasal hyperresponsive rats was markedly augmented as compared with that of control animals. Interestingly, the venodilatation induced by sodium nitroprusside, an NO donor, was also augmented in nasal hyperresponsive rats. Therefore, not only increased NO production but also enhanced NO responsiveness might be involved in the development of nasal hyperresponsiveness in allergic rhinitis.

key words: nitric oxide, NOS, allergic rhinitis, nasal hyperresponsiveness

P260141

Pharmacological studies with STW33-I, a polyphenol-rich willow bark extract used in back pain, show multiple mechanisms of action

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The pharmacological profile of the willow bark extract STW33-I was studied in vitro and in vivo for elucidating its clinical effects. In IFN/LPS treated monocytes, STW33-I reduced expression of iNOS, COX-2, Bcl2, Il1beta, Il6 and TNF-alpha, with IC50 between 10 and 200 ug/ml, and inhibited PGE_2, Il6 and MMP3 in chondrocytes. Activities of 5-LOX, hyaluronidase, elastase (HLE), COX-1 and -2 and oxidation in AAPH and XOD reactions were inhibited. In vivo, STW33-I (50 to 150 mg/kg b.w.) were effective in writhing test in mice, Randall-Sellito model, brewers yeast model, paw edema, adjuvant arthritis and air pouch model in rats. In the latter, PGE_2 and LTB4, Il1beta, Il6, TNF-alpha, TxB4, COX-2 and the antioxidative parameters MDH were decreased, GSH increased. These multiple mechanisms, including anti-inflammatory, -oxidative, -pyretic, joint protecting, and analgesic actions were mainly not due to salicylates, but to polyphenols, relevant for the proven therapeutic efficacy of STW33-I in back pain.

Key words: inflammation, pain, willow bark

P260142

Proteasome inhibition ablates liver injury induced by the intestinal ischemia-reperfusion

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BACKGROUND AND AIMS: To investigate the role of proteasome in the pathogenesis of liver injury caused by intestinal ischemia/reperfusion (I/R) METHODS: Thirty-two Wistar rats were randomized into (1) sham-operated (2) I/R, (3) and (4) lactacystin pretreated group (0.2, 0.6 mg/kg). Liver and intestine histology were observed. Serum levels of ALT, AST, LDH and TNF-α were measured. The expression of liver NFκB and ICAM-1 were assayed. RESULTS: Liver and intestine injury was induced by intestinal I/R, characterized by the significant rising of serum ALT, AST and LDH levels. As compared with control group, MPO activity in the liver and intestine tissues and serum TNF-α level increased significantly. Strong positive expression of liver ICAM-1 and NF-κB p65 was observed. Administration of lactacystin (0.6 mg/kg) markedly ameliorated liver and intestine injury and the liver NF-κB and ICAM-1 expression decreased significantly. CONCLUSION: This is the first study to demonstrate proteasome inhibitor ablates liver injury induced by intestinal I/R.

Key words: proteasome; liver injury; intestinal ischemia/reperfusion; NFκB;

P260143

VLO5, A HETERODIMERIC LIGAND OF alpha9/alpha4beta1 INTEGRIN INHIBITS NEUTROPHIL APOPTOSIS: INVOLVEMENT OF Bcl-2 FAMILY

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In this study we evaluated the effect of VLO5 on human neutrophil apoptosis and the involvement of integrin coupled signaling pathways in this process. VLO5 potently inhibited spontaneous apoptosis apparently through interaction with alpha9beta1 integrin and activation of Erk-2 and PI3K pathways as VLO5 induced Erk2 nuclear translocation, FAK-PI3K association, and LY294002, a PI3K inhibitor, and PD95059, an Erk2 inhibitor, reverted VLO5 effects. Accordingly VLO5 induced Bcl-xL expression and Bad degradation. Moreover VLO5 modulates Bax mitochodrial insertion and prevents cytochrome c release. These data suggest that interaction of VLO5 with alpha9beta1 integrin on human neutrophils might be related with its anti-apoptotic effect, which is dependent on PI3K and Erk2 activation.

P260144

IMMUNOMODULATING PROPERTIES OF TIBETAN MEDICINE MULTICOMPONENT PHYTOPREPARATIONS

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In connection with wide distribution of various pathological conditions related with disturbed immune system, search of new means increasing immunological reactivity of the body is currently one of the urgent problems of contemporary medicine. The objective of the given research was to identify immunomodulating properties of the multicomponent phytopreparations of Tibetan medicine: infusions «gumbrum» (Calendula officinalis L., Glycyrrhiza glabra L., Bistorta major S.F. Gray, Cinnamomum camphora (L.) Noes et Eberm, Vidis vinifera L.), «shizhid» (Rheum tanguticum Maxim, Sanguisorba officinalis L, Inula helenium L., Zingiber officinale Roscoe) and decoction «dig-da-shi-tan» (Gentianopsis barbata (Froel.) Ma, Odontites vulgaris Moench, Malus baccata (L.) Borkh, Sophora flavescens Soland) in experimental immunodepression induced by azatioprin. Based on the research conducted examinations, it is found that the means tested restore indices of the humoral, cellular and macrophagal chains of the immune response under azatioprin immunosupression. The means tested may be placed following the diminishing order of their immunomodulating activity as follows: «shi-zhid» → «dig-da-shi-tan» → «gum-brum». Efficiency of plant means is likely to be stimulated by a high variety of biologically active substances available in their composition, mainly polyphenolic compounds, polysaccharides, saponins, vitamins, micro- and macronutrients. Thus, the multicomponent plant means of Tibetan medicine may have good prospects for creation of new plant immunocorrecting preparations.

P260146

Effect of angelica A3 active component on isolated rat uteruses cyclooxygenase - 2 expression

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Objective To study the effect of Angelica A3 active component (A3) on lipopolysaccharides (LPS) induced rat uteruses cyclooxygenase - 2 (Cox - 2) gene expression up - regulation. Methods RT - PCR and Western blot were used to analyze the uteruses cyclooxygenase - 2 mRNA and the protein expression level. Results LPS 1 μg/mL could significantly increase the level of Cox - 2 mRNA and protein expression respectively from normal control group 0.159 ±0.021 and 122.2 ±19.7 to 0.381 ±0.141 and 183.6 ±16.7 (n = 8). Angelica A3 10, 20, 40, 80, 160, 320 mg/L could concentration - dependently inhibit increased Cox - 2 mRNA and protein expression stimulated by LPS respectively from tween - 80 control group 0.462 ±0.164 and 187.8 ±13.5 to 0.408 ±0.136 and 162.6 ±16.3; 0.368 ±0.126 and 155.0 ±17.0, 0.306 ±0.065 and 148.4 ±14.3, 0.250 ±0.084 and 133.6 ±13.3, 0.138 ±0.016 and 125 ±15.4, 0.008 ±0.003 and 119.4 ±14.4 (n = 8). Conclusion The mechanism of the effects of A3 on anti - inflammatory, analgesic and anti - dysmenorrhea may be related with the inhibition of the Cox - 2 gene expression up - regulation stimulated by LPS.

P260147

Effect of Aegiceras corniculatum (stem) extracts on arachidonic acid metabolism: A mechanistic study of its anti - inflammatory activity

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Aegiceras corniculatum, a mangrove is tradtionally used against rheumatism. Cyclooxygenase - 1 (COX - 1) and 5 - lipoxygenase (5 - LOX) are major arachidonic acid (AA) metabolizing enzymes that play a crucial role in inflammatory diseases. In present study, glycogen - induced rat neutrophils upon stimulation with Ca^{+2} - ionophore A23187 formed 5 - LOX products, leukotriene B_4 (LTB_4) and 5 - hydroxyeicosatetraenoic acid (5 - HETE). Similarly in human platelets, COX - 1 and 12 - LOX catalyzed the formation of 12 - hydroxyheptadecatrienoic acid (12 - HHT) and 12 - hydroxyeicosatetraencic acid (12 - HETE). All these metabolites were quantified by high performance liquid chromatography. Hexane and ethyl acetate extracts of the plant were found to suppress the formation of LTB_4 and 5 - HETE (IC_{50} = 0.8 μg/ml and 3.0 μg/ml). In human platelets, hexane extract inhibited 12 - HETE (IC_{50} = 0.36 μg/ml). Ethyl acetate extract dually inhibited the COX - 1 and 12 - LOX pathways implying specificity towards COX - 1 (IC_{50} = 0.086 μg/ml). A. corniculatum derived extracts can inhibit 5 - LOX, COX and 12 - LOX, suggesting its therapeutic potential in inflammatory and allergic diseases thereby justifying the traditional use of the plant.

Key words: Aegiceras corniculatum, anti - inflammation, eicosanoids

P260148

INHIBITION OF CLASSICAL PKC ISOENZYMES DOWN - REGULATES STAT1 ACTIVATION AND iNOS EXPRESSION IN MACROPHAGES

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In inflammation, high amounts of nitric oxide (NO) are produced by inducible nitricoxide synthase (iNOS) and it acts as a proinflammatory mediator. Protein kinase C (PKC) pathway represents a major signaling system in inflammation. The aim of the present study was to investigate the role of classical PKC (cPKC) isoenzymes (α, β and βI) in the regulation of iNOS expression and NO production in activated macrophages. LPS induced iNOS expression and NO production in J774 murine macrophages. PKC inhibitors RO318220 (inhibits PKCβ, γ and ε), G 6976 (inhibits PKCα, β and γ) and LY333531 (inhibits PKCβ) reduced LPS - induced iNOS expression and NO production in a dose - dependent manner. This was seen also with 6 h preincubation with 1 μM PMA, which down - regulated PKC expression. PKC inhibitors had no effect on iNOS mRNA half - life or NF - κB activation. In contrast, PKC inhibitors reduced STAT1 activation which may well explain their inhibitory action on iNOS expression. These results suggest that cPKCs, especially PKCβ, are involved in the up - regulation of iNOS expression and NO production in activated macrophages possibly through the activation of transcription factor STAT1.

P260149

DOWN - REGULATION OF TRISTETRAPROLIN EXPRESSION RESULTS IN ENHANCED IL - 12 AND MIP - 2 PRODUCTION AND REDUCED MIP - 3α SYNTHESIS IN ACTIVATED MACROPHAGES

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In inflammation the post - transcriptional regulation of transiently expressed genes provides a potential therapeutic target. One of the factors regulating cytokine expression at posttranscriptional level is tristetraprolin (TTP), which is known to destabilize TNF - α and IL - 6 mRNAs. The aim of the present study was to identify cytokines, whose expression is regulated by TTP. We established a TTP knock - down cell line by expressing shRNA against TTP (shTTP cell line). A cytokine antibody array was used to measure cytokine production in macrophages exposed to LPS. The LPS - induced production of five cytokines (IL - 6, IL - 12, MIP - 2, MIP - 3α and TNF - α) was altered in shTTP cells as compared to control cells suggesting that the expression of these five cytokines is regulated by TTP. Cytokines IL - 6, IL - 12, TNF - α and MIP - 2 (a homologue to human IL - 8) were expressed at higher levels whereas MIP - 3α was produced at lower levels in shTTP cells than in control cells. The present data provides novel inflammatory cytokine targets for TTP - mediated mRNA decay. Understanding the mechanisms controlling the mRNA stability of cytokine genes provides targets for treatment of inflammatory diseases.

Key words: tristetraprolin, inflammation, cytokine

P260150

THE IMPROVING OF METHYLPREDNISOLONE POTENCY AFTER INCORPORATED WITH LIPOSOME. AN ANTIINFLAMMATION STUDY IN CULTURE OF MICE'S SPLENOSIT

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Longterm utilisation at high dose of glucocorticcid is associated with serious side effects. By incorporating the drug into its vehicle such as liposome, the systemic side effect can be minimized. The aim of the study are to learn the pharmacological effect of L - MPLP, especially on antiinflammatory effect of this novel preparation, compared with the standard methylprednisolone (MPL). The parameter was the potency of L - MPLP in reducing gamma - interferon production in T - lymphocyte culture after stimulation with concanavalin A in vitro as well as in vivo. Gamma - interferon was assayed by ELISA method. The reduction of gamma interferon, in vivo, after the administration of L - MPLP at the dose of 2, 8 and 16 mg/kgBW respectively, showed significantly difference than a control group, while MPL did not. The addition of both L - MPLP and MPL in in vitro culture at the concentration of 5.10^{-3}, 5.10^{-2} and 5.10^{-1} mM have proved to suppress the gamma - inteferon production, where the supresion of L - MPLP has more effective than MPL, significantly.

Key words: antiinflammation, gamma interferon, liposome - methylprednisolone palmitate

P260151

The effect of ephedrae decoction on DNA damage in mice peripheral lymphocyte by single cell gel electrophoresis and with orthogonal design

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The effects of ephedrae decoction on DNA damage in mouse peripheral lymphocytes were investigated by using single cell gel electrophoresis (SCGE, also called comet assay) and with orthogonal design. The results showed that chronic treatment (administered once a day for consecutively 7 days) of ephedrae decoction showed no genotoxicity in mouse lymphocytes. The single herb Ephedra sinica Stapf could induce significantly DNA damage in mouse lymphocytes. While the

other single herbs Cinnamomum cassia Presl, Prunus mandshurica (Maxim.) Koehne, Glycyrrhiza uralensis Fisch showed no genotoxicity. Further studies showed that DNA damage in lymphocytes induced by Ephedra sinica Stapf was significantly inhibited by Cinnamomum cassia Presl and Glycyrrhiza uralensis Fisch.

Key words: ephedrae decoction; orthogonal design; single - cell gel electrophoresis; DNA damage

P260152

Activation of cerebral peroxisome proliferator - activated receptors gamma (PPARγ) inhibits brain inflammation after cerebral ischemia in the rat

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The accumulation of macrophages and activated microglia and up - regulation of cyclooxygenase (COX - 2) in neurons considerably contribute to the expansion of brain injury and neuronal death after cerebral ischemia. We studied the neuroprotective function of cerebral peroxisome - proliferator - activated receptor(s) gamma (PPARγ) in the rat brain after middle cerebral artery occlusion (MCAO) for 90 min followed by reperfusion. Intracerebroventricular infusion of pioglitazone (3 nmol/h), an agonist of the PPARγ, over a 5 - day period before, and 2 days after MCAO, reduced the infarct size and attenuated the invasion of macrophages and activated microglia in the peri - infarct regions. Pioglitazone also reduced the expression of COX - 2 and the number of cells positively stained for COX - 2. In primary cortical neurons expressing the PPARγ, pioglitazone suppressed COX - 2 induction in response to oxidative stress. This protective effect was reversed after co - treatment with GW9662, a selective antagonist of the PPARγ, demonstrating a PPARγ- dependent mechanism. Our results demonstrate that activation of cerebral PPARγ inhibits inflammatory reactions and contributes to the neuroprotection after cerebral ischemia.

Key words: brain, PPARγ, ischemia, neuroprotection

P260153

Effect of Triterpene Acids of Eriobotrya japonica (Thunb.) Lindl. Leaf on inflammatory mediums expression from alveolar macrophages of chronic bronchitis rats

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Objective To evaluate the effect of triterpene acids of eriobotrya japonica (thunb.) lindl. Leaf (TAL) on inflammatory mediums expression in alveolar macrophages (AM) of chronic bronchitis (CB) rats. Methods CB model was established by BCG + LPS injection and the in vitro experiments were used to investigate the effect of TAL on inflammatory mediums expression in AM of CB rats. IL - 1β, TNF - α and PGE_2 levels in the incubated supernatants were measured by thymocyte co - stimulating assay and radioimmunoassay. Immunocytochemistry staining was used for NF - κB detection. LTB4 level was analyzed by RP - HPLC. Results The level of TNF - α, IL - 1β, NF - κB, PGE_2 and LTB4 expression in AM of TAL groups were significantly decreased than that of CB group ($P < 0.05$ or $P < 0.01$), and there was a dose dependent trait. Conclusion TAL could inhibit NF - κB activation and led to down regulation of TNF - α, IL - 1β, PGE_2 and LTB4 expression in AM, which might be one mechanism of its anti - inflammatory effects in CB rats.

Key words: chronic bronchitis; alveolar macrophage; inflammatory mediums
Natural Science of China No. 30371766 and No. 30572355

P260154

Effect on passive cutaneous anaphylaxis and flat musculature of Plectrhanthus Amboinicus (Lour) Spreng 100 mg oral tablet

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We studied the anti - allergy properties conferred to Plectrhanthus amboinicus (Lour) Spreng (PAS) over the anaphylactic mediators and the flat musculature, so we carry out the evaluation of the influence of PAS 100 mg oral tablet in the histaminic, cholinergic and adrenergic transmission in vitro for that we determined the contractions of the isolated organ with a isotonic transducer coupled to a polygraph and over the passive cutaneous anaphylaxis tests in rats. As result we proved that the PAS 100 mg oral tablet increase the adrenergic transmission and inhibit the contraction induced by histamine on isolated guinea - pig ileum and we observed that PAS causes immediate contraction of ileum and later showed anticholinergic activity, besides the contraction induced by PAS was blocked with atropine $3X10^{-12}$ M; we speculate that PAS 100 mg oral tablet have cholinergic and anticholinergic activity, with prevalence of a blockade of the receptor. Also, we can affirm that PAS 100 mg oral tablet inhibit the passive cutaneous anaphylaxis in rats and conclude that the tablet can be used in the treatment of allergic disorder type I.

Key words: Plectrhanthus amboinicus (Lour) Spreng; allergy, preclinical studies.

P260155

3,4 - oxo - isopropylidene - shikimic Acid Protects Vascular Endothelial Cells from Lipid Peroxidation in vitro

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3,4 oxo - isopropylidene - shikimic acid (ISA) was one of the derivatives of shikimic acid which was extracted from the Chinese herb Bajiao Huixiang. We investigated the action of ISA on lipid peroxidation of human umbilical vein endothelial cells (HUVEC) induced by H_2O_2 in vitro. Malonaldehyde(MDA), superoxide dismutase(SOD) and catalase(CAT) in HUVEC were detected by colorimetric assays. The scavenging of free radicals were tested with xanthine oxidase system and Fenton reaction. Preincubation of HUVEC with ISA significantly alleviated the increased MDA production and the reduced activities of SOD and CAT caused by H_2O_2. But the activities of SOD and CAT were unchanged after incubation HUVEC with ISA only. Besides, ISA dose dependently decreased the superoxide anion radical and hydroxyl radical. Taken together, our results demonstrate that ISA protected endothelial cells from lipid peroxidation through scavenging the free radicals whereas had no direct effect on the activities of SOD and CAT. (Project supported by the State Science and Technology Commission grant, No2001BA701A07 - 14, P.R. China)

Key Words: 3,4 - oxo - isopropylidene - shikimic acid; vascular endothelium; hydrogen peroxide

P260156

Effects of Geraniin on Osteoporosis and Osteoclastic Bone Resorption

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Effects of geraniin were evaluated on tretinoin - induced rat osteoporosis and osteoclastic bone resorption. The model of female rat osteoporosis was induced by tretinoin. The concentrations of calcium, phosphorus and activity of alkaline phosphatase in serum were measured by colorimetric method. The concentrations of osteocalcin, calcitonin and estradiol in serum were measured by competitive radioimmunologic method. Bone density of femur metaphysic of rats was determined by QCT. Changes of the bone resorption ability of the osteoclasts were observed in 3rd and 7th days. The results showed that intragastric geraniin (50 and 100 mg/kg) increased the bone density of femur metaphysic of osteoporotic rats and uterus weight. The alkaline phosphatase activity and inorganic phosphorous content in serum were decreased. The levels of estradiol, osteocalcin and calcitonin in serum were also increased, but no effect on calcium concentration in serum. Geraniin significantly decreased the number and area of bone resorptive pits on bone slices. It is suggested that geraniin had anti - osteoporosis due to its suppression of osteoclastic bone resorption.

Key words geraniin; osteoporosis; osteoclasts, bone resorption
Acknowledgement: This project was supported by the natural science foundation of Yunnan Province (2004C0044M), the United Cultivation Base of Yunnan

Province for Innovative Talents of Medicine & Biotechnology and Pharmacological Innovative Group Foundation of Kunming Medical College.

P260157

Comparison of norepinephrine responsiveness of mucosal veins in vivo with that of isolated mucosal tissue in vitro in guinea pig nasal mucosa

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The vascular responsiveness of nasal mucosa has been frequently determined by using isolated whole mucosal tissues, although it is not clear whether the response of the whole tissue truly reflects the response of the vascular blood vessels (especially veins) in mucosa. In the present study, the in vivo responsiveness of mucosal veins was compared with in vitro responsiveness of isolated mucosal tissue in guinea pig nasal septa. The in vivo venous responsiveness to norepinephrine (NE) of guinea pig nasal septal mucosa was measured by changes in the diameters of mucosal veins, stereomicroscopically. The in vitro responsiveness to NE of isolated nasal septal mucosa was also determined by standard organ-bath technique. Application of NE induced concentration-dependent contractile responses both in vivo and in vitro with the pD2 values of 5.23 ±0.29 and 5.00 ±0.17, respectively. The equal potencies obtained by the in vivo and in vitro experiments suggest that an increase in tension of isolate nasal mucosal tissue might be due to the contraction of mucosal veins.

Key words; nasal mucosa, mucosal veins, norepinephrine, contraction

P260158

Antiinflammatory activity of Ampelopsis grossedentata in experimental animals

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Ampelopsis grossedentata (Hand.-Mazz) W. T. Wang grows wild in the southern region of China. Healthy tea has been made from the stems and leaves for treating common colds and pyretic fever, inflammatory, pain-swelling of pharynx and larynx as well as jaundice hepatitis, with a long history of several hundred years among the Yao people in China. The anti-inflammatory effects of the extract of Ampelopsis grossedentata (AGE) and its mechanism in experimental animals are studied in the present study. The data showed that AGE (5, 10g/kg, p.o., for 5 d) markedly inhibited hindpaw edema induced by carrageenin in rats, ear edema induced by dimethylbenzene, and increased capillary permeability in the mouse abdominal cavity induced by acetic acid. Moreover, the chemotaxis of WBC induced by CMC, the weight of cotton-pellet grahuloma in mice and the weight of croton oil-gas cyst in rats are suppressed markedly. The present study provided evidence that AGE has significant anti-inflammatory activities, suggesting the benefit action of Ampelopsis grossedentata for health.

Keywords: Ampelopsis grossedentata; antiinflammation

Acknowledgement This study is supported by the project of Key-Laboratory for New Drug Screen of Liaoning Province.

P260159

Protective Effect of Total Flavonoids of Chrysanthemum Indicum on Joint Damage in Adjuvant Arthritis Rats

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The protective effect of Total Flavonoids of Chrysanthemum indicum (TFC) on joint damage was studied by measuring the volume of non-injected hind paw, plasma malondialdehyde (MDA) content, superoxide dismutase (SOD) activity of red blood cells, nitrite and tumor necrosis factor (TNF) released from peritoneal macrophages (PMΦ) of adjuvant arthritis (AA) rats in different periods. The results showed that treatments of AA rats with TFC (84, 168, 336 mg.kg^{-1}.d^{-1}, ig 12-22d) could not only markedly inhibit paw swelling in AA rats, but also down-regulate their elevated MDA, nitrite and TNF contents as well as up-regulate their diminished SOD activity to normal levels. Correlative analysis suggested that suppression of arthritis of TFC could be associated with its reduction of elevated lipid peroxidation and restoration of anti-oxidative enzymes and the secretion of PMΦ.

KEY WORDS: TFC; arthritis adjuvant; lipid peroxidation; tumor necrosis factor

ACKNOWLEDGEMENTS: This research was supported by the Research Foundation of Anhui Medical University and the Key National Basic Research Foundation under grant 2002CCCC02900, these supports are gratefully acknowledged.

P260160

Recombinant human endostatin suppressed proliferation of fibroblast-like synoviocytes from adjuvant arthritis in vivo

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Objective: To investigate the effect of recombinant human endostatin on proliferation of fibroblast-like synoviocyte from adjuvant arthritis (AA) in vivo. Methods: AA rat model was induced by injection of intradermal complete Freund,s adjuvant (CFA). Three groups of AA rats received 1.25、2.5、5 mg/kg/d of endostatin respectively for 7 days after the secondary inflammation appeared. Synoviocytes from rat knees were excised and dispersed with sequential incubation of collagenase type II and trypsin. The proliferation of synoviocytes in vivo was measured by MTT assay. Hind paw volume of rat was measured by volume meter and the activity of IL-1β, TNF-α produced by synoviocytes was estimated with radioimmunoassay. Results: Recombinant human endostatin significantly reduced the secondary paw swelling and decreased the production of IL-1β and TNF-α from synovial supernatants. Endostatin resulted in a dose-dependent reduction in the number of synoviocytes and inhibited the proliferation of synovial fibroblasts in vivo. Conclusion: The systemic administration of recombinant human endostatin had an inhibiting effect on the proliferation of fibroblast-like synoviocytes from AA rat model in vivo.

Key words: recombinant human endostatin, synoviocyte, proliferation, adjuvant arthritis

Acknowledgement: Supported by National Natural Science Foundation.

P260161

Genotoxic Studies on Panax Ginseng, Polygonum multiflorum **and their Compatibility in Mouse Peripheral Lymphocyte Cells**

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The single cell gel electrophoresis (comet assay) was employed to assess the genotoxicities of Panax ginseng C. A. Meyer, Polygonum multiflorum Thumb and their compatibility on peripheral lymphocyte cell DNA in the mice. P. ginseng, P. multiflorum and their compatibility were orally administered to mice in low, middle and high doses for consecutive seven days. Cyclophosphamide was used as a positive control. Blood samples were drawn from the vein cluster behind the eyeball of the mice 2 hours after drug administration on the first, third and seventh days, respectively. P. ginseng (0.65 g/kg (low dose), 1.3 g/kg (middle dose), and 3.9 g/kg (high dose), p.o.) was found to have no harmful effect on peripheral lymphocyte cell DNA. P. multiflorum (3.9 g/kg (middle dose) and 11.7 g/kg (high dose), p.o.) was noted to have harmful effects on peripheral lymphocyte cell DNA on the first, third and seventh days as shown in changes of tail DNA (%), olive moment, tail length, or tail moment. The compatibility of these two herbs (5.2 and 15.6 g/kg, p.o.) showed harmful effects on peripheral lymphocyte cell DNA on the first day, as observed in tail DNA (%), tail length, tail moment or olive moment. However, the harmful effects were diminished on the third and seventh days. The above results demonstrated that P. ginseng, but not P. mulitiflorum, has no genotoxic effect in vivo on peripheral lymphocytes, and the combination of these two herbs could decrease the potential genotoxic effects induced by P. multiflorum, suggesting the rationality of the use of compatibility of herbs in the Traditional Chinese Medicine.

Key words: Panax ginseng; comet assay; genotoxic

P260162

The antiinflammatory and immunostimulating activities of Ampelopsis grossedentata

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The healthy tea made from the stems and leaves of Ampelopsis grossedentata (Hand.-Mazz) W. T. Wang is considered to be benefit for common colds and

pyretic fever, inflammatory, pain-swelling of pharynx and larynx as well as jaundice hepatitis. Our previous study on the activity of the extract of this healthy tea showed that AGE significant antiinflammatory activity was found in various experimental animal models. The purpose of this study was to examine further the antiinflammatory and immunostimulating activities. The results showed that AGE (5, 10g/kg, p.o., 5 days) markedly inhibited ear edema induced by dimethylbenzene in adrenalectomyized mice, the hindpaw edema and the levels of MDA and PGE_2 of extravasate induced by egg white in rats. Apart from these actions, AGE (0.3、0.6、1.2、2.5、5g/kg, p.o., for 15 days) showed a number of immunostimulating actions such as increasing the phagocytosis of monocyte of mice and potentiating the immune function hydroxyurea-treated mice. These results indicated that Ampelopsis grossedentata possesses significant anti-inflammatory and immunostimulating activities, which implies that it would be a potential candidate for further investigation as a new botanical drug for humans.

Key words: Ampelopsis grossedentata; antiinflammation; immunostimulation

Acknowledgement: This study is supported by the project of Key-Laboratory for New Drug Screen of Liaoning Province.

P260163

Prescribing Pattern of NSAIDs and Gastro Protective Drugs in Orthopedic patients in a Tertiary Care Indian Hospital

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Gastrointestinal (GI) toxicity is often associated with the use of NSAIDs, depending upon the agent, dose and concomitant risk factors such as age, corticosteroids, anticoagulants, alcohol etc. The aim of the present study was to investigate prescribing pattern of NSAIDs and gastroprotective drugs in orthopedic patients (340) suffering from low backache, polytrauma, arthritis etc. Non-selective COX inhibitors were more frequently prescribed (56%) than COX-2 inhibitors (44%) but gastro protective drugs were co-prescribed to 28% patients only. Forty-eight patients (14%) received two NSAIDs with GI protection with Proton pump inhibitors (PPI). Twenty per cent patients on selective COX-2 inhibitors received H2-blocker or PPI, whereas 74% patients on high dose of non-selective COX inhibitors were not prescribed any gastro protective agent leading to increased incidence of GI symptoms, gastric ulceration and bleeding in 65% patients.

Conclusion: Co-prescription of NSAIDs and gastro protective drugs is recommended.

Key Words: NSAID Pain Prescribing pattern

Acknowledgements: Authors are thankful to Indian Council of Medical Research, New Delhi, India for their financial support.

P260164

The study of Forsythoside in cleaning heat and toxins

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Objective: To ascertain whether Forsythoside(FOS) is one of the active substances in cleaning heat and toxins in the herb Forsythia suspense(Thunb.) Vahl. Method: use two feverish models (endotoxin-induced fever of rabbit, barm-induced fever of rat) to observe whether FOS have the antifebrile effect;establish three kinds of infection models respectively with coliform, bacillus pyocyaneus, staphylococcus aureus; in vitro, some research was done in antivirus, the content of endotoxin acted with FOS was tested. Result: body temeperature of feverish animals is lowered; and animals was protected against infection of bacterium, and living time of animals injured with toxin was prolonged. some kinds of virus were inhibited in multiplication, also the content of endotoxin was decreaseed. Conclusion: FOS has notable function in cleaning heat and toxins and has the value for further more study.

Key words: Forsythoside; cleaning heat and toxins; anti-infection; anti-inflammation

P260165

TIMP-1 Promoter Regulation In Astrocytes During Chronic Neuroinflammation.

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The pathogenesis of many neurodegenerative disorders is exacerbated by an imbalance between metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs). We previously reported differential TIMP-1 expression in acute versus chronic astrocyte activation, and in brain tissue of patients with HIV-1-associated dementia (HAD). To investigate TIMP-1 promoter regulation we used TIMP-1-luciferase reporter constructs in transfected astrocytes and interleukin (IL)-1β as a model proinflammatory stimulus. Our results demonstrated that promoter regulation is an important mechanism for TIMP-1 chronic downregulation in astrocytes. IL-1β downregulated TIMP-1 promoter activity through previously identified silencer regions. Other factors including tumor necrosis factor (TNF)-β and interferon (IFN)-β and HIV-1 enhanced the effects of IL-1β on TIMP-1 promoter regulation. The minimum TIMP-1 promoter demonstrated the strongest downregulation in promoter activity following activation of transfected astrocytes, suggesting the location of a silencer element. These data are important for unraveling the mechanisms underlying astrocyte responses during chronic inflammation.

Key words: TIMP-1, IL-1β, HAD

P260166

Effects of AST and AS-I on metabolism of oxygen free radical in senescent rats treated by hydrocortisone

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To explore the effects of Astragalosides (AST) and Astragals Saponin I (AS-I) on metabolism of oxygen free radical in senescent rats treated by hydrocortisone. Free radicals were believed to be one of the main causes of aging. Hydrocortisone (HC) induced obvious memory impairment of senescent mice accompanied with atrophy of the thymus and hippocampus. The results showed that the function of memory acquisition and the proliferation and interleukin-2 production of splenocytes induced by ConA in HC treated senescent rats are much less than those of normal control of the same age rats. The content of MDA and GSSG in cytoplasm and mitochondria from liver and brain of the rats are higher than that of normal control while reduced GSH content, the activities of Mn-SOD and CAT are significantly lower than those of normal control. Treatment with AST or AS-I could restore the cellular immunity, lower the MDA content, and restore activities of Mn-SOD in HC treated senescent rats.

Key Words: AST, AS-I, MDA, Mn-SOD, Hydrocortisone

Acknowledgments: Supported by the NSF of Anhui Province (No. 00144414), the "Sliwu" Technology Special Foundation of Anhui Province (No. 01803016) and Department of Anhui Province Education (No:2005hbz18)

P260167

Effect of Bavachin on Oxidation Damaging Endothelial Cell Induced by Endotoxin

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Objective To study the effect of Bavachin on oxidation damage and gene expression of endothelial cells apoptosis induced by endotoxin(ET). Methods: The third ~ fifth passages of the cultured ECs were divided into groups as follows: control, ET ($10\mu g \cdot mL^{-1}$), Bavachin (2×10^{-1} ~ 2×10^{-3} $mg \cdot mL^{-1}$) + ET ($10\mu g \cdot mL^{-1}$). Oxidizing injury Model was performed by treating cultured endothelial cells (BAECs) with ET in medium. Cell viability was determined by MTT assay. MDA content was determined by TBA assay. SOD was determined by xanthine oxidase and visible light. NO content was determined by Griess. The expression of Bcl-2, Bax in endothelial cells was detected by immunocytochemical method. Results: In ET group, NO, MDA content and DNA fragmentation rate were increased. SOD vigor was decreased. Expression of Bax was increased. Expression of Bcl-2 was decreased. In Bavachin + ET group, NO, MDA content and DNA fragmentation rate were decreased. SOD vigor was increased effectively. Expression of Bax was decreased. Expression of Bcl-2 was increased significantly. Conclusion: Bavachin may protect ECs by inhibiting oxi-

dation damage induced by ET.

Key Words: Endothelial cells; Bavachin; ET; effect

P260168

Dissecting the anti-inflammatory effect of rosiglitazone

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Recent studies suggest that the thiazolidinedione class of PPAR-gamma ligands, may be clinically beneficial in several inflammatory diseases, even if the molecular mechanisms responsible for these activities have not yet been clarified. In this study, by using J774 cell line lacking PPAR-gamma, we demonstrate that PPAR-gamma expression is not essential for rosiglitazone anti-inflammatory activity which seems to depend on its ability to activate glucocorticoid receptor (GR) nuclear translocation as demonstrated by using different cell lines (J774 and GR conditional cell line). Furthermore, we found that in GR conditional cell line rosiglitazone induces nuclear co-immunoprecipitation of GR and NF-kappaB p65 subunit. This observation may further explain the molecular mechanism underlying the anti-inflammatory activity of rosiglitazone.

Keywords: Rosiglitazone, anti-inflammatory activity, glucocorticoid receptor, nuclear factor-kB.

Acknowledgments: We thank Dr. Vedeckis for providing E8.2 and E8.2/GR3 cell lines. Supported by the Italian PRIN 2003.

P260169

Impact of duration and severity of persistent pain on programmed cell death

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Programmed cell death is a highly regulated form of cell death mostly distinguished by the activation of a family of cystein-aspartate proteases (caspases) that cleave various proteins resulting in morphological and biochemical changes characteristic of this form of cell death. Several recent studies have addressed the role of programmed cell death in inflammatory and chronic pain states. Caspase-3 plays a central role in mediating nuclear programmed cell death including chromatin condensation and DNA fragmentation as well as cell blebbing. The aims of this study were to investigate the effect of duration and severity of persistent pain on induction of programmed cell death. Formalin was administered subcutaneously in the Wistar rat hind paws 1, 4 or 7 consecutive days, and then the activity of caspase-3 was measured in both rat liver and brain cells. Morphological changes characterizing programmed cell death was also studied using Sigma's Apoptosis Detection kit, Annexin V-Cy3. Our findings showed that caspase-3 activity and apoptotic phenotype significantly increased in liver but not brain cells following the increase in duration and severity of formalin induced persistent pain.

Key words: Inflammatory pain, Reactive oxygen species, Caspase, programmed cell death, Glia, Hepatocytes, Rat.

P260170

Marked deficiency in neutrophil recruitment during polymicrobial sepsis is dependent to TLR2 and TLR4 signaling

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We demonstrated failure of neutrophil migration into the infectious focus in severe sepsis, which is mediated by nitric oxide, which release is mediated by circulating cytokines. This study aimed to investigate the role of TLR2 and TLR4 on the failure of neutrophil migration to infection focus in mice subjected to polymicrobial sepsis. TLR2 deficient (TLR2$^{-/-}$), C57BL/6, C3H/HePas and TLR4 mutated C3H/HeJ mice were subjected to sub-lethal or lethal polymicrobial sepsis induced by cecal ligation and puncture. Mice were killed 6h after sepsis induction and neutrophil migration, bacteremia, lung neutrophil sequestration, cytokines were evaluated. It was observed that TLR2 and TLR4 signaling are not essential to display neutrophil migration in sub-lethal CLP, but they are crucial to establish the impairment of neutrophil migration in lethal CLP, since TLR2$^{-/-}$ and C3H/HeJ mice did not present failure of neutrophil migration. As consequence, these animals presented low bacteremia and high survival and low systemic inflammation determined by levels of circulating cytokines and lung neutrophil sequestration. These results highlight the harmful role of TLR2 and TLR4 signaling in polymicrobial sepsis.

P260171

Lipopolysaccharide induces upregulation of glyceraldehyde-3-phosphate dehydrogenase in rat liver and lungs

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Bacteria endotoxin or lipopolysaccharide (LPS) could trigger inflammatory responses and cause damages in organs such as liver and lungs when introduced into mammals, but the exact molecular events that mediate these responses had remained obscure. In this study, we found that both protein and mRNA levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were significantly increased in rat liver and lungs after treatment with LPS when analyzed in 2-D gel electrophoresis and cDNA microarrays. The results were further confirmed by Western blots and Northern blots. Given the known role of GAPDH in inducing apoptosis, our results suggest that LPS-induced GAPDH upregulation might be an important mechanism responsible for Gram negative bacteria-induced mammalian tissue damage and GAPDH might be involved in LPS signaling pathway. Our results also demonstrate that GAPDH is not a suitable internal control in gene expression studies, especially when bacteria infection is involved.

Key words: LPS; GAPDH; Liver; Lungs; Rat.

Acknowledgement: This work is supported by the Natural Science Foundation of China (Project No.30271658, 30472286).

P260172

Modulatory Effects of Food Supplements on Iodoacetate-Induced Osteoarthritis on Joint of Knee Rat

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Histopathological alterations following consumption of glucosamine (GA) and chondroitine were studied on in vivo model of osteoarthritis in tibiofemoral joint of male rats. Single intraarticular injection of iodoacetate (1 mg/knee) was administered to left knee. GA and chondroitine, given orally, were examined for their ability to affect histopathological changes in damaged cartilage. Disorganization of chondrocytes, erosion of cartilage surface, subchondral bon exposure, and reduction in proteoglycan diffusion in cartilage were observed in iodoacetate-injected knee after staining with hematoxilin/eosine and toluidine blue. GA alone or in combination with chondroitine prevented negative effects of iodoacetate on chondrocytes, and proteoglycan and led to a more pronounced intensity of glycosaminoglycans reactions, however, chondroitine alone did not produced significant improvement. The present study revealed that in iodoacetate model of osteoarthritis, which mimics the OA in human, glucosamine has the ability to modify histopathological changes while the efficacy of chondroitine may not be pronounced after injuries has been established.

Key words: osteoarthritis, animal model, food supplements

P260173

NOVEL MECHANISM OF ACTION OF DICLOFENAC ON CYCLOOXYGENASE

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It is generally accepted that NSAIDs exert pharmacological actions by inhibiting the formation of prostaglandins. However, it has been reported that NSAIDs (diclofenac) induces cyclooxygenase (COX) activity in transformed monocyte/macrophage cell line (Simmons et al 1999). We report the effects of diclofenac on the expression of brain and spinal cord COX-2 mRNA and protein in the rat tail ischaemia-reperfusion-hyperalgesia model, and in the A549 cell line. COX-2 mRNA was quantitated by RNAse protection assay while COX-2 protein was analysed by Western blot. We found that diclofenac (sc 40 mg/kg) effectively abolished the hyperalgesia from the injury and that COX-2 mRNA and protein levels in the brain and the spinal cord were elevated following hyperalgesia. Surprisingly diclofenac significantly further increased (300% - 800%) the expression of COX-2 mRNA in the brain and spinal cord. When incubated with A549 cell line, diclofenac exhibited both inhibitory (5 to 10 μM) and inducing ($\geq$50 μM) effects on PGE_2 production. We conclude that an antihyperalgesic dose of diclofenac upregulates, not inhibits as predicted, COX-2 expression in vivo and diclofenac exhibits a biphasic effect in vitro. These findings challenge the current understanding of NSAID mechanisms of action.

Key words: NSAIDs, diclofenac, cyclooxygenase, hyperalgesia

P27. Cytokine and Autacoids

P270001

The effects of COX inhibitors on endothelial cell proliferations activated by Human Cholangiocarcinoma cells

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Cholangiocarcinoma is a malignant epithelial neoplasm arising within the biliary tract. Significant progress has been made over the past several years in defining the link between COX pathway and this cancer. We investigated the effects of COX inhibitors on cell proliferations of HUVEC activated with conditioned medium (CM) from Human Cholangiocarcinoma cells (HuCCA) culture. Cell proliferations were measured by using MTT, Thymidine and crystal violet assay. COX protein expression was measured by immunoblotting. CM from HuCCA can significantly induce cell proliferations and COX-2 expression in HUVEC. Interestingly, NS-398, paracetamol, dipyrone and phenacetin could inhibit cell proliferations in a dose dependent manner. SC-560, but not by VSA, can also significantly inhibited cell proliferations. At higher dose of aspirin and indomethacin can also inhibit cell proliferations. Thus, CM from HuCCA can induce cell proliferation of HUVEC through the expression of COX-2. These cell proliferations can be inhibited by various COX inhibitors suggesting the roles of each COX isoform and potential use of NSAIDs in initial step of cancer metastasis through angiogenesis pathways.

P270002

PGE_2 causes endothelium-dependent vasodilatation through EP4-receptor-mediated stimulation of NO synthesis in mouse aorta

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In the present study we validated isolated mouse aortic rings for isometric force measurements and addressed pathways by which PGE_2 causes vasodilatation. Phenylephrine (PE, 10^{-5} M) followed by acetylcholine (10^{-6} M) was added at the start of each experiment to test the viability of the smooth muscle and the endothelium, respectively. All experiments were conducted in the presence of the COX-inhibitor Indomethacin ($5x10^{-6}$ M) and the TP-receptor antagonist S18886 (10^{-7} M). PGE_2 relaxed PE-constricted aortic rings ($IC_{50} = 5x10^{-8}$ M). The PGE_2 mediated relaxation was blocked by the NO-synthase inhibitor L-NAME (10^{-4} M) and by the inhibitor of soluble guanylate cyclase, ODQ (10^{-6} M). The PGE_2 mediated relaxation was absent in segments without endothelium as in the aorta from $eNOS^{-/-}$ mice. The EP4-receptor blocker AE3-208 (10^{-8} M) abolished the PGE_2-mediated relaxation while the EP_4 agonist AE1-329 (10^{-7} M) mimicked the effect of PGE_2. Butaprost, an EP_2-agonist had no effect on vasoreactivity and PGE_2 dilated rings from the $EP_2^{-/-}$ mice. The PGE_2-mediated relaxation was significantly attenuated in rings from $EP_4^{-/-}$ mice. PGE_2 causes an endothelium-dependent EP_4-receptor mediated vasodilatation through NO.

P270003

Endogenous prostaglandins (PGs) regulate spontaneous contractility of non-pregnant porcine myometrium

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We have already demonstrated that prostanoid receptor populations (EP_1, EP_2, EP_3, FP, TP, IP, DP) similar to those in the human uterus are present in the porcine uterus. The aim of this study was to clarify the physiological roles of endogenous PGs and coupled prostanoid receptors in the regulation of spontaneous contractility. Western blotting and immunohistochemical studies revealed the expression of cyclooxygenase (COX)-1, but not COX-2, in myometrial cells. The level of expression was dependent on the muscle layer (longitudinal muscle, LM > circular muscle, CM). Treatment with COX-1 inhibitors significantly decreased tissue PGs contents and the amplitude of spontaneous contraction in the LM. However, the inhibitors were ineffective in the CM. PGE_2 and $PGF_{2\alpha}$ caused phasic contraction resembling spontaneous contraction in the LM but not in the CM. These results suggest that endogenous PGs liberated from myometrial cells regulate spontaneous contractility of the LM of the porcine uterus in an autocrine or paracrine manner.

Key words: prostaglandins, myometrium.

Acknowledgement: this work was supported by grant-in-aid for JSPS fellows from the Japanese Ministry of Education, Culture, Sports, Science.

P270004

Gastrointestinal-motility stimulating action of ghrelin in isolated chicken gastrointestinal tract

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Objective Ghrelin, the endogenous peptide for the growth hormone secretagogue receptor (GHS-R), stimulates GH release, food intake and gastrointestinal (GI) motility in mammals. Ghrelin also stimulates GH release but inhibits food intake in chickens. The different actions of ghrelin prompted us to examine the effects of ghrelin on GI motility of the chicken in vitro. Results Among rat, human and chicken ghrelin (ch-ghrelin), only ch-ghrelin caused transient contraction. The amplitude of contraction was highest in the crop and colon, moderate in the oesophagus and proventriculus, and weak in the small intestine. Desacyl-ch-ghrelin was ineffective. The contractile response to ch-ghrelin in the crop was not affected by tetrodotoxin (TTX), but that in the proventriculus was decreased by TTX and atropine to the same extent. D-Lys3-GHRP-6 (a GHS-R antagonist) attenuated the response to ch-ghrelin. Ch-ghrelin enhanced the EFS-induced contraction in the proventriculus. Conclusion GHS-R which is highly sensitive to ch-ghrelin was present in the chicken GI tract in a region-dependent manner. The location of the GHS-R differed in the crop and proventriculus.

Key words: ghrelin, chicken, stomach.

P270005

Up-regulation of histamine H1 receptors in nasal mucosa of allergy model rats and elucidation of the mechanism

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[Aim] Histamine H1 receptor (H1R) up-regulation in nasal mucosa of allergy model rats was examined and the molecular mechanism the up-regulation was studied. [Methods] Allergy model rats were developed by the treatment of toluene diisocyanate (TDI) to nasal mucosa of Brown-Norway rats. H1R mRNA was determined by real-time PCR. [Results] Both H1R up-regulation and preceding H1R mRNA elevation were induced in the nasal mucosa of allergy model rats after the provocation by TDI. H1R mRNA elevation was partially suppressed by antihistamines and completely suppressed by dexamethasone. H1R-

mediated H1R up-regulation and preceding H1R mRNA elevation was observed in HeLa cells. The H1R promoter was also activated. PKCχ isoform was suggested to mediate the up-regulation. [Conclusion] H1R mediated H1R up-regulation through the activation of H1R gene expression in HeLa cells. PKCχ isoform was involved in the up-regulation. H1R upregulation was induced in the nasal mucosa of allergy model rats partially through H1Rs. The mechanism of H1R gene expression is the target of dexamethasone.

P270006

7-Ketocholesterol induces death in human aorta smooth musde cell by TNF-α

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We found that 7-ketocholesterol changed the viability of human aorta smooth muscle cells (HAoSMC) not by cytotoxicity but by activation of tumor necrosis factor-α receptor (TNFR)-mediated death. Whereas TNF-α did not affect the viability in the presence of 7α-hydroxycholesterol or cholesterol, the cytokine induced HAoSMC death in the presence of 7-ketocholesterol as detected by morphology, viability, and fragmentation of chromosomal DNA. The HAoSMC death was inhibited by a neutralizing anti-TNF receptor 1 (TNFR1) antibody and by the caspase inhibitors of z-VAD and z-DEVD. Activations of caspase-8 and -3 were detected from dying HAoSMCs. 7-Ketocholesterol inhibited translocation of the nuclear factor kB (NF-kB) subunits of p65 and p50 from the cytosol into the nucleus, increase of NF-kB activity, and expression of caspase-8 homolog Fas ligand interleukin-1-converting enzyme inhibitory protein by TNF-α. We also found that X-chromosome-linked inhibitor of apoptosis protein was degraded in dying HAoSMC.

P270007

Effect of macrophage migration inhibitory factor on expression of MMP-2 and MMP-9 in cultural macrophage

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OBJECTIVE The study was designed to investigate whether the macrophage migration inhibitory factor (MIF) can affect the expression of matrix metallcproteinase 2, 9 (MMP-2, MMP-9) in cultural human macrophage. METHODS 28SC human macrophage cell line was used in the study. Experiment divided six groups equally: In test group, MIF with different final concentration as 3.12 mg/L, 6.25 mg/L, 12.5 mg/L, 25 mg/L, 50 mg/L were added respectively in cultural human macrophage. In control group added nothing. After culture 24h together, all cells were extracted RNA using 1.0 ml of Trizol reagent. Reverse transcribtase polymerase chain reaction (RT-PCR) was applied to evaluate the mRNA expression level of MMP-2 and MMP-9. RESULTS Compared with the control group, the mRNA expression level of MMP-2 and MMP-9 significant increased in 12.5 mg/L, 25 mg/L, 50 mg/L MIF groups ($p < 0.05$). CONCLUSION MIF cytokine might play an important role in the progress of atherosclerosis by up-regulate the mRNA expression level of MMP-2 and MMP-9 in macrophage.

KEY WORDS: Macrophage, MIF, MMPs.

ACKNOWLEDGMENTS: This work was supported by NSFC (30300421) and GDNSF (015015, 04102307, 033189).

P270008

Effects of asymmetric dimethylarginine on erythrocyte deformability in streptozotocin-induced diabetic rats

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Objective: To investigate the relationship between erythrocyte deformability and endogenous inhibitors of nitric oxide (NO) synthase asymmetric dimethylarginine (ADMA) in streptozotocin-induced diabetic rats. Methods: Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 65 mg/kg) in male Sprague-Dawley rats. Results: (1) Erythrocyte deformability was significantly decreased concomitantly with the elevated levels of ADMA in both plasma and erythrocytes in diabetic rats. The contents of NO in erythrocytes were decreased at 8-week duration while those in plasma remained unchanged all along. The contents of MDA in erythrocytes were increased time-dependently. (2) The incubation of erythrocytes with ADMA (1 μM) decreased erythrocyte deformability. ADMA increased NO, MDA and ROS production in erythrocytes, which was reversed by L-arginine or vitamin E. Conclusion: Impairment of erythrocyte deformability is associated with elevated levels of ADMA in STZ-induced diabetic rats, and ADMA decreases erythrocyte deformability by triggering oxidative stress.

Key words: Asymmetric dimethylarginine; erythrocyte deformability; oxidation stress

P270009

Circular gastrotomy in rat: a new healing modd. Stable gastric pentadecapeptide BPC 157, atropine, cimetidine, omeprazde.

Zoricic Ivan, Sever Marko, Radic Bozo, Jakir Ana, Ercic Luka, Anic Tomislav, Seiwerth Sven, Sikiric Predrag[*]. Medical Faculty

Like gastric folds for stomach integrity, large flat areas without any fold could be particular for injured stomach and muscle damage. Circular gastrotomy was at 1cm bellow rat cardia, only 5mm at small curvature remained intact. Therapy (mg/kg) (gastric pentadecapeptide BPC 157 (in IBD, PLD-116, Pliva, Croatia) (0.01), cimetidine (50), atropine (10), omeprazole (50)) was i.p. once daily, first immediately after surgery, last at 24h before sacrifice (at 1h, 2h, 6h, 24h, 5 days, 7 days, 14 days). Results. Largely flatted stomach and only 40% area with thin gastric folds are along with poor healing of transected muscle, grossly and microscopically. Also, desmin immunohistochemistry/muscle regeneration shows sharp demarcation of positive fibers on the musclegranulation tissue border with only very scant immunoreactivity in vessel walls in the granulation tissue. All agents improved folds presentation, but only BPC 157 approaches to nearly 100%, with desmin immunoreactive cell clusters (muscle regeneration) penetrating the granulation tissue, and stronger immunoreactivity in vessel walls throughout the granulation tissue. Conclusion. BPC 157 is valuable for major stomach resection.

P270010

Gastric pentadecapeptide BPC 157-effective therapy of musde crush injury in rat, given intraperitoneally or applied locally as a cream.

Noviscak Tomislav, Staresinic Mario, Jukic Ivana, Kokic Neven, Pevec Damira, Mise Stjepan, Ercic Luka, Batelja Lovorka, Banic Tihomir, Jakir Ana, Buljat Gojko, Anic Tomislav, Romic Zeljko, Seiwerth Sven, Sikiric Predrag[*]. Medical Faculty

Stable gastric pentadecapeptide BPC 157 GEPPPGKPADDAGLV, M.W. 1419, PL10/PLD116/PL14736 Pliva, Croatia, in trials for inflammatory bowel disease, wound treatment, no toxicity reported, effective alone without carrier, also heals rat Achilles tendon or quadriceps muscle after transection. Therefore, after crush throughout 14 days (rat gastrocnemius muscle complex, impulse force 0.4653 Ns, kinetic energy 0.7217 J, force delivered 0.727 Ns/cm2), BPC 157 (without carrier, i.p. (10ug, 10ng, 10pg/kg) or locally (1.0 or 0.01ug dissolved in distilled water/g commercial neutral cream) as a thin layer) given only immediately after injury (sacrifice at 2h), and/or once daily (final application 24h before sacrifice) improves muscle healing (i) function (walking recovery, motor function index returned towards healthy values), (ii) microscopy (regenerating myofibres with centralized nuclei and desmin immunoreactivity), (iii) macroscopy (decreased injury severity (haematoma + edema + hyperaemia), surface haematoma, maximum circumference, muscle weight; no postinjury leg contracture), (iv) increased serum enzymes (CK, LDH, AST) values decreased. Thus, it should be a peptide for muscle healing.

P270011

Gastric pentadecapeptide BPC157 maintains esophageal mucosal integrity and sphincters pressure in esophagitis rats with pyloric sphincter failure

Petrovic Igor, Dobric Ivan, Drvis Petar, Shejbal Drazen, Batelja Lovorka, Brcic Luka, Boban Blagaic Alenka, Kokic Neven, Tonkic Ante, Mise Stjepan, Baotic Tomislav, Staresinic Mario, Anic Tomislav, Seiwerth Sven, Sikiric Predrag*. Medical Faculty

A stable anti-ulcer gastric pentadecapeptide BPC 157 is in IBD trials (PLD116, PL14736, Pliva). It recovers rat esophagitis, duodenogastroesophageal reflux, pyloric-/lower esophageal-sphincters (PS/LES) failure that otherwise appear with 1 week-tube into pylorus. Assessed at 1 or 2 weeks of esophagitis: (i) saline or BPC 157 (directly into the stomach 1 ml/rat; 5 ml/kg, 10ug/kg;, 10ng/kg) 5 min before LES-, PS-pressure (cm H_2O) (water manometer connected with drainage port of Foley catheter implanted into the stomach either through esophageal or duodenal incision) assess in esophagitis-rats constantly lessened both PS- and LES-pressure (controls), but prompt increase till the level in healthy and maintained pressure preserved at the healthy level in rats with potential esophagitis situation (BPC 157). BPC 157 in normal rats increases LES-, but decreases PS pressure. (ii) BPC 157 (10 ug/kg;, 10ng/kg) i.p. once daily or in drinking water in reflux esophagitis attenuates (both macro-(0 (normal) to 4 (the worst)) and micro-scopically esophageal lesion at either region, or either interval. Thus, this peptide recovers esophagitis and PS/LES malfunction.

P270012

Stable gastric pentadecapeptide BPC 157 heals gastrocutaneous fistula in rats.

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For gastrocutaneous (GC) fistula anaesthetized rats were subjected to laparotomy and gastrotomy, the open defect through the stomach (2 mm diameter) fixed by two stitches to front abdominal wall getting full communication between the lumen of the stomach and the skin defect (3 mm diameter). Therapy (stable gastric pentadecapeptide BPC-157 (in inflammatory bowel disease PL-10, PLD-116, PL-14736, Pliva, Croatia, heals external and internal wounds) compared with anticholinergics, H2-blockers, and PPIs) was given intraperitoneally (/kg), first application 30 min following surgery, last 24 h before sacrifice (at 1, 2, 3, 7, 14, 21 days postoperatively). Results. Pentadecapeptide BPC 157 (10ug, 10ng, 10pg) strongly improves both skin and stomach mucosa healing, and closure of fistulas since the earliest period, macro-/micro-scopically, and functionally (fistula does not leak upon volume application). Contrary, atropine (10 mg), cimetidine (50 mg), omeprazole (50 mg) improve firstly skin healing, and then stomach mucosal healing, but regularly fail to affect fistula leaking and bursting strength. Conclusion. Pentadecapeptide BPC 157 could solve complex healing of GC fistula.

P270013

Distended and filled stomach in rat and alcohol. Stomach, esophageal and duodenal lesion-omeprazole, ranitidine, atropine, pentadecapeptide BPC157

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Hyperemic response of left gastric artery along with fully stomach distention is studied in rat alone or with 96% alcohol ingestion (2 ml/stomach) into fully distended and filled stomach (12 ml water, 12 ml of air) that increases damaging potential of gastro-esophageal and-duodenal reflux, presenting lesion in proximal esophagus and duodenal bulbous besides stomach (as gray areas at 2, 5 and 15 min intervals, assessed (% of total area) at 2, 5 and 15 min intervals, digital compact camera, morphometry). Therapy (mg/kg, 2 ml/stomach) was immediately before. Results. Omeprazole (50) and stable gastric pentadecapeptide BPC 157 (0.01) (in IBD (PLD116, PL14736, Pliva)) increased, ranitidine (50) and atropine (10) decreased presentation of left gastric artery major branches of exposed stomach (% of initial value, at 5s intervals for 2 min). Alcohol antagonizes hyperemic response, an effect reversed with BPC 157. Lesion inhibition was in stomach (BPC 157), duodenum (BPC 157, omeprazole), esophagus (BPC 157, omeprazole, ranitidine, atropine). Conclusion. With increased hyperemic response, only BPC 157 protects stomach, esophagus and duodenum against damaging gastro-esophageal and-duodenal reflux.

P270014

Gastroesophageal reflux disease (GERD) associated osteopenia

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We determine osteopenia-gastroesophageal reflux disease (GERD) association in 131 subjects (no therapy for osteoporosis, not different nutrition, physical activity, alcohol consumption), randomly assigned, 62 with endoscopically determined GERD (35 female (F), 27 male (M)), and 69 rheumatic (RH) patients with normal endoscopy findings examined because of degenerative rheumatic disorders and needed NSAIAs (32 F, 37 M). They (min/med/max) had not different ages (GERD 34-65-84, RH 30-53-82), high (GERD 146-166-186 cm, RH 151-165-190 cm), weight (GERD 47-72-117 kg, RH 48-76-107 kg), menarche (GERD 12-14-19 y, RH 11-14-18 y), menopause (GERD 35-49-57 y, RH 38-49-55 y). Densitometry analysis (lumbosacral (LS) spine and left hip, > -1 (normal), -1.0/-2.5 (osteopenia), < -2.5 (osteoporosis)) shows dominating osteopenia in GERD: LS spine: GERD -4.6/-1.3/2.1, RH -3.2/-0.3/2.6, $P < 0.0002$, left hip: GERD -2.9/-0.9/2.8, RH -2.5/-0.2/2.2, $P < 0.003$. Osteopenia frequency: RH: left hip 22%, LS spine 10.1%, GERD: left hip 43.5%, $P < 0.008$, LS spine 22.6%, $P < 0.05$. Sex relation in GERD: LS spine: M -2.7/-0.6/1.4, F -4.6/-1.4/2.1, $P < 0.014$, left hip: M -1.5/-0.4/2.8, F -2.9/-1.1/1.1, $P < 0.028$.

P270015

Stable gastric pentadecapeptide BPC 157 heals ileoileal-anastomosis and counteracts corticosteroid-negative effect in rat.

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In inflammatory bowel disease (PL10/PLD116, Pliva, Croatia) pentadecapeptide BPC 157 should be valuable after resection for anastomosis healing. Rat ileoileal anastomosis healing (after 1, 2, 3, 4, 5, 6, 7, 14 days) was assessed in normal and impaired conditions: (i) adhesions (0-7 (neighboring loops, stomach, liver packed)), loop diameters, anastomosis arcade vessels, (ii) leak induction (the time (sec), the volume (ml) (through syringe perfusion pump system 1 ml/10sec) and the pressure (mmHg) (catheter (BD Careflow 5Fr 200 mm, Becton Dickinson, USA) connected with chamber (BD Gabarith PMSET 1DT-XX, Becton Dickinson, USA) and monitor Sirecust 732 (Siemens, Germany) at 10 cm proximal to anastomosis), and (iii) microscopy. Treatment was once daily (first after surgery, last at 24 h before sacrifice), saline, BPC 157 (10ug, 10ng, 10pg/kg i.p.) and/or 6-alpha-methylprednisolone (1 mg/kg i.p.). Results. BPC 157 clearly improves all parameters of anastomotic wound healing. Morevoer, the low dose of pentadecapeptide BPC 157, without effect by itself, is effective confronted with corticosteroids treatment that adversely affects healing of anastomoses in the rat. Thus, it is an effective peptide therapy.

P270016

The presentation and the organization of stomach-duodenum-colon adaptive cytoprotection in rat

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We define adaptive cytoprotection in the whole GI tract. With adaptive cytoprotection appeared, and lesion attenuated, challenged were stomach, duodenum or

colon , in various combinations , with initial and/ or final challenge throughout two weeks period . In rat , the specific challenges - mild / strong irritants were 25 % or 96 % ethanol i . g . 1 ml/ rat (stomach) , cysteamine 40 mg or 400 mg/ kg s . c . (duodenum) , or intrarectally (colon) . For prostaglandin relation known in Robert 's cytoprotection and adaptive cytoprotection , indomethacin (1 mg/ kg s . c .) was given simultaneously with second challenge . Results . Presenting mild and strong irritant protocol within the same part of GI tract , adaptive cytoprotection presents in stomach - stomach (i . e . , 1h - 14 days) , duodenumduodenum (i . e . , 2h - 14 days) , while not in colon - colon . With mild and strong irritant protocols that affect the different parts of GI tract to generate adaptive cytoprotection , cross - react stomach - duodenum , duodenumstomach (1h - 14 days , or 2h - 14 days) , stomach - colon , duodenum- colon (both 2 - 24h) , but not colon - stomach or colon - duodenum . This is fully antagonized with indomethacin . Conclusion . Evidenced for day - weeks , this is a new defensive phenomenon .

P270017

EFFECTIVE THERAPY OF TRANSECTED QUADRICEPS MUSCLE IN RAT : GASTRIC PENTADECAPEPTIDE BPC 157

Staresinic Mario , Petrovic Igor , Noviscak Tomislav , Jukic Ivana , Damira Pevec , Kokic Neven , Batelja Lovorka , Brcic Luka , Zoric Ivan , Seiwerth Sven , Sikiric Predrag [*] . Medical Faculty , University of Zagreb

Stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV , M. W. 1419 , PL - 10 , PLD - 116 , PL 14736 Pliva , Croatia , in trials for inflammatory bowel disease , wound treatment , no toxicity reported , effective alone without carrier , also heals Achilles tendon after transection . Therefore , after rat quadriceps muscle complete transection , BPC 157 (10ug , 10ng , 10pg/ kg) is given intraperitoneally , once daily , the first application at 30 minpost - transection , the final at 24 h before sacrifice . Throughout 72 days , it consistently improves muscle healing(i) biomechanic (load of failure increased) , (ii) function (walking recovery and extensor postural thrust/ motor function index returned toward normal healthy values) , (iii) microscopy (i . e . , always mostly muscle fibers connect muscle segments , absent gap , significant desmin positivity for ongoing regeneration of muscle , larger myofibrils diameters at both sides , distal and proximal (i . e . , normal healthy rat - values reached)) , (iv) macroscopy (stumps connected ; subsequently , atrophy markedly attenuated ; finally , presentation close to normal non - injured muscle , no post - surgery leg contracture) . Thus , it should be a peptide for muscle healing .

P270018

New Insight into the Mechanism of Hair Follicle Development : Protective Effect of Wnt5a against Apoptosis in Dermal Papilla Cells

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Wnt5a has been reported to be expressed in mature mouse anagen follicles while its function remains completely unknown . We here report that Wnt5a suppresses apoptotic death of primary dermal papilla cells (DPC) induced by serum deprivation (SD) . To examine the effect of Wnt5a on viability of DPC , we co - incubated DPC with Wnt5a - containing conditioned medium (CM) that had been prepared by culturing CHO cells stably overexpressing Wnt5a in a serum - free CD CHO Medium . Replacement with the control CM caused decrease in viability of DPC mainly because CM was deficient in growth factors . In contrast , replacement with Wnt 5a - CM did not result in loss of cell viability . In support of this observation , purified recombinant Wnt5a prevented death of DPC induced by SD in a dose - dependent manner . Furthermore , we found that induction of apoptosis markers in DPC by SD was suppressed by treatment with Wnt5a . Taking altogether , we have concluded that the Wnt5a suppresses cell death induced by SD . This novel anti - apoptotic function of Wnt5a will serve as an important initial clue to clarify how Wnts regulate hair development .

Key words : Wnt , dermal papilla cells , development , anti - apoptotic function

P270019

Inhibition of Gastric H^+/K^+ - ATPase K^+ - Site Is Invdved in Epidermal Growth Factor - Induced Suppression of Acid Secretion in the Mouse Stomach In Vivo and In Vitro

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We examined whether EGF suppresses histamine - stimulated acid secretion by the inhibition of gastric H^+/K^+ - ATPase K^+ - site via polyamines in vivo and in vitro . Gastric acid secretion was measured in anesthetized mice with pylorus ligation (in vivo) and isolated mouse stomachs (in vitro) . EGF significantly and dose - dependently suppressed stimulated acid secretion in vivo and in vitro , whose suppression in acid secretion in vitro was abolished in the presence of ornithine decarboxylase inhibitor , alpha - difluoromethylornithine . Exogenous polyamine spermine also significantly and dose - dependently suppressed stimulated acid secretion in vivo . Those suppressions with EGF and spermine , however , were significantly reversed in the luminal side of medium with the increased concentration of KCl from 5 .9 to 40 mM in vitro , which were quite similar to the suppressant fashion with SK&F 96067 , a competitive K^+ - site inhibitor of gastric H^+/K^+ - ATPase . These data suggested that the suppression of EGF on histamine - stimulated acid secretion , at least partly , involves the inhibition of gastric H^+/K^+ - ATPase K^+ - site via polyamines .

Key words : Gastric acid secretion , EGF , Polyamines , SK&F 96067

P270020

PLASMA ENDOSTATIN LEVELS AFTER PARTIAL OR SALVAGE LIVER RESECTION IN MICE

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Endostatin is a potent endogenous angiogenesis inhibitor which induces tumor regression . The aim of the study is to evaluate the effects of partial and salvage liver resections on endostatin levels . The Swiss Albino mice were anaesthetized with thiopental sodium . In control group (C) liver was mobilized following median laparotomy . Blood samples were withdrawn from the 40 % (salvage) hepatectomized mice , groups I and II , on the 1st and 15th day of surgical procedure , respectively , similar to the 25 % (partial) hepatectomized mice , groups III and IV . Plasma endostatin level was detected by using a sandwich immunoassay technique . There is no significant difference in endostatin levels between groups C and I . Plasma endostatin levels were greater in groups II , III and IV than that in controls ($p < 0.05$) . The most elevated endostatin levels were determined in group IV ($p < 0.01$) . Partial liver resection might be considered as an alternative to nonsurgical modalities or salvage hepatectomy , by inducing a progressive increase in endostatin level .

Key words : Endostatin , Hepatectomy Supported by B . U . Research Grant .

P270021

Cytokines effects on intracellular calcium from area postrema neurons .

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Besides their immunological actions , cytokines elicit some important neurological effects . Symptoms ranging from fever and anorexia to major psychosis had been reported after the administration of cytokines . Moreover , the systemic and local application of these cytokines modifies the electrophysiological pattern of neurons in several SNC sites . Since the main effect induced is a long - lasting increase of the excitability , this could represent an action mediated through calcium channels . Rat cultured neurons from area postrema were used . Intracellular calcium concentration was measured using fura - 3 after the addition of several concentrations of interferon - alpha and interleucine - 1beta and calcium influx through voltage - dependent calcium - channels was determined using patch - clamp techniques . Both cytokines increase the intracellular calcium concentration in a dose - dependent faslion . In addition , the currents mediated by low voltage activated calcium channels but not by high voltage activated were increased by both cytokines . These results show the mechanisms used by cytokines to modify the excitability of neurons .

P270022

The role of interleukin-1 family in the development of Leishmaniasis

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To assess the role IL-1 family in the development of Leishmaniasis, IL-1 gene family knockout (IL-1/IL-1Ra KO) and BALB/c mice were injected with murine L. Major promostigotes. Most progressive form of disease was observed in IL-1Ra KO mice, in which unattenuated level of IL-1 exists, whereas in mice deficient in IL-1 genes, there was significant delay in disease development and mortality. Injection of rIL-1 to IL-1 deficient mice induced exacerbation of the disease, while, injection of IL-1Ra to mice deficient in IL-1Ra led to a delay of wound development and mortality. In IL-1 KO mice more pronounced Th1 response was observed compared to control and IL-1Ra KO mice. In opposite, expansion of immature myeloid cells (CD11b$^+$ and Gr-1$^+$-double positive) was found in IL-1Ra KO mice that can increase Leishmania-mediated suppression and exacerbation of the inflammation. The lack of these molecules induces immunological switch with the increase of Th1 response that leads to delay of the disease progression. Further studies are aimed to assess the possibility of therapeutic intervention in Leishmaniasis by manipulating the IL-1 molecules.

P270023

The effects of IL-5 on the differentiation of dendritic cells ex vivo

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Objective: To investigate the direct effects of IL-5, a Th2 cytokine, on inducing DC differentiation from mouse Bone marrow (BM) progenitors. Methods: BM progenitors were cultured with GM-CSF alone or combined with different cytokine, such IL-4 (GM/4 DCs), IL-5 (GM/5 DCs), or IL-4 and IL-5 (GM/4, 5 DCs) in vitro. The cell number, the purity, surface molecules and the capability to stimulate allogenic T cell proliferation and antigen presenting ability were detected by FCM and MLR. Results: IL-5 significantly inhibited the differentiation of DCs induced by GM-CSF or GM-CSF/IL-4. GM/5 DCs expressed high level of CD11c, but lower level of MHC class II molecules and CD40. GM/5 DCs had much more potent antigen-presenting capability and displayed poor immunogenicity to allogeneic T cells in MLR assays, compared with DCs generated with GM/4 DCs or GM/4, 5 DCs. Conclusions: These data suggest that IL-5 inhibits the development of DCs in vitro. DCs induced in the presence of IL-5 showed unique phenotype and function.

Key word: IL-5, cytokine, Dendritic cells

Acknowledgement: This work was supported by grants from National Natural Science Foundation for Distinguished Young Scholar (C03020504, Y.Z.), the National Basic Research Program (973 Program, 2003CB515501, Y.Z.)

P270024

PRO-INFLAMMATORY CYTOKINE ACTIVATES AIRWAY NOCICEPTORS

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Increasing evidence suggests the immune and neural systems interact with one another, and that this interaction is propagated by cytokines released during inflammatory processes, such as sepsis and acute tissue injury. The present studies test the hypothesis the vagus nerves link the lungs' immune and neural systems by transmitting information through pulmonary nociceptors. Single unit activities from pulmonary C fiber receptors (CFRs) and high threshold A-delta fiber receptors (HTARs) were recorded from the cervical vagus nerve in anesthetized, open-chest, and mechanically ventilated rabbits. Interleukin1β was then injected into the nociceptor field (IL-1β, 10μg/ml, 20μl. Both CFRs and HTARs were stimulated by the local injection; their activities increased from 0.20 ±0.09 to 1.48 ±0.51 imp/s (n=8; p<0.05), and from 0.25 ±0.14 to 1.08 ±0.14 imp/s, respectively (n=6; p<0.01). These increases were greatly attenuated by simultaneous administration of IL-1β with IL-1ra, a natural IL-1 receptor antagonist. Our data demonstrate that nociceptors can be activated by pro-inflammatory cytokines — supporting the hypothesis that airway nociceptors transmit immune signals from the lung to the brain. (supported by NIH HL-58727)

P270025

Aristolochic acid I targeted to adenine nucleotide translocator sensitizes mitochondrial permeability transition pore opening in vitro: a possible mechanism for toxicity of aristolochic acid I[1]

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Aristolochic acid (AA) is an extract derived from nature manshuriensis. To study the mechanism of nephrotoxicity and possible hepatotoxicity, fifty C57BL/6J mice were used to test LD50, HepG2 cell line was used to test the cellular toxicity and rat liver mitochondria was isolated to detect mitochondria function. The LD50 of AA in mice was 29 mg/kg. Kidney and liver injury were shown by quantification of plasma transaminase activities and histological analysis. For the mitochondria study, a lower AA concentration (5~25uM) strongly induced cyclosporin A-sensitive mitochondrial swelling. AA promoted both calcium and GSH release from the matrix of isolated mitochondria. AA also decreased greatly the mitochondrial membrane potential (△Ψm). In addition, AA significantly inhibited mitochondrial adenine nucleotide translocator (ANT). This inhibition of ANT likely facilitates the AA-induced MPT pore opening which mimicked the effect of atractyloside, a specific inhibitor of ANT, induced clear mitochondrial swelling. It is suggested that inhibition of ANT may mediate, in part, the AA-induced MPT pore opening, which may be an important mechanism for AA toxicity.

Key words aristolochic acid (AA); MPT; ANT

[1] Project supported by National Science and Technology Foundation of China "863 project" (No 2004AA2Z3779).

P270026

EFFECTS OF INFLIXIMAB ON CYTOKINES AND SOLUBLE ADHESION MOLECULES IN PATIENT WITH JUVENILE IDIOPATHIC ARTHRITIS

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TNF-α modulators are proved to be effective and well-tolerated in the treatment of rheumatoid arthritis (RA) and also in juvenile idiopathic arthritis (JIA). We measured cytokine concentrations and soluble adhesion molecule levels in patients with JIA during a treatment with a chimeric monoclonal anti-TNF-α antibody, infliximab. Eight patients refractory to standard treatment were included. Infliximab (3-4 mg/kg) was given intravenously at weeks 0, 2, 6 and thereafter at 4-8 week intervals. All patients (n=8) responded to the treatment, and after six weeks the number of active joints had been reduced from 16 ±4 to 4 ±1 (mean ±SEM, p<0.01) and CRP levels from 31 ±8 to 8 ±3 (p<0.001). IL-6 concentrations decreased by about 50% from 14.6 ±3.4 to 7.2 ±1.3 pg/ml (p<0.01) and MPO levels about 35% from 584 ±121 to 368 ±41.1 ng/ml, (p<0.01) in 12 weeks treatment. In addition, the levels of soluble adhesion molecules ICAM-1 and E-selectin reduced during infliximab treatment. TNF-α levels tended to increase while the endogenous TNF-α antagonists (soluble TNFRI and TNF-RII) reduced in most of the patients during treatment. Treatment with TNF-α antagonist reduced inflammatory mediators along good clinical response.

Key words: TNF-α, cytokines, TNF-α antagonist, rheumatoid arthritis

P270027

Aristolochic acid I targeted to adenine nucleotide translocase sensitizes mitochondrial permeability transition pore opening in vitro

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Aristolochic acid (AA), a naturally occurring nephrotoxin, has been associated with a tubulointerstitial nephropathy. We hypothesized that mitochondria may be involved in this process. To elucidate affects of aristolochic acid I (AAI) on mitochondria, kidney mitochondral were isolated, then permeability transition pore (MPTP) opening, calcium fluxes, ROS generation and adenine nuclectide translocase (ANT) activity were determined. A low AAI concentration (5 ~ 25μM) strongly induced cyclosporin A-sensitive mitochondrial swelling. AAI also promoted calcium and cytochrome c release from mitochondria. However, exogenous thiol groups like GSH and DTT application could not inhibit the MPTP opening induced by AAI. And no change occurred in mitochondria ROS production after AAI added. Meanwhile, AAI significantly inhibited mitochondrial ANT, which likely facilitated the AAI-induced MPT pore opening since application of atractyloside, a specific inhibitor of ANT, induced significant mitochondrial swelling. It implies that inhibition of ANT may mediate, in part, the AAI induced MPT pore opening, which may be responsible for the toxicity of AAI.

Key words: Aristolochic acid, MPTP, ANT

P270028

The compatibility of cytokines across species: a preliminary study on Porcine and human Interleukin-2

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Interleukin 2 (IL-2) is a potent growth factor, vital to a productive immune response and critical for the development and expansion of $CD4^+CD25^+$ regulatory T cells, which promotes self-tolerance by suppressing T cell responses in vivo. In order to investigate whether porcine IL-2 (pIL-2) or human IL-2 (hIL-2) functions well on both porcine and human PBMCs (peripheral blood mononuclear cells), crystal structures of pIL-2 and hIL-2 was rebuilt by ESyPred3D. This study showed hIL-2 had a time and dose-independent effect on porcine and human PBMCs, whereas pIL-2 works on porcine PBMCs, but not well on human PBMCs, as determined by [^{3}H]thymidine incorporation, cell dividing cycle, and apoptosis. These results may contribute to understand the compatibility between pIL-2 and hIL-2, and have significance on xenogeneic bone marrow transplantation.

Key words: pIL-2; hIL-2; compatibility; xenotransplantation; cytokines

Acknowledgements: This work was supported by grants from National Natural Science Foundation for Distinguished Young Scholars (C03020504, Y.Z.) and 100 Quality Vocational Colleges of Chinese Academy of Sciences (2003-85, Y.Z.).

P28. Diabetes, Metabolism, Endocrine Pharmacology

P280001

Effects of berberine on diabetes induced by alloxan and a high cholesterol diet in rats

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To investigate the effect and mechanisms of berberine (Ber) on diabetic rats, diabetic rats induced by vein injection of alloxan 55 mg/kg were treated with Ber 100 and 200 mg/kg. After rats were treated for 3 weeks, the fasting blood glucose and NO were determined. Antihyperlipidemic and antioxidative activities of Ber were also investigated. Pancreas tissue sections were stained with HE and examined under a light microscope. Results showed the damage of pancreas tissues was restored in Ber treated. The hypoglycemic effect of Ber was confirmed by decreased fasting blood glucose levels in Ber treated group. Moreover, the treatment with Ber reduced serum content of total cholesterol, triglyceride and low density lipoprotein cholesterol, it also increased high density lipoprotein cholesterol. Furthermore, Ber treatment significantly blocked the increase in malondialdehyde, associated with a partial elevation of superoxide dismutase and glutathione peroxidase in heart. Meanwhile Ber increased the NO level in diabetic rats. Ber has a hypoglycemic effect, modulating lipids metabolic effects, and can protect the myocardium of rats with diabetes.

key words: Berberine; Diabetes; Hypoglycemic; Hypolipidemic; Antioxidant

P280002

Meal-Induced Peripheral Insulin Sensitization is Regulated by Hepatic Parasympathetic Nerves

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Glucose disposal induced by insulin is doubled in response to a meal. Insulin sensitization results from insulin acting on the liver, in the presence of a permissive parasympathetic feeding signal, to release Hepatic Insulin Sensitizing Substance (HISS). Eliminating the feeding signal, using atropine to block hepatic muscarinic receptors, eliminates HISS release. The parasympathetic signal can be restored in the denervated liver by intraportal infusion of acetylcholine. The capacity of indirectly-acting cholinergic agonists to restore insulin sensitivity was tested using a rodent model of 75%-Atropine-Induced HISS-Dependent Insulin Resistance. Insulin action, determined using a rapidly-sampled transient euglycemic clamp in response to a 50 mU/kg bolus, was decreased in a dose-dependent manner by atropine to a maximum 55% inhibition. Following a 75%-max atropine dose, potentiation of remaining parasympathetic effect using intraportal neostigmine, restored insulin sensitivity with a peak dose 0.1 μg/kg/min. The data suggest the use of either direct or indirect acting cholinergic agonists for treatment of impaired postprandial insulin sensitivity.

Key words: HISS, insulin sensitivity, neostigmine, atropine

P280003

Prevention of Free Fatty Acid-induced Apoptosis by Glargine in pancreatic beta Cells and the Mechanisms

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Aims: (1) to test anti-apoptotic effects of Insulin Glargine in fatty acid-induced apoptosis in pancreatic beta cells. (2) to investigate the role of NF-κB in fatty acid-induced apoptosis and if the protection of Glargine is via NF-κB pathway.

Methods: Apoptosis was characterized by morphology as well as Hoechst 33342 staining and quantified by flow cytometry and DNA fragmentation. NFκB activity was determined by western blotting of Phospho-NF-kB p65(Ser536).

Results: Glargine treatment lessened apoptosis in fatty acid-incubated beta cells at 500 nmol/L and this antiapoptotic effect was dose-dependent. NF-κB activity was elevated in fatty acid-incubated cells and specific NF-κB inhibitor Bay-117082 potently increased apoptosis in fatty acid-incubated cells. Bay-117082 completely abolished the antiapoptotic effect of Glargine. No changes in NF-κB activity was detected in fatty acid-incubated cells treated with Glargine compared with fatty acid-incubated cells.

Conclusions: Our data suggest Glargine exerted dose-dependent counteraction against free fatty acid-induced apoptosis in pancreatic beta cell. It is indcated that NFκB activation is stimulated in free fatty acid-induced apoptosis in pancreatic beta cell and an anti-apoptotic role of NFκB. Furthermore, to our knowledge we report the first time that cytoprotective effects of Glargine and regular insulin might be mediated via NFκB pathway.

P280004

EFFECT OF SOME CALCIUM CHANNELS BLOCKERS IN EXPERIMENTALLY INDUCED DIABETIC NEPHROPATHY IN RATS

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Aim: Diabetic nephropathy (DNP) is considered a CRD (The present study was designed to illustrate the role of CCBs (amlodipine and diltiazem) in prevention and treatment of DNP in rats. Materials & Methods: Eighty male albino rats weighing (130-180gm) were used in this study. These animals were subdivided into five equal groups. Insulinopenic diabetes was induced by STZ, two weeks later, 30 minutes of complete ischaemia was induced in the left kidney to induce diabetic nephropathy then treatment was started for 12 weeks. Results: Combination of renal ischaemia with DM produced a significant increase in rat weight, rat kidney weight, BUN level, K/B ratio, random blood glucose, 24 hrs urine proteins, and 24 hrs urine volumes and creatinine clearance. Treatment with diltiazem or amlodipine significantly lowered elevated SBP and elevated 24 hrs urine volumes. Conclusion: It can be concluded that, renal ischaemia hasten the progression of DNP, diltiazem and amlodipine have a tendency to reverse of changed parameters toward normal values except biochemical parameters

Key words: Diabetic Nephropathy, Diabetes Mellitus, Ischaemia

P280005

To find novel therapeutics for the T1D prevention

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Because of genetic variations, human population can be divided to two groups by insulin expression level in thymus (higher vs. lower). Low insulin expression in fetal thymus will promote autoimmune reaction against β-cells of the pancreas and the development of type 1 diabetes (T1D) in childhood. Using a high throughput drug screening platform, this study is to utilize a unique resource developed in our laboratory, clonal cell lines of insulin-producing medullary thymus epithelial cells, to find new therapeutics for T1D. These cells are rare (~1% of the thymus stroma) and no cultured lines or identification markers existed to date. Using pancreatic β-cells as control, drugs can promote specifically insulin expression in thymus can be identified. Any drug(s) that can promote the insulin product of the thymus cells will be potentially valuable to develop novel therapy for the T1D prevention. The insulin production will be detected by stable transfection of INS-promoter reporter gene (for high-throughput screening) and by ELISA (for replication of drug's effect in thymus cells). After the drug screening, futher study in vitro and in vivo will be performed to clarify the positive drug's mechanism.

P280006

The effects of insulin on rat liver mitochondria

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The purpose of the present study was to explore the effects of insulin on the function of rat liver mitochondria. Rats were sacrificed by decapitation and liver mitochondria were isolated by differential centrifugation. Mitochondria were pre-incubated with 0.004, 0.02, 0.04 IU/ml insulin respectively and saline for control in a respiratory medium for 5 minutes before substrate (10 mM/L-glutamate plus 5 mM/L-malate) and ADP addition to initiate state 3 respiration. The respiratory parameters were determined at 25 °C with a respirometer. Results showed that when mitochondria were incubated with insulin, there was an increase in state 4 respiration and oligomycin resistant respiration rate (V_{Oig}) ($p < 0.05$), but with no significant change in state 3 respiration and uncoupled respiration rate (V_{FCCP}) ($p > 0.05$), thus resulted in a decrease in the respiratory control rate (RCR, V_3/V_4) and the uncoupled respiratory control rate (UCR, V_{FCCP}/V_{Oig}). These results suggest that insulin may affect ion channel function of the mitochondria membrane or increase the proton leak and can promote the oxidative phosphorylation under normal conditions.

Key words: mitochondria; oxidative phosphorylation; insulin

P280007

Cytochrome P450 2C9 (CYP2C9) polymorphism influences hypoglycaemic attacks induced by sulphonylurea treatment

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CYP2C9 is a genetically polymorphic enzyme that plays an important role in the metabolism of several widely used drugs, including oral antidiabetics. The aim of our study was to evaluate the impact of CYP2C9 polymorphism on hypoglycaemia in patients with sulphonylurea treatment. Eighty-four Turkish diabetic patients (40 males, 44 females), treated with oral antidiabetics (glipizide, n=10, glimepiride, n=36 gliclazide n=38 for at least 10 weeks, were included in the study and genotyped by RT-PCR for the most common CYP2C9 alleles, CYP2C9*1, *2 and *3. Eleven patients (5 males, 6 females) experienced hypoglycaemic attacks (also diagnosed by their home glucose measurements). The frequency of subjects carrying CYP2C9*2 and *3 alleles was significantly higher ($p = 0.0074$) among patients who experienced hypoglycaemic attacks than in patients who did not (*1/*1 36% vs 58%, *1/*2, *1/*3 55% vs 37% and *2/*2, *2/*3, *3/*3 9% vs 5%, respectively). Polymorphisms of CYP2C9 thus seem to be associated with occurance of hypoglycaemia during treatment with sulphonylurea compounds metabolized by this enzyme.

P280008

BLOOD PRESSURE LOWERING & ANTIOXIDANT POTENTIAL OF AQUEOUS EXTRACT OF EMELIA RIBES IN DIABETIC RATS.

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Objective: To evaluate the blood pressure lowering & antioxidant activity of aqueous extract of Embelia ribes burm (Myrsinaceae) in streptozotocin (STZ)-induced diabetes in rats.

Method: Diabetes was induced by streptozotocin (40 mg/kg single, i.v, through tail vein) In all the groups of adult male rats (150-200gm Body wt). Aq. extract of Embelia ribes (200 mg/Kg) fed orally for 20 days in diabetic rats. The control rats received normal saline for the same duration. The pathogenic diabetic rats received on STZ administration rats sacrificed and blood samples were collected from the overnight fasted of all the rats. Animals were sacrificed on 22nd day and heart, pancreas and liver tissues were collected for biochemical analyses.

Result: There was a significant increase ($P < 0.01$) in blood glucose, serum glycosaylated Hb, heart rate, systolic BP and decrease in GSH, increase in LDH, CK levels and increase in tissues (heart, liver and pancreas) GSH, SOD and lipid peroxide levels in pathogenic diabetic rats as compared to normal healthy control rats. Furthermore, drug treatment in diabetic rats reversed the above parameters ($P < 0.01$) as compared to pathogenic diabetic rats.

Conclusion: The aqueous extract of Embelia ribes possesses the significant antioxidant & blood pressure lowering potential.

Key words: Embelia Ribes, Blood Pressure.

Acknowledgement: We acknowledge University Grant Commission, India for providing major research grant for this research work.

P280009

Establishment of Type 2 Diabetes Mellitus Model on Rabbit and Studies of the Effect of Total Flavone of Ampelopsis on Diabetes Rabbit

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Objective: To establishment Type 2 Diabetes Mellitus (T2DM) Model of Rabbit and study the effect and mechanism of total flavone of Ampelopsis TFA on diabetes rabbit. Methods: The rabbits were intravenous injected streptozotocin (STZ) after received high fat and high sucrose diet to induce T2DM. The level of nitricoxide synthase (NOS) and nitricoxide (NO) in serum was detected before and after taking medicines respectively. The morphology changes of their kidneys were observed by HE stained. Result: Successful T2DM rabbit model can be induced by the methods above. TFA can decline T2DM rabbits blood sugar. It could significantly inhibit both earlier increase and latterly decrease of NO level in serum. High dosage of TFA effected more obviously than low doseage. At the same time, the value of iNOS and total NOS in the serum of T2DM rabbits could be decreased by TFA and the decrease of iNOS was more obviously. Pathological sections showed TFA could release kidney damages on T2DM model. Conclusion: TFA has certain treatment function on T2DM.

Key word: Ampelopsis diabetes flavone NO/NOS

P280010

Effect of Tangweikang on leptin, TNF-a and C-peptide in experimental insulin resistance rats

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Objective: To study the effect of Tangweikang on blood glucose, serum insulin, leptin, TNF-a and C-peptide in experimental insulin resistance rats. Methods: The insulin resistance rats were induced by administrated high-fat and high-sugar diet. At the same time, the different dose of Tangweikang was given to the rats. After 10 weeks, Tested are the blood glucose, serum insulin, leptin, TNF-a and C-peptide of the rats. Results: Tangweikang 1.5g/ml、0.75g/ml increased the insulin resistance rats' insulin sensitivity index (ISI) ($P < 0.01$), decreased serum leptin ($P < 0.05$, $P < 0.01$). Combining Tangweikang 1.5g/ml and Metformin 104 mg/kg decreased the IR rats' serum leptin and TNF-a ($P < 0.01$), increased C-peptide ($P < 0.05$). Tangweikang 0.75g/ml increased insulin resistance rats' C-peptide ($P < 0.05$). Conclusion: Tangweikang improved on

insulin resistance rats' ISI, decreased serum leptin, increased Met's effect to decreased serum TNF-a and increased C-peptide.

Key words: insulin resistance, Tangweikang, leptin, TNF-a, C-peptide

P280011

Effect of Cimicifuga extract on bone histomorphometry in ovariectomized rats

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Objective To observe the effect of Cimicifuga extract on estradiol(E2) in serum and bone histomorphometry in ovariectomized rats. Methods: 60 SD rats were divided into 6 groups randomly: the control group(Sham), the model group (OVX), the Cimicifuga extract groups at dose of 20 mg/kg, 40 mg/kg, 80 mg/kg and the positive control group. The osteoprosis model was induced by ovariectomy. Three months after ovariectomy, the Cimicifuga extract was administered to rats once daily for 3 months. The concentrations of E2 in serum was measured by electric radiation immunologic method. The proximal tibiae of rats were processed to undecalcified sections at 5μm thickness for histomorphmetric analysis. Results In ovariectomized rats, TBV% in proximal tibiae reduced markedly, but TFS%, AFS%, MAR, OSW, mAR and TRS% increased remarkably. It shows that the osteoprosis induced by ovariectomy is high transformation type which bone absorption exceeds bone formation. In contrast, treatment of OVX rats with Cimicifuga extract, TBV% in proximal tibiae heightened evidently, and TFS%, AFS%, MAR, mAR and TRS% decreased significantly. However, there is no effect on OSW, the level of E2 in serum and index of uterus. Conclusion The Cimicifuga extract has an antiosteoporotic effects on ovariectomized rats. The Cimicifuga extract exerts estrogen-like effects in the bone, particularly in osteoblasts, but not in the uterus of variectomized rats. The extract appears to contain rat organ-specific selective estrogen receptor modulators (SERMs), and if these findings can be approved in human, it may be an alternative to hormone replacement therapy (HRT).

Key words: Cimicifuga extract; osteprosis; E2; bone histomorphometry

P280012

Attenuation of Bone Mass and Increase of Osteoclast Formation in Decoy Receptor 3 Transgenic Mice

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Decoy receptor 3 (DcR3), a soluble receptor for FasL, LIGHT and TL1A, induces osteoclast formation from monocyte, macrophage and bone stromal marrow cells. However, the function of DcR3 on bone formation remains largely unknown. To understand the function of DcR3 in bone formation in vivo, transgenic mice overexpressing DcR3 were generated. Bone mineral density (BMD) and bone mineral content (BMC) of total body were significantly lower in DcR3 transgenic mice compared with wild-type controls. The number of osteoclast increased in DcR3 transgenic mice. Osteoclastogenesis and resorption activity of osteoclast increased in cultured bone marrow stromal cells derived from DcR3 transgenic mice. In addition, local administration of DcR3 into the metaphysis of rat tibia via the implantation of a needle cannula significantly decreased the BMD, BMC and bone volume of secondary spongiosa in tibia. These results indicate that DcR3 may play an important role in osteoporosis or other bone diseases.

Key words: DcR3; bone formation; osteoclast; osteoclastogenesis.

Acknowledgement: This work was supported by grants from NSC.

P280013

Effect of polysaccharide sulfate for the treatment of diabetic dyslipidemic rats

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AIM: To investigate the therapeutic efficiency of ploysaccharide sulfate (PSS) on lipometabolism and glycometabolism in diabetic dyslipidemic rats. METHODS: The rat model of diabetic dyslipidemia was established by streptrozotocin and high fat diet. Then the effects of PSS on fast blood glucose, insulin and lipids concentrations were studied. RESULTS: Before PSS administered, all diabetes groups had higher glucose concentrations to normal control group, and significantly higher triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C). PSS (treated for four weeks) reduced TG, TC, LDL-C and increased HDL-C in PSS groups compared with group. PSS groups had a somewhat lower glucose and insulin concentrations, but had significantly higher insulin sensitivity index (ISI) to diabetes control group. However, treated for four weeks, none of them showed sufficient effects on the clinic syndrome of diabetes mellitus, such as body weight, food consumption and water intake. CONCLUSIONS: PSS can correct the dislipidemia and improve insulin resistance in diabetic dyslipidemic rats.

KEY WORDS experimental diabetes mellitus streptozotocin dyslipidemia

P280014

Increased oxidative stress in the streptozotocin-induced diabetic apoE-deficient mouse.

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Objective: Investigate oxidative stress in the streptozotocin (STZ)-induced apoE (STZ-apoE--/-) deficient diabetic mouse.

Methods: Oxidative stress was assessed in aorta and small mesenteric arteries (SMA) by immunofluorescence labeling with dihydroethidium and levels of NADPH oxidase subunits were determined by a real-time polymerase chain reaction protocol and Western blotting.

Summary of results: Blood glucose levels and oxidative stress were significantly increased 4, 8 and 16 weeks after STZ in both STZ-apoE-/- aorta and SMA compared to the time- and age-matched citrate (CIT)-treated nondiabetic apoE-/-. In the SMA the expression of Nox4 (4 wks) and gp91 (8 wks) subunits of NADPH oxidase from STZ-apoE-/- were enhanced as was eNOS mRNA ($P < 0.05$). Oxidative stress was increased in mouse aortic endothelial cells treated with high glucose (HG) compared to normal low glucose medium; oxidative stress in HG was lowered by treatment with sepiapterin and eNOS mRNA and protein were increased significantly Conclusions: Increased oxidative stress in the vasculature of STZ-apoE-/- mice is linked to changes in eNOS and NADPH oxidase expression.

Key words: oxidative stress, NADPH oxidase, eNOS, diabetes.

P280015

Establishment of a mice nutritional non-alcoholic fatty liver disease model

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Aims: To set up a non-alcoholic fatty liver disease (NAFLD) mice model resembling clinical features. Method: Male C57BL/6n mice were fed rodent chow (control) and western diet (model) for 26 weeks. The bodyweight, and blood glucose, total cholesterol (TC) and insulin was measured. The insulin sensitivity was evaluated by oral glucose tolerance test (OGTT), insulin tolerance test (ITT) and homeostasis model assessment of insulin resistance index (HOMA-IR). The NAFLD were estimated by histopathology, content of triglyceride (TG) and malondialdehyde (MDA) in liver, and aminotransferase (ALT and AST) in serum. Results: In model mice, comparing with control, the bodyweight, glucose, TC, and insulin was elevated by 50%, 74%, 110% and 490% respectively; the insulin resistance was validated by OGTT, ITT, and HOMA-IR; the levels of ALT, AST, MDA, and TG were increased by 124%, 20%, 40%, and 75%, separately; the severe steatosis and ballooning in the liver was observed. Conclusion: The model mice induced by western diet developed a syndrome that shares metabolic and histopathologic characteristics compatible with human NAFLD.

Key words: NAFLD, mice model

Acknowledgement: We thank Prof. Liu Geng-tao for his instruction, and the sustentation funds from NSFC (90209057) and State Administration of Traditional Chinese Medicine, PRC (Guo Zhong Yi Yao Ke 02-03 ZP11).

P280016

Ability of cyclohexenonic long-chain fatty alcohol to reverse diabetes-induced cystopathy in the rat

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Objective: We investigated the ability of n-hexacosanol to reverse diabetes-induced cystopathy in the rat. Methods: Eight-week-old male SD rats were di-

vided randomly into three diabetic and age-matched control groups. Four weeks after the induction of diabetes (i.p., 50 mg/kg streptozotocin), then received another four weeks of treatment by n-hexacosanol (0, 2, or 8 mg/kg, i.p. every day). The serum glucose and insulin levels were determined, and the bladder functions were estimated by voiding behavior studies, cystometric studies, and functional studies. The participation levels of M2 and M3 receptors were investigated by real-time PCR. Results: Treatment with n-hexacosanol did not alter the rats' diabetic status, but did significantly improve the diabetes-induced dysfunction of the detrusor in a dose-dependent manner. Furthermore, n-hexacosanol significantly reversed the up-regulation of muscarinic M2 and M3 receptor mRNAs in STZ-diabetic rats. Conclusion: These results indicate that n-hexacosanol has a beneficial effect on hyperreactivity in the diabetic detrusor by ameliorating over-expression of muscarinic M2 and M3 receptor mRNAs.

Key Words: n-hexacosanol, cystopathy, muscarinic receptor mRNA

P280017

The antihiperglicemics in the glicemic control of the diabetic patient. El FZ (Glicolit) an antihiperglicemic of natural origin.

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Introduction: The FZ(Glicolit); a derived product of the natural zeolites has shown that it slows the intestinal absorption of glucose, avoiding the elevation of its level in blood in the periods post pandriales. Objectives: To deepen in the knowledge of the mechanism of action of the Glicolit like antihiperglicemiante, Material and methods: 15 rats were studied, It was administered in fast a preparation of marked glucose with 14 C; at 6 of them they were administered alone (group 1, control) and to the other 9 with Glicolit (group 2). They were carried out extractions of blood at different times to carry out mensurations of glucose in plasm by means of radio-active counts. Results: Differences were observed between both in the areas under the curves of absorption of glucose groups of rats, that which demonstrates the utility of the method used to measure the effect antihiperglicemiante of the Glicolit. Conclusions: The results obtained by the method radioisotópico with marked glucose with 14 C corroborate that observed in previous works and they allow us to advance in the study of this possible medication, when having an effective method.

Key words: Diabetes, Antihiprgligemiants

P280018

AN UNSUSPECTED ROLE OF ATRIAL NATRIURETIC PEPTIDES IN THE CONTROL OF LIPID MOBILIZATION IN HUMANS: EXISTENCE OF SEX-RELATED DIFFERENCES

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Atrial natriuretic peptides (ANP and BNP) stimulate human fat cell lipolysis through a cGMP-dependent activation of hormone-sensitive lipase. The lipid-mobilizing mechanisms were studied in the subcutaneous adipose tissue (SCAT) of overweight men and women, using in situ microdialysis. Importance of catecholamine and ANP-dependent pathways was delineated using beta- and alpha2-adrenergic receptor antagonists (alone or associated) added to dialysis probes. Extracellular glycerol concentration (EGC) was determined to assess lipolysis. Exercise-dependent increment in EGC was observed in both sexes but the contribution of catecholamine and ANP-dependent pathways was strikingly different. Overweight women mobilize more lipids than men during exercise. Alpha2-antilipolytic effect was only functional in SCAT of men; less in women. The striking finding of the study is that during low-to-moderate exercise periods, lipid mobilization in SCAT is not related to catecholamine-dependent stimulation of beta-adrenergic receptors by but rather to a decrease in plasma insulin and an increase in plasma ANP concentrations.

Key words: lipolysis, catecholamines, adrenergic receptors, atrial natriuretic peptides

P280019

Effects of Diabetes Mellitus and High Glucose on Brain-Pancreas Relative Protein(BPRP)

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Brain-Pancreas Relative Protein (BPRP) is a novel protein identified in our Lab. It was primarily localized in brain neurons and islet cells, which implies its function in these tissues. We examined the effects of alloxan-induced diabetes in rats on the level of the BPRP in the brain. Diabetes resulted in significant increase in blood glucose, and decrease in BPRP levels in the brain at both 4 and 8 weeks of diabetes duration. To investigate whether the changes of blood glucose could regulate the alterations of BPRP, we use the PC12 cells to examine the effects of high glucose on the level of BPRP. Treatment of PC12 cells with different concentration of glucose significantly decreased BPRP level in the dose-dependent and time-dependent manners. The effect of glucose couldn't be mimicked by mannitol. In addition, high glucose-induced down-regulation of BPRP was reversed by ALLN, an inhibitor of calpain and not affected by treatment with the MG132, a specific proteasome inhibitor. These results suggest that this protein was probably destroyed by proteolytic degradation and the down-regulation of BPRP and the activiy of calpain may contribute to the complications of diabetes in Central Nervous System.

P280020

Advanced Glycation Endproduct (AGE) is Linked to Cardiomyocyte Contractile Dysfunction in Streptozotocin-Induced Diabetic Mice

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Although clinical manifestation of diabetic cardiomyopathy has been identified, its pathogenesis and, in particular, the causative mechanisms behind advanced glycation endproduct (AGE) have not yet elucidated. This study was designed to examine the potential role of AGE in the pathogenesis of diabetic cardiomyocyte dysfunction. Mechanical properties were evaluated in ventricular myocytes from streptozotocin-induced diabetic mice including peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR90). AGE formation was evaluated by immunohistochemistry and ELISA. Cardiomyocytes from diabetic mice displayed prolonged TPS and TR90 compared to those from normal group. Cardiac AGE was significantly enhanced in diabetic mice. To further validate the role of AGE in the pathogenesis of diabetic cardiac dysfunction, cardiomyocytes were incubated with methylglyoxal-derived AGE (MG-AGE, 0.1-5.0 micromol/l, 2 hrs). MGAGE directly led to contractile dysfunction in myocytes, the response of which was exaggerated by diabetes. Collectively, this study supports a role of AGE in the pathogenesis of diabetic cardiomyopathy.

Key words: Diabetes, advanced glycation endproduct, cardiac myocytes, contraction

P280021

ACTIVATION OF THE AMP-ACTIVATED KINASE BY ANTI-DIABETES DRUG METFORMIN IMPROVES NITRIC OXIDE BIOACTIVITY IN VIVO

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Metformin, one of most commonly used drugs for the treatment of type II diabetes, improves vascular endothelial functions and reduces cardiovascular events in patients with Type II diabetes although its mechanisms remain unknown. The present study was aimed to elucidate how metformin improves endothelial functions. Exposure of cultured bovine aortic endothelial cells to clinically relevant concentrations of metformin (50 to 500 μM) dose-dependently increased the serine 1179 phosphorylation (equal to human serine 1177) of eNOS as well as its association with heat shock protein (hsp) 90, resulting in increased activation of eNOS and NO bioactivity (cyclic GMP, cGMP). These effects of metformin were mimicked or completely abrogated by adenoviral overexpression of a constitutively active AMP-activated kinase (AMPK) mutant or a kinase-inactive AMPKα, respectively. Further, administration of metformin as well as AICAR, an AMPK agonist, significantly increased eNOS serine 1179 phosphorylation, NO bioactivity, and co-immunoprecipitation of eNOS with hsp-90 in the wild type C57BL6 mice but not AMPK-α1 knock out mice, suggesting that AMPK is required for metformin-enhanced eNOS activation in vivo. Finally, incubation of BAEC with clinically relevant concentrations of metformin dramatically attenuated high glucose (30 mM)-induced reduction in the association of hsp90 with eNOS, which resulted in increased NO bioactivity with a reduction in overexpression of adhesion molecules and endothelial apoptosis caused by high glucose exposure. Taken together, our results indicate that metformin might improve vascular endothelial functions in diabetes by increasing AMPK-dependent, hsp90-mediated eNOS activation.

P280022

Hydrogen Sulfide Abrogates Insulin Secretion from Insulin Secreting (HIT-T15) Cells

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Hydrogen sulfide (H_2S), a naturally occurring gas exerts physiological effects by opening K-ATP channels. Antidiabetic drugs (e.g. glibenclamide) block K-ATP channels and abrogate H_2S-mediated physiological responses which suggests that H_2S may also regulate insulin secretion in pancreatic cells. To investigate this hypothesis, insulin secreting (HIT-T15) cells were exposed to NaHS (100uM) for 12h. Subsequently, insulin secreted into the media was determined. Cell viability and intracellular ATP and reduced glutathione (GSH) levels (known regulators of insulin secretion) were also determined. The concentration of insulin secreted from HIT-T15 cells decreased significantly from to 33.9 +/- 7.7ng/ml/mg protein (untreated control) to 14.1 +/- 5.5ng/ml/mg protein after NaHS exposure. Cell viability and levels of intracellular ATP and GSH remained unchanged suggesting that changes in insulin secretion are not metabolically linked. This data shows that H_2S abrogates insulin secretion perhaps by directly opening K-ATP channels in HIT-T15 cells. This study also provides molecular insight into a recent observation of increased pancreatic H_2S production in the streptozotocin diabetic rat. H_2S, Insulin, HIT-T15

P280023

Protective Effects of Astragalus Saponin I against Development of Diabetic Nephropathy

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To study if Astragalus Saponin I (ASI) has ability to prevent diabetic nephropathy (DN). In the presence of high glucose and H_2O_2, the total antioxidative capability, catalase, reduced glutathione (GSH), and superoxide dismutase (SOD) level of rat mesangial cells were significantly decreased, and transforming growth factor β1 (TGF-β1) mRNA level, collagen IV and laminin level were significantly increased. When compared with those in the high glucose group, these 4 indexes of cells incubated in 2.0 μmol/L and/or 20 μmol/L of ASI were significantly enhanced, and levels of TGF-β1 mRNA, collagen IV and laminin were statistically decreased. By flowcytometry, percentages of S phase of cells incubated in high glucose and H_2O_2 were lowered, while those in ASI were increased. Furthermore, the physical behaviors of rats treated with 12 mg/kg of ASI restored with vigor and weight gaining, while the level of HbA1C was significantly reduced. Thus, ASI has antioxidative effects and is a potential compound worth further study in preventing the development of DN.

Keywords: Astragalus Saponin I, diabetic nephropathy, antioxidative effect, mesangial cells

P280024

Resistin, TNF-α, Insulin, Glucose serum levels and Body Weight-Increasing Rate changes in rats fed with High-Fat Diet under Sibutramine treatment.

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Resistin (R) an adipocyte hormone, has been implicated in the pathogenesis of obesity-mediated insulin resistance. The aim of this study was to investigate serum R levels and its correlations with insulin resistance parameters and body weight-increasing rate (BW-IR) changes in rats fed a High-Fat Diet (HFD) under Sibutramine (S), an antiobesity drug. Male Wistar rats (n=42) were fed with HFD or standard diet (SD) for 13 weeks. The last 3 weeks each group divided into 3 subgroups received: S 5mg/kg, S 10mg/kg or vehicle. Daily food intake, BW-IR, serum resistin, TNFα, insulin and glucose levels were measured. HFD intake increased BW-IR, R and TNF-α levels compared to SD. Sibutramine at 10 mg/kg decreased HFD intake, BW-IR and insulin without changes on R, TNF-α and glucose levels compared to vehicle. A positive correlation between R and TNF-α and BW-IR was found. Results suggest that S exerts its observed effects without involvement of TNF-α and R changes caused by HFD intake.

Keywords: Resistin, Sibutramine, High-fat diet.

Acknowledgement: Project is co-financed within Op. Education by the ESF and National Resources.

P280025

The long-acting glucagon-like peptide-1 prodrug, Pro-GLP-1, ameliorates glycemia and stimulates insulin secretion in mice

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Objective To study the effects of Pro-GLP-1, a long-acting pro-drug glucagon-like peptide-1, on regulations of blood glucose levels and insulin secretion in mice. Methods Pro-GLP-1 was administered via ip or sc route. Blood glucose levels were measured using a blood glucose meter. Plasma insulin concentrations were determined by ELISA. Results In C57BL/6J mice, native GLP-1 and Pro-GLP-1 decreased blood glucose, but Pro-GLP-1 had a more evident action. A single injection of Pro-GLP-1 dose dependently reduced higher glucose following glucose load at least 3h, and it had no effect on normal blood glucose resumed at 90 min postdose. Moreover, it dose dependently stimulated insulin secretion and significantly improved glucose tolerance after glucose challenge. Conclusions These demonstrated that Pro-GLP-1 facilitates a significant and prolonged glucoselowering effect and glucose dependently stimulates insulin secretion in mice.

Key Words: GLP-1; pro-drug; diabetes

Acknowledgement: This work is supported by Shaanxi Province International Cooperation Foundation. We wish to thank Dr. Zhen-guo Liu and Hai-fang Wang for excellent technical assistance.

P280026

An herbal cocktail serves as a novel insulin sensitizer but shares divergent mechanisms with those of thiazolidinedione agents

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Diabetes mellitus has been recognized as a major health problem in the world. Under the guidance of theories of tradtional Chinese medicine, we re-combined an herbal cocktail (FF-V) from Coptis chinensis, Radix Astragali and Lonicera japonica Thunb, and evaluated its anti-diabetic effects in monosodium glutamate (MSG) obese mice model with insulin resistance and tried to elucidate some of the mechanisms. FF-V has been administered orally for 28 days. It significantly mitigated abnormal glucose and insulin tolerance; inhibited gluconeogenesis; lowered fasting serum glucose and insulin concentration but didn't increase body weight of animals. Hepatic glycogen and muscle free fatty acid content were reduced while IRS-1 and GLUT-4 protein expression in muscle were increased. FF-V enhanced glucose utilization in cell lines in vitro. Furthermore, FF-V significantly reduced PPAR αt γgene expression as Rosiglitazone did but had no effect on 3T3-L1 cell differentiation. In conclusion, FF-V is a novel insulin sensitizer but without the side effect of weight gain. It shares, in some aspects, similar pharmaceutical effects with those of thiazolidinedione agents with mechanisms which deserve further elucidation.

P280027

Heterologous Expression of Human Dipeptidyl Peptidase-IV in Pichia pastoris and Screening of the DPP-IV Inhibitor

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Inhibitor of dipeptidyl peptidase-IV (DPP-IV) is a kind of novel potential drug for the treatment of type 2 diabetes. A full-length coding sequence of human DPP-IV was obtained from cultured human Caco-2 (colon adenocarcinoma) cells by RT-PCR method, and cloned into the expression vector pPIC9K. The recombinant plasmid pPIC9K-dpp-iv was introduced into Pichia pastoris GS115 strain (invitrogen) by electroporation. After the selected transformant was grown at 30 °C for 6 days in YPM medium (2% methanol), the supernatant of the culture broth was freeze-dried and dialyzed. The crude or purified enzyme was dissolved in Tris-Cl buffer (pH8.0) and the activity was determined by the hydrolysis of the substrate Gly-Pro-p-nitroanilide hydrochloride (Sigma). The drug screening system including the high throughout screening (HTS) model for the DPP-IV inhibitors was preliminarily set up. Over 1,600 compounds have

been tested for their in vitro inhibiting activity against the DPP-IV and other fifty thousand compounds are under screening.

Key words: Dipeptidyl peptidase-IV, heterologous expression, Pichia pastoris, DPP-IV inhibitor, screening

P280028

The PPARalpha/gamma Dual Agonist Chiglitazar Ameliorates Insulin Resistance and Hyperglycemia While Improving Dyslipidemia In Predinical Models

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AIM: The aim of this study is to investigate the capacity and mechanisms of chiglitazar, a novel PPAR (Peroxisome proliferator-activated receptor) alpha/gamma agonist to improve insulin resistance and dyslipidemia in MSG obese rats and hyperglycemia in diabetic KKA-y mice,

METHORDS: KKA-y mice were divided into three groups that received chiglitazar (20 mg kg-1 day-1), rosiglitazone (2 mg kg-1 day-1), or vehicle for 14 days. MSG obese rats were sorted into five groups that received chiglitazar (5, 10 and 20 mg kg-1 day-1), rosiglitazone (5 mg kg-1 day-1) or vehicle for 40 days. Experiments about insulin resistance and dyslipidemia were performed during these days.

RESULTS: Chiglitazar reduced the hyperglycemia in diabetic KKA-y mice. Moreover, the compound improved the impaired insulin and glucose tolerance. Unlike rosiglitazone, chiglitazar showed significant increase of mRNA expression invdlved in FFA oxidation.

CONCLUSION: Chiglitazar may have better effects on lipid homeostasis in diabetic patients than selective PPARgamma agonist.

Key words: peroxisome proliferator-activated receptor; type 2 diabetes; insulin sensitizer

P280029

Ionic mechanisms of antiherglycemic agent, LS-NTU-A, in enhancing insulin secretion of pancreatic beta cells

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Our previous study found that LS-NTU-A, an aporphine derivative, dose-dependently lowered plasma glucose in normal rats, nicotinamide-streptozotocin (STZ)-induced, and STZ-induced diabetic rats. The mechanism of the anti-hyperglycemic activity of LS-NTU-A was partly due to enhancing insulin secretion. The present study was aimed to investigate the ionic mechanisms of LS-NTU-A in pancreatic beta cells. LS-NTU-A dosedependently increased insulin secretion in isolated rat islets, and the maximum effect reached at the concentration of 3 micromole/l. PI3K inhibitors and PKC inhibitors was unable to abolish the effect of LS-NTUA. Whole-cell voltage clamp study in pancreatic beta cells revealed that LS-NTU-A significantly inhibited ATP-sensitive K^+ current at 3 micromole/l, and voltage-gated K^+ currents at 100 micromole/l. In conclusion, LSNTU-A acted as an insulin-secretagogue through IKATP inhibition.

Key words: insulin, beta-cell, LS-NTU-A, KATP

P280030

Dietary Influence of High Fat and Marginal Copper Deficiency on Cardiac Contractile Function in Isolated Cardiomyocytes

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High fat and copper deficient diet trigger cardiac hypertrophy, increased myocyte lipid droplet volume and compromised contractile function. This study examined the interaction between high fat and copper defiency diet on cardiomyocyte contractile function. Rats were fed diets low or high fat diet (10% or 45% of kCal from fat) with adequate (6 mg/kg diet) or deficient (1.5 mg/kg) copper for 12 wks. Contractile function was determined including peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR90), maximal velocity of shortening and relengthening ($\pm$dL/dt) and intracellular Ca^{2+} handling. High fat induced obesity and glucose intolerance. High fat or copper deficiency depressed PS, $\pm$dL/dt and frequency response, with no additive effect. Cardiac protein expression of phospholamban but not SERCA2a was increased by either diet. Elevated cardiac triglyceride levels were observed in high fat group with no oxidative injury or lipid peroxidation.

Ceramide levels were similar among all groups. Our data suggests high fat diet and copper deficiency depressed cardiomyocyte function through similar mechanism without obvious oxidative damage.

P280031

Expression and effects of anion exchangers (AEs) on HUVECs apoptosis induced by hyperglucose

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Dysregulation of endothelial cells (ECs) is an initial step of angiopathy resulted in by diabetes mellitus (DM). Anion exchangers (AEs) are likely to play an important role in angiopathy. The study is to examine the expression of AEs in human umbilical vein cells (HUVECs) and investigate if AEs participate in HUVECs apoptosis induced by high glucose. HUVECs were treated with DMEM containing glucose (5.5, 27.8, 40.0, 55.6 mmol·L^{-1}, respectively) for 72 h. Apoptosis was detected by TUNNL assays and AEs mRNA levels were examined by RT-PCR The results showed that glucose exposed for 72 h resulted in expression up-regulation of AE2 mRNA and apoptosis rate enhancement in a concentration-dependent manner, while corresponding PH and osmotic pressure did not affect these changes. In additional, expression of AE1 and AE3 mRNA failed to detect in case of hyperglucose. The results suggest that hyperglucose may up-regulate AE2 expression and AE2 may play a critical role in ECs apoptosis induced by hyperglycemia.

Keywords anion exchangers; endothelial cells; apoptosis; diabetes mellites
Acknowledgement This work was supported by a grant from Natural Science Foundation of China (No.30560049).

P280032

Role of caveolin-1 in regulatory effect of estrogen on osteoblastic differentiation

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OBJECTIVE: To explore the role of caveolin-1 in the regulatory effect of estrogen on osteoblastic differentiation. METHODS: 17β-Estradiol was administrated in two osteoblast line, MC3T3-E1 or MG-63, to investigate the influence of estrogen on the expression of caveolin-1 mRNA and protein. Transfection of cavedin-1 antisense oligo-deoxynucleotides (ASODN) and Pcl-neo-cav-1 were used to evaluate the role of caveolin-1 in estrogen regulation of osteoblastic differentiation shown by the expression of cbfa1 mRNA. RESULTS: Treatment with 17β-estradiol up-regulated the expression of caveolin-1 mRNA and protein in MC3T3-E1 ($p < 0.05$), but had no effect in MG-63. The transfection of caveolin-1 ASODN abolished the estrogen up-regulation of MC3T3-E1 differentiation as shown by reversing the increased expression of cbfa1 mRNA by 17β-estradiol ($p < 0.05$).

CONCLUSION: Caveolin-1 is related to the up-regulation of MC3T3-E1 differentiation by estrogen.

KEY WORDS: estrogen, osteoblastic differentiation, caveolin-1

P280033

Enhancement of 3T3-L1 preadipocyte differentiation and adiponectin expression by new compound GY3

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Thiazolidinediones (TZDs) such as rosiglitazone could improve diabetes by increasing insulin sensitivity, and they could enhance the differentiation of preadipocytes into adipocytes that is relative to their antidiabetic activities. In this study, we aimed to identify whether GY3, a new synthesized non-TZD but indole compound, enhance adipocyte differentiation in 3T3-L1 cells as rosiglitazone do. Further more, we compared the effect of GY3 on the expression of adiponectin, an insulin-sensitizer released by adipocytes, with that of rosiglitazone. It is found that although both of GY3 and rosiglitazone increased the lipid accumulating of 3T3-L1 adipocytes induced by isobutyl methylxanthine, dexamethasone and insulin (IBMX-DEX-INS), but GY3 could not increase the accumulating of lipid induced by insulin only, whereas rosiglitazone could. However, Western blot analysis showed that GY3 could significantly increase the expression of adiponectin as well as rosiglitazone did in both conditions above. These

results indicated that GY3 could be developed as a new agent for the improvement of type 2 diabetes and might have less possibility of body weight gain.
Key words: GY3; 3T3-L1; differentiation; adponectin

P280034

Experimental Studies on HBP over flux induced by the hyperglycaemia or insulin resistance

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Aim: To investigate the effects of hyperglycaemia (HG) or insulin resistance (IR) on glutamine:fructose-6-phosphate amidotransferase (GFAT) activity, the key enzyme of hexosamine biosynthesis pathway (HBP). Methods: GFAT activity was measured by enzyme method. IR was validated by Insulin tolerance test (ITT) or insulin-induced glucose uptake (IGU). HG mice were induced by alloxan in ICR mice, IR-mice were induced by western diet in C57BL/6N mice, IR-HIRc cells were induced by long-action insulin in HIRc. Results: Comparing with control, GFAT activity was increased 87% in kidney, 95% in muscle, and revised 24% and 27% by treated with insulin. In IR-mice, comparing with control, the area under glucose-time curve in ITT was elevated by 38%; GFAT activity was raised 27% in kidney. In IR-HIRc cells, IGU was reduced by 21%; GFAT activity was increased 47% and reversed by azaserine to almost normal. Conclusions: HBP over flux was correlativity with HG and IR in vivo and in vitro.
KEY WORDS: HBP, GFAT, HG, IR
Acknowledgement: This work was supported by Natural Sciences Funds of China (90209057) and State Administration of Traditional Chinese Medicine, PRC (Guo Zhong Yi Yao Ke 02-03 ZP11).

P280035

GRK-2 differentially regulates insulin-induced glycogen synthesis and mitogenesis

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The insulin via activation of the insulin receptor (IR) regulates metabolic pathway to maintain glucose homeostasis and mitogenic pathway leading to cell growth. Although IR is a tyrosine kinase receptor, it also interacts with G-protein coupled pathways. In the present study, we investigated the role of G-protein coupled receptor kinase-2 (GRK2) on IR signaling and functions i.e., glycogen synthesis and mitogenesis. The GRK-2 was down-regulated by 90% in hepatocytes using GRK2 siRNA. D-[U-14C] glucose incorporation into glycogen and [^{3}H]-methyl thymidine incorporation was measured. The GRK-2 deficiency caused an increase in the insulin induced glycogen synthesis and a decrease in the insulin induced [^{3}H]-methyl thymidine incorporation. The tyrosine phosphorylation of IRS1 was increased and the activity of GSK3-alpha and GSK3-beta was decreased in GRK2-deficient compared with control hepatocytes. The phosphorylation of ERK1/2 was reduced in GRK2 deficient cells. The data suggest that GRK2 negatively regulates insulin-induced metabolic pathway, but positively regulates insulin-induced mitogenesis in mouse hepatocytes.

P280036

Stability and reproducibility assessment of liver-specific GK gene knockout mice and the role of hepatic GK in the pathogenesis of diabetes mellitus

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An ideal animal model may provide valuable clues to understanding pathological mechanism of human diseases and assist in designing optimum therapeutic approaches. We have generated a diabetic animal model with liver-specific glucokinase gene knockout. To assess the stability and reproducibility of this model, we evaluated the phenotype of three generations of mice. All mice showed elevated fasting blood glucose and impaired glucose tolerance consistent with that of diabetes patients. Furthermore, some mice displayed dyslipidemia and hepatic steatosis. Both protein expression and enzyme activity of glucokinase in liver decreased in model mice; as well there was a corresponding decrease in liver glycogen contents, suggesting glucokinase played a key role in glycogenesis. Additionally, Insulin receptor expression in the liver also reduced in all generations, indicating insulin resistance. These results suggest this model may be ideal for researching diabetes pathogenesis and screening anti-diabetic drugs.
Keywords: stability; animal disease model; diabetes mellitus; glucokinase; glycogen

P280037

Mechanisms for abnormalities of nitric oxide-mediated vasorelaxations in SHR/NDmcr-cp (cp/cp) rats, an animal model of metabolic syndrome

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The aortas of SHR/NDmcr-cp (SHR-cp) rats display impaired vasorelaxation via the nitric oxide (NO)/cyclic guanosine monophosphate system although NO production from the endothelium increases. We examined whether the vasorelaxant dysfunction can be improved by treatment with antihypertensive drugs, amlodipine, a calcium channel blocker, and telmisartan, an angiotensin II type 1 receptor blocker. Treatment with these drugs for 9 weeks showed significant antihypertensive effects, with no difference between their potencies. Telmisartan ameliorated the impaired relaxation in response to acetylcholine and the increased protein expression of eNOS in thoracic aortas, but amlodipine did not display these effects. The protein expression of gp91phox, a component of NADPH oxidase, and the contents of 3-nitrotyrosine, a biomarker of peroxynitrite, in aortas were decreased by treatment with telmisartan. These findings in SHR-cp rats suggest that increased oxidative stress, probably involved in angiotensin II, in the metabolic syndrome disturbs the NO-mediated vasorelaxation, and thus leads to a compensatory increase in NO production from the endothelium.
Key words: metabolic syndrome, angiotensin II, nitric oxide

P280038

Vascular α_1-adrenergic responsiveness in early diabetic stage in the rat

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α_1-Adrenoceptor-induced contraction in models of diabetes mellitus (DM) has been reported to be heterogeneous. We analyzed phenylephrine (PHE) effects on α_1-adrenoceptors in blood vessels, during early DM stages (2 and 4 weeks). Male Long-Evans 6 weeks-old rats were DM-induced by streptozotocin (65 mg/kg, i.p.). Two and 4 weeks after aorta, mesenteric and tail arteries were stimulated by PHE alone and with α_1-adrenoceptor antagonists. pD_2 values increased 3-10 times in 2 weeks DM in all arteries, compared to controls, while E_{max} did not change. At 4 weeks of DM pD_2 values were similar in DM and controls, but decreased compared with 2 weeks DM. Antagonists showed α_{1D}-adrenoceptor decreased pIC_{50} at 4 weeks of DM in all arteries. It will be important to study the progression of DM on α_1-adrenoceptor expression and function.
Key words: Diabetes mellitus, blood vessels, α_1-adrenoceptors
Conacyt grant 47481, Fundación Miguel Alemán and PAPIIT IN322005

P280039

Euonymus alatus prevents a high fat diet-induced hyperglycemia and hyperlipidemia

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This study investigated the preventive effect of Euonymus alatus (EA) ethanol extract on high fat diet-induced hyperglycemia and hyperlipidemia. ICR mice were randomly divided into five groups: control mice were to receive either a regular diet (RD) or high-fat diet (HFD), and treatment groups were fed a high fat diet with either 350 mg/kg, 700 mg/kg of EA or 250 mg/kg of metformin for a 10-week period. EA not only reduced body weight in a dose dependent manner, but also corrected associated hyperinsulinemia and hyperlipidemia. EA exerted beneficial effects on glucose and lipid homeostasis in diabetes that are not secondary to its ability to decrease food intake but its specific effects on hepatic lipogenesis related genes (SREBP1a, FAS, GAPT) and PPAR-gamma gene expression in periepididymal fat. Taken together, the combined effect of EA to reduce plasma glucose and lipid levels, and reduce the deposition of triglyceride in the liver are indicative of a marked improvement in high fat diet-induced hyperglycemia and hyperlipidemia.
Key words: Euonymus alatus; high fat diet; lipogenesis; PPAR-gamma. This work was funded by Plant Diversity Research Center of 21st Century Frontier Re-

search Program.

P280040

Pharmacology of GGT-1 (a new incretin mimetic): a potential therapeutic for improved glycemic control of diabetes

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AIM: To study the glycemic control of GGT-1, a new polypeptide, in normal and diabetic mice. METHODS: ICR mice, MSG-treated mice, and KKAy mice were used to estimate the acute glycemic control of GGT-1. After a range of doses of GGT-1 (from 0.2 to 1.8 μg/kg) were sc injection, followed by glucose challenge or not, blood glucose levels were monitored and serum insulin concentration were assayed. After alloxan-diabetic mice were injected sc once daily for 4 weeks with GGT-1, pancreas were weighed and the insulin in pancreas were assayed. Charcoal meal assay were performed in ICR mice and the effect on duodenal delivery of GGT-1 were estimated. RESULTS: The plasma glucose excursion was reduced significantly by GGT-1 with a dose-dependent pattern. In contrast with sulfonylurea, the glucose lowering effect of GGT-1 is due to glucose-dependent insulinotropism and inhibition in the gastrointestinal motility. GGT-1 at dose of 1.8 μg/kg could enhance the insulin content of pancreas in alloxan-mice (20.5 ±5.8 vs. 9.4 ±6.2 IU/g tissue, $p < 0.01$), suggesting GGT-1 may improve the function of βcell. CONCLUSION: We suggest that GGT-1 have therapeutic potential in diabetes.

Key words: GGT-1; incretin mimetic

P280041

1α,25-D3 and TLR2 agonist Improve Insulin Sensitivity in MSG Obese Rat by regulation of regulatory T cells and Th1/Th2 immune responses

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The underlying cause of metabolic syndrome is a chronic inflammatory response characterized by enhancement of Th1 immune response that may be responsible for pathogenesis of insulin resistance. We wonder if shift of Th1/Th2 balance toward to regulatory T (Treg) cells or Th2 responses improves insulin sensitivity in MSG obese rats. The insulin sensitivity was determined by body weight, the insulin sensitivity index, oral glucose tolerance test, insulin tolerance test, and hyperinsuline mic-euglycemic clamp. The expression and activity of TLRs and their signal pathways were determined by PCR or western blot. We found that 1,25-D3 (1,25-D3), an immunomodulator, significantly improved the insulin sensitivity via increasing the Treg cell number leading to polarization of T cell development toward Treg direction. Interestingly, a TLR2 agonist peptidoglycan (PGN), but not TLR4 agonist, markedly improved the insulin sensitivity because PGN stimulated TLR2 leading to a Th2 immune response. In summary, 1,25-D3 and PGN improve insulin sensitivity via elevation of Treg cells or shift of Th1/Th2 balance toward Th2 immune response.

Key Words: metabolic syndrome, MSG obese rat, TLR2, Th1/Th2/Treg

P280042

Role of H_2S in the Development of Diabetes and the Underlying Mechanisms

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Physiological importance of H_2S, generated by cystathionine gamma-lyase (CSE) in vivo, has been known. In the present study, we demonstrated that pancreatic H_2S production rate was 58.1 + 9.2 % higher in streptozotocin (40 mg/kg/day for 3 days)-induced diabetic mice ($n = 6$) than that in control mice ($n = 5$, $p < 0.05$). Injection of propargylglycine (50 mg/kg/day for 30 days) to inhibit CSE gene expression significantly decreased glucose level to 16.2 mM in streptozotocin-induced diabetic mice ($n = 8$) whereas streptozotocin-treated mice without propargylglycine treatment had a glucose level of 30.6 mM ($n = 6$, $p < 0.05$). Furthermore, H_2S at 100 micromolar induced 8.2 + 1.5 % apoptosis ($n = 4$, $p < 0.05$) of cultured INS-1E cells, an insulin-secreting beta cell line. CSE overexpression with a recombinant defective adenovirus increased endogenous H_2S production and stimulated INS-1E cell apoptosis as well. It is concluded that abnormally high activity of CSE in the pancreas would increase endogenous H_2S production, leading to diabetes by inducing pancreatic beta cell apoptosis. (Supported by NSERC and Heart and Stroke Foundation of Canada).

Key words: H_2S; Cystathionine gamma-lyase; Diabetes; Apoptosis

P280043

Microvascular endothelial dysfunction following transient high glucose is related to oxidative stress and reduced glutathione levels.

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Objective: Investigate effects of high glucose (HG, 30 mM) on vascular function in mouse aorta and small mesenteric artery (SMA).

Methods: Aorta and SMA function was measured with a wire myograph following normal glucose or HG for 0, 2, 4, 6, 20 h. Superoxide by lucigenin chemiluminescence and fluorescence microtopography, and glutathione (GSH-endogenous antioxidant) were also assessed.

Summary of results: HG did not impair endothelium(E)-dependent vasodilatation (EDV) to acetylcholine or E-independent vasodilatation (EIDV) to sodium nitroprusside in aorta. In contrast, EDV and EIDV were impaired in the SMA following 20h HG, whereas contractile responses to potassium chloride and phenylephrine were unchanged in both aorta and SMA. In both aorta and SMA superoxide was significantly elevated and GSH significantly decreased by HG for 20h.

Conclusions: Microvessels are more susceptible to HG compared to conduit vessels. HG-induced endothelial dysfunction (ED) may be downstream of HG-induced oxidative stress. Repeated episodes of HG may lead to permanent ED and thereby development and progression of vascular complications in diabetes.

Key words: hyperglycaemia, oxidative stress, endothelial dysfunction.

P280044

Increased macrophage migration inhibitory factor (MIF) expression in non hypertension and cardiovascular disease 2 type diabetes patients with LVDD

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OBJECTIVE: Recent studies showed that inflammation factors play a crucial role in diabetes. The aim of this study is to investigate the association between the pro-inflammation factor, macrophage migration inhibitory factor (MIF) and diabetes cardiomyopathy. METHODS: To observe 63 patients with type 2 diabetes who were aged 38-60 years without evidence of hypertension, coronary artery disease, congestive heart failure, diabetic complications. Left ventricular diastolic dysfunction (LVDD) was evaluated by Doppler echocardiography, E'/A' > 1 regarded as LVDD. Systolic function was normal in all subjects. RESULTS: LVDD patients was found in 33 subjects (52.4%). Patients with normal left ventricle diastolic function were used as controls. Plasma MIF concentrations in the patients with LVDD were significantly higher, and MIF-mRNA level in lymphocytes was increased. These increases in MIF are related to plasma glucose and FFA concentrations. CONCLUSION: Plasma MIF concentrations and MIF mRNA expression in the lymphocytes are elevated in type 2 diabetes mellium patients with non-persistent hypertension and cardiovascular disease, therefore it is independent prognostication factor for type 2 diabetes.

Key words: Atherosclerosis, ERK MAP kinase, MIF, MMPs.

ACKNOWLEDGMENTS: This work was supported by NSFC (3030042) and GDNSF (015015, 04102307, 033189).

P280045

A Novel Biosynthetic Pathway for Anandamide

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The endocannabinoid anandamide (AEA) is thought to be generated from its membrane precursor, N arachidonoyl phosphatidylethanolamine (NAPE), through cleavage by a phospholipase D (NAPE-PLD). Here we document a novel biosynthetic pathway responsible for LPS-induced AEA production. In RAW264.7 macrophages, LPS unexpectedly down-regulates NAPE-PLD expression by 60% but increases 3.2-fold the expression of PTPN22, a protein tyrosine phosphatase also present in brain. siRNA knockdown of NAPE-PLD does not modify cellular AEA levels or prevent their increase by LPS, whereas the PLC inhibitor neomycin or the tyrosine phosphatase inhibitor NaVO3 blocks LPS-induced AEA synthesis. Endogenous phospho-AEA (pAEA) is increased by NaVO3 treatment. Incubation of synthetic pAEA with macrophage or brain ho-

mogenates or with recombinant PTPN22 leads to time-dependent, heat- and NaVO3-sensitive generation of AEA, which is increased in cells overexpressing PTPN22. We conclude that the regulated biosynthesis of AEA from NAPE proceeds through the PLC-catalyzed generation of pAEA and its dephosphorylation by PTPN22. This pathway may represent a novel pharmacotherapeutic target for modulating the endocannabinoid system.

P280046

Anti-hyperglycemic activity of an α-glucosidase inhibitor TD-01

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To investigate the effect and mechanism of TD-01, a chemical synthesis compound, on antihyperglycemic activity in vitro and in vivo. The activities of TD-01 and Acarbose against sucrase, maltase and α-amylase are compared in vitro. Normal and alloxan-induced diabetic mice were used to study the effects of TD-01 on the tolerances of sucrose, starch and glucose in vivo. TD-01 was also given to Streptozotocin (STZ) diabetic rats with the chow for chronic experiment. The findings showed that TD-01 has strong inhibitory activities against sucrase and maltase and no inhibition on α-amylase. In fasting normal and alloxan-induced diabetic mice, TD-01 can lower and prolong the zenith of blood glucose concentration after sucrose or starch loading and stabilize blood glucose levels. When STZ diabetic rats fed with high calorie chow were tested with TD-01, the hyperglycemic symptoms and the blood lipid levels were improved. These results indicate that TD-01 has strong property of α-glucosidase inhibition and may be useful for treating diabetes and its complications.

Key Words: α-glucosidase inhibitor, oral carbohydrate tolerance test, hyperglycemic symptoms

P280047

Role Of The Transcription Factor NFkB In Myocardial Ischemia Reperfusion In Diabetic Mice

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Diabetic patients frequently suffer from accelerated atherosclerosis with increased mortality for coronary artery disease and myocardial infarction. Loss of endothelial function with increased expression of endothelial adhesion molecules plays a key role in the development of diabetic vasculopathy. In endothelial and vascular smooth muscle cells both hyperglycemia and advanced glycosilation end products, induce cellular oxidant stress leading to activation of the transcription factor Nuclear Factor Kappa B (NFkB). Myocardial ischemia-reperfusion in rats is an experimental model useful to study cell-cell interaction and leukocyte accumulation in the ischemic tissues, phenomena that play a key role in the development of end-organ damage and are NF-kB mediated. With the aim to investiagate on these mechanisms, we study the effects of NFkB inhibitors clasto-Lactacystin Lactone and Epoxomicin and tyrosine kinase inhibitor Genistein on myocardial ischemia-reperfusion in genetically diabetic mice. We used diabetic C57BL/KsJ db (db/db) male mice and their controls (db/mice). Myocardial ischaemia reperfusion injury was produced by the occlusion of the left descending coronary artery for 45 min. The occlusion was then released and reperfusion lasts 5 hours. We also compared the effects of intraperitoneal injection of clasto-Lactacistin Lactone (3 mg/kg), or epoxomicin (0.5 mg/kg) or genistein (1 mg/kg), both in diabetic and non diabetic mice subjected to myocardial ischaemia-reperfusion injury. Myocardial injury was evaluated with the triphenyl tetrazolium-chloride-Evans-blue technique; neutrophil accumulation was measured by determining myeloperoxidase (MPO) activity, and NFkB activity was investigated with western blot analysis. Result showed in db/db mice treated with clasto-Lactacistin Lactone, a reduction of area-at-risk of 35%; NFkB inhibition was of 70% vs control mice db/db. In area-at-risk and in necrotic, clasto-Lactacistin Lactone, reduced leucocyte accumulation of 38,5% vs controls. Treatment with epoxomicin, caused an inhibition of NFkB activity of 75% vs db/db mice not treated; reduction of leucocyte accumulation of 37%; reduction of area-at-risk of 33%. In db/db not treated mice, we observed an activation of NFkB of 75%; MPO activity was in area at risk of 70.4 ± 4.7 nmol/g tissue and in necrotic area of 87.8 ± 4.8 nmol/g tissue. These results suggest that NFkB inhibitors clasto-Lactacystin Lactone, Epoxomicin and tyrosine kinase inhibitor Genistein, could protect from myocardial ischaemia, occurring in diabetes.

P280048

Characterization of the role of the adenosine A1 receptor (A1R) in metabolism using A1R knock-out (-/-) mice

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Chronic consumption of caffeine, known to act by blocking adenosine receptors (AR), can decrease the risk of type 2 diabetes. In this study, the role of the A1R has been evaluated in congenic A1R(-/-) mice. In young mice, an i.p. injection of glucose gave a transient rise of plasma insulin levels in A1R(+/+) mice, but in A1R(-/-) mice the rise was prolonged. In A1R(+/+), glucose suppressed glucagon levels, whereas they were increased in A1R(-/-) mice. In young mice, plasma glucose levels were unaltered and tolerance to i.v. glucose was not changed when A1R was deleted. In addition, insulin- and contraction-stimulated glucose transport in skeletal muscle, HbA1c values and body weight were essentially the same in young A1R(-/-) and A1R(+/+) mice, but A1R(-/-) males above 5 months had higher body weight than A1R(+/+). A1R(-/-) mice had also higher mortality rates than A1R(+/+) mice. In vivo and in vitro data showed that the antilipolytic effect mediated by adenosine is lost in the A1R(-/-) mice. In conclusion, the A1R is involved in different metabolic pathways, and may be particularly important in older animals.

Key words: metabolism, adenosine, A1 receptor

P280049

Effects of 17beta-estradiol on the progression of glioblastomas

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Glioblastomas are the most common and aggressive type of glioma and one of the most aggressive of all malignancies. Epidemiology studies have found that female glioblastoma patients had a decreased length of survival, suggesting estrogen could affect the progression of glioblastoma. In the present study, the effects of 17beta-estradiol (E2) on the progression of a gliobastoma cell line, C6 cell, were determined using both in vitro and in vivo approaches. In the cell culture, effect of E2 on C6 cells proliferation was determined by trypan blue exclusion method, and the effect of E2 on glutamate-induced C6 cell death was determined. E2 has no effect on C6 cells growth, while, E2 significantly decreased cell death induced by glutamate. In the animals study, C6 cells were injected subcutaneously in ovariectomized female rats, which simultaneously received E2 replacement or vehicle. The animals were sacrificed two weeks after tumor implantation for tumor evaluation. E2 replacement significantly promoted tumor growth. Our studies indicated that estrogen could change the balance of glioblastoma growth events by decrease of cell death, hence promote the tumor progression.

Key words: glioblastoma, estrogen.

P280050

Antidiabetic activity of flowers of *Nelumbo nucifera* **extract in streptozotocin-nicotinamide induced type 2 diabetic rats**

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Objective: The aim was to evaluate the effect of methanolic extract of *Nelumbo nucifera* flowers (MNF) in streptozotocin-nicotinamide induced type 2 diabetes in rats. *N. nucifera* has been used in Unani medcine for treating diabetes. Method: Diabetes was induced in rats by streptozotocin (65 mg/kg) and nicotinamide (230 mg/kg) (i.p) 15 minutes later. MNF (500 mg/kg) was administered for 21 days (p.o). Insulin tolerance test (on 21st day) and blood glucose, Serum insulin serum lipid profile, hepatic hexokinase and phosphoend pyruvate carboxykinase (PEPCK) were determined. Results: The administration NMF decreased the blood glucose levels significantly ($P < 0.001$). It increased the insulin

sensitivity and decreased the serum cholesterol levels. HDL/total cholesterol ratio was increased. Serum insulin levels were not altered. Hexokinase activity was increased ($P < 0.01$) and activity of PEPCK decreased. The possible insulinomimetic action of the extract at the cellular levels requires further study. Conclusion: MNF was found to possess antidiabetic activity in streptozotocin-nicotinamide induced type 2 diabetic rats.

Key words: Nelumbo nucifera; diabetes; streptozotocin; nicotinamide

Acknowledgement: Authors acknowledge DBT (Govt. of India) for financial assistance.

P280051

Recombinate Human Ciliary Neurotrophic Factor Reduce Weight by Regulating Nuclear Respire Factor 1 and Components of Mtochondrial

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The effects and mechanisms of recombinate human Ciliary Neurotrophic Factor (rhCNTF) were studied in genetically obesity and diabetic KK-Ay mice. Semi-quantitative RT-PCR demonstrated that, the gene expression of nuclear respire factor (NRF)-1, mitochondrial transcription factor A (Tfam), uncoupling protein (UCP)-1 were up-regulated, and the content of cytochrome C enhanced in brown adipose tissue (BAT) from KK-Ay mice given rhCNTF for 3 days. Also, the activity of mitochondrial complex IV were increased after rhCNTF administration. Also, rhCNTF (0.1, 0.3, 0.9 mg/kg/day S.C.) administrated to KK-Ay mice for 30 days manifest powerful weight reduction effect. The stimulation of NRF-1, TFam, UCP-1 and enhanced activity of mitochondrion complex IV might be closely related to the anti-obesity effects of rhCNTF.

Key words: recombinate human ciliary neurotrophic factor, obesity, UCP-1, NRF-1, TFam, mitochondrion respiratory chain

P280052

Hypoglycemic Effects of Exo-biopolymers Produced by Five Different Medicinal Mushrooms in STZ-induced diabetic rats

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Hypoglycemic effects of exo-biopolymers (EBP) produced by submerged mycelial cultures of Coriolus vesicolor, Codyceps sinensis, Paecilomyces japonica, Armillariella mellea, and Fomes fomentarius were investigated in stereptozotocin-induced diabetic rats. All the experimental group were orally administrated with EBP (100 mg/kg body weight) for 2 weeks. Hypoglycemic effect was achieved in the all experimental group, however, C. vesicolor EBP proved to be most potent one. The administration of the C. vesicolor EBP substantially reduced plasma glucose level by 24.2% as compared to the saline administrated group. It also reduced the plasma total chdlesterol, triglyceride, aspartate aminotransferase and, alanine aminotransferase levels, respectively. The sugar and amino acid composition of C. versicolor EBP were also analyzed in detail.

This work was supported by RRC program of MOIEC

P280053

Effects of bis(a-furancarboxylato) oxovanadium(IV) on non-diabetic and streptozotocin-diabetic rats

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Aim To study the effects of bis(a-furancarboxylato) oxovanadium(IV) (BFOV) on carbohydrate and lipid metabolism in normal and diabetic rats. Methods Diabetic rats were induced by a injection of streptozotocin (STZ, 50 mg/kg; i.p). BFOV was given intragastrically to normal and STZ-rats for 4 weeks. Blood glucose, oral glucose tolerance test (OGTT), glycohemoglobin, serum insulin, lipid levels and glycogen content were observed. Results Administration of BFOV (0.1, 0.2 and 0.4 mmol/kg) to STZ-rats dose-dependently reduced blood glucose level, while did not influence blood glucose in normal rats. Serum insulin levels were not increased in the BFOV treated diabetic groups, and, in contrast, significantly lowered in the 0.2 mmol/kg BFOV treated normal group. BFOV markedly reduced glycohemoglobin level, improved OGTT and dyslipidemia in STZ-rats, in a dosedependent manner, but had no significant effect on normal rats. Conclusion The complex was effectively attenuate diabetic alterations in STZ-diabetic rats.

Key words Bis(a-furancarboxylato) oxovanadium(IV); STZ-rats; Blood glucose; Lipid.

Acknowledgement: This work was supported by National Natural Science Foundation of China (30260118).

P280054

Insulin Mimetic Effects of Bis(a-furancarboxylato) oxovanadium(IV) in Isolated Rat Adipocytes and Alloxan-Diabetic Mice

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Aim: To study the insulin-like effects of bis(a-furancarboxylato) oxovanadium (IV) (BFOV) in vivo and in vitro.

Method: Glucose uptake and lipogenesis in isolated rat adipocytes were determined using 2-deoxy-D-[^{3}H]-glucose and D-[^{3}H]-glucose, respectively. Lipolysis was assayed by free fat acid (FFA) released from isolated rat adipocytes treated with epinephrine. Diabetic mice were induced by injection of alloxan. Blood glucose was measured after given BFOV i.g. to normal and alloxan-mice for 14 days. Result BFOV increased the uptake of glucose and the transformation from glucose to lipid in isolated rat adipocytes, with the EC50 values of 0.31 ± 0.08 mM and 0.49 ±0.12 mM, respectively, which were enhanced in the presence of insulin. BFOV inhibited FFA release from adipocytes treated with epinephrine, with the IC50 value of 1.20 ±0.23 mM. BFOV (0.2, 0.4 mmol/kg i.g.) decreased blood glucose levels, food and water intake in alloxan-mice, but not in normal mice. Conclusion BFOV had insulin-like effect in vivo and in vitro.

Key words: bis(α-furancarboxylato) oxovanadium(IV); glucose uptake; lipogenesis; lipolysis

Acknowledgement: This work was supported by National Natural Science Foundation of China (30260118).

P280055

Effects of bis(a-furancarboxylato) oxovanadium(IV) on type 2 diabetic rats

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Aim: To study the effects and mechanism of bis(a-furancarboxylato) oxovanadium(IV) (BFOV) on glucose and lipid metabolism in type 2 diabetic rats.

Method: Type 2 diabetic rat was induced by high fat and sucrose feeding + STZ injection. Given i.g. BFOV for 4 weeks, blood glucose, OGTT, serum insulin, and associated parameters were measured; hepatic glycogen, activities of HK and PK and phosphoenolpyruvate carboxykinase (PEPCK) mRNA in liver were determined. Result: The type 2 diabetic rats have been established, with hyperglycemia, hyperinsulinemia, hypertriglyceridemia and high level of FFA. BFOV reduced the blood glucose, but did not improve OGTT of diabetic rats. BFOV reduced serum TG and FFA, increased hepatic glycogen and the activities of HK and PK, and decreased PEPCK mRNA of liver in diabetic rats. BFOV had no effect on liver and kidney function. Conclusion: BFOV has antidiabetic effect in type 2 diabetic rats, which mechanism was related to increase hepatic glycogen and decrease gluconeogenesis.

Key words: bis(α-furancarboxylato) oxovanadium(IV); rat; glycometabolism; lipid metabolism

Acknowledgement: This work was supported by National Natural Science Foundation of China (30260118).

P280056

Cardonat and regulation of metabolic processes

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The aim of the presented study was to investigate physiological activity of

metabolic agent Cardonat.

Methods: Acute toxicity and rats' endurance under physical loading were studied. Acute toxicity was investigated with Prosorovsky method (1998). Physical loading was done with "swimming test" and others.

Results: It was shown that Cardonat preventive usage significant increase physical endurance (prolong "swimming test" in 1.5 times), glucose utilization (in 1.5 times), decrease level of blood metabolic acidosis (acidum lacticum was decreased in 2 times) and others tests.

Conclusions: Cardonat can be useful in normalization of organism metabolic regulation, remove physical and mental overloading (for example, sportmans), muscle dystrophy and atone. As a part of complex therapy it eliminate pathological processes of heart and vessels. Cardonat can be useful for treatment of asthma, chronic bronchitis, acute and chronic disorders of cerebral blood circulation, liver and renal deseases, and others pathological processes, that demand metabolic regulation improving.

Key words: Cardonat, metabolic regulation, physical and mental overloading.

P280057

Mediation of endogenous β- endorphin by serotonin to lower plasma glucose in Streptozocin- induced diabetic rats

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Serotonin had multiple pharmacological and physiological actions. We investigate the mechanisms of plasma glucose lowering action of serotonin in streptozocin- induced diabetic rats (STZ- rats). Serotonin produced a dose- dependet hypoglycemic action in STZ- rats after i.p. injection. In STZ- rats, pretreatment with pimozide(5HT7) or dihdroergotamine, two selective antagonists of serotonin receptor, abolished the hypoglycemic effect of serotonin. Similar antagonism of the hypoglycermic effect of serotonin was observed in STZ- rats treated with naloxone. Moreover, bilateral adrenalectomy in STZ- rats eliminated the activities of serotonin, including the plasma glucose- lowering effect and the plasma β- endorphin (BER) effect. Naloxone inhibited the plasma glucose- lowering activity of serotonin at dose sufficient to block opioid receptor. In adrenal medulla isolated STZ- rats, serotonin- induced BER secretions were abolished by pretreatment with serotonin receptor antagonists. In conclusion, our results suggested that serotonin may activate 5HT7 receptor to enhance the secretion of BER, which can stimulate the opioid receptor to increase glucose utilization, resulting in decrease of plasma glucose in STZ- rats.

P280058

Fructose, methylglyoxal, and peroxynitrite production in vascular smooth muscle cells

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The aims of present study were to investigate whether fructose, a precursor of methylglyoxal (MG), induced ONOO- generation and whether this process was mediated via MG formation. The intracellular production of MG was significantly increased by 118 ~23% or 373 ~32% after vascular smooth muscle cells (VSMCs) were treated 6 hours with fructose (15 or 30 mM), compared with that from untreated cells ($p < 0.01$, $n = 4$ in each group). Levels of ONOO-, NO, and $O_2^{\cdot-}$ were also significantly increased in VSMCs treated with either fructose or MG. ONOO- generation induced by fructose or MG was significantly inhibited by reduced glutathione or Nacetyl- l- cysteine, and by $O_2^{\cdot-}$ scavengers (diphenylene iodonium and superoxide dismutase) or NOS inhibitor (N- nitro- L- arginine methyl ester). Moreover, iNOS expression was enhanced in the cells treated with MG and it was significantly inhibited when co- application with N- acetyl- l- cysteine. Our results demonstrated that fructose induces a significant increase in ONOO- production, which is mediated by an increase in endogenous MG formation in vascular smooth muscle cells.

Key words: Methylglyoxal; Fructose; Smooth muscle cell; Peroxynitrite (Supported by CIHR & HSFS)

P280059

α- glucosidase inhibitor for the treatment of diabetes

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In order to find a new α- glucosidase inhibitor for the treatment of diabetes, we screened a lot of extracts of Chinese medicinal herbs using α- glucosidase method in vitro and found that the aqueous extract from suregade glomerulata (BL) had the inhibitory effect with IC50 value of 0.01 mg/ml. The effect of BL- ex on the postprandial rise in blood glucose level was investigated. We performed starch, sucrose and glucose tolerance tests in normal mice, and acarbose was used as a positive control during these tests. The increase in plasma glucose level in response to the oral administration of starch or sucrose was significantly suppressed in mice when BL- ex or acarbose was given, respectively. However, BL- ex or acarbose had no effect on plasma glucose level when glucose was administered orally. Those results suggested that the antihyperglycemic effect of BL- ex is due to inhibition of α- glucosidase in the small intestinal epithelium.

Key words: diabetes; α- glucosidase inhibitor; suregade glomerulata

P280060

Establishment of the glucokinase activators screening model and hetero- expression of human glucokinase

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Glucokinase(GK) is a novel potential drug target for the treatment of type 2 diabetes, and has a highly control strength in glucose homeostasis. GK activators (GKAs) will facilitate insulin secretion and decrease hepatic glucose production. We have established an in vitro screening model of GKAs, investigated 12 oriented synthesis compounds and 1,600 non- oriented synthesis compounds. One oriented synthesis compound had been found which increased GK activity 1.5 fold at a concentration 10 mM, and its other characters have being experimented. In the other hand, we amplified the cDNA fragment of GK by RT- PCR from human liver, the DNA fragment was cloned into pPIC9K vector(invitrogen). A transformart of highest activity from 8 transformants was obtained. After the selected transformed P. pastoris cell line was fermented with YPM medium, the supernatant was collected and freeze- dried, followed by a series of purification steps. The recombinant enzyme will be used in screening model.

Key word: Glucokinase, hetero- expression, activator, screening

P280061

Annexin 1: a mediator of glucocorticoid action at the neuroendocrine- immune interface.

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Glucocorticoids (GCs) play an essential role in the maintenance of homeostasis and aberrations in the mechanisms which control their secretion and/or activity are strongly implicated in the pathogenesis of a number of common diseases including depression, hypertension, diabetes/obesity and immune/inflammatory disease. Annexin 1 (ANXA1), a protein mediator of GC action, is a key regulator of GC secretion, acting within the brain and pituitary gland to depress the release of the hormones which normally drive GC production. Its mode of action is unusual as it acts by a juxtacrine/paracrine mechanism and, following secondary processing, appears to interact with formyl peptide receptors (FPRs). Ligands for FPRs include bacterial peptides, mediators of the resolution of inflammation and peptides concerned with the pathogenesis of Alzheimer's disease, suggesting a complex interaction between GCs and inflammatory mediators in the brain and pituitary gland. Early life events (e.g. stress) exert long- term effects on ANXA1 expression and function in adulthood. ANXA1 may thus contribute to the altered disease susceptibility linked to adverse events in perinatal life.

Supported by the Wellcome Trust.

P280062

Green Tea Extract Modulates Adipocytokines and Activates PPAR Protein Expression in Insulin Resistant Hamsters

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A formulation of green tea extract (GTE) was evaluated for its potential to modulate adipocytokines and improve lipid and glucose homeostasis in fructose- induced insulin resistance hamsters. The effects of GTE on the activation of peroxisome proliferator- activated receptors (PPAR) were also investigated. Following the oral supplementation with GTE(150 mg/kg/day) for 4 weeks, triglyceride in plasma, liver and heart tissues were significantly decreased. GTE reversed the

metabolic defects by decreasing in insulin level and an improving in glucose tolerance. GTE modulated adipocytokines by significantly suppressing TNF-, IL-1 and IL-6 expression and increasing adiponectin. GTE significantly increased PPAR (330%) and PPAR-540%) protein expression in the liver. This study suggests that GTE could ameliorate hypertriglyceridemia and its antidiabetic effects might occur as a consequence of adipocytokine modulation and PPAR and PPAR activation.

Key words: green tea extract; adipocytokines; PPAR.

Acknowledgments: The project was supported by the NIH-National Center for Complementary and Alternative Medicine (R21 AT001286-02).

P280063

Streptozotocin-induced experimental diabetes causes an impairment in Ca2+-calmodulin dependent contractions in rat aorta: Effect of insulin treatment

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Experimental diabetes causes various long-term changes in smooth muscles(1). In the present study, the effect of STZ diabetes and insulin treatment on the reactivity of isolated rat aorta to KCl and calmidazolium, specific calmodulin blocker, were examined.

After 8 weeks of STZ diabetes, the contractile effect of KCl and the noncompetitive antagonistic effect of calmidazolium against KCl on isolated aorta were found to be decreased. Calmodulin levels were also found to be decreased in aorta from STZ diabetic rats. Both impaired reactivity to KCl and decreased calmodulin levels in diabetic rat aorta were not corrected by the treatment with insulin (10 IU/kg for 20 days). Only a partial correction following the insulin treatment was observed in the antagonistic effect of calmidazolium as observed by the increase in non-competitive antagonist affinity constants.

From the findings obtained in the present study, it was concluded that STZ diabetes causes an impairment in calcium/calmodulin dependent contractile process of aorta which seems to be resistant to insulin therapy.

Key words: Diabetes, aorta, calcium, calmodulin

P280064

Age-dependency of Amadori-induced Effects. An in Vitro Study.

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We previously described that; early product of non-enzymatic glycation of proteins (Amadori-adducts) can have a pivotal role on several diabetes-associated complications; inducing oxidative stress, inflammation, and apoptosis in human cells or impairing endothelium-dependent relaxations in human microvessels. As those effects can be also observed during the aging process in both experimental models, the aim of the present study was to evaluate whether ageing may modulate Amadori-induced effects, in either human peritoneal cells or human microvessels, isolated from individuals with different ages (range 21-86 yrs), and using different cellular, molecular biology, and vascular reactivity approaches.

We found that, above-described effects decreased according to the age of the donor, becoming practically absent in cells or vessels from old people (over 65 yrs-old). Thus, the age-dependency of Amadori-induced effects in vitro raises the hypothesis that the mechanisms underlying the involvement of target organs in diabetes will be different, depending upon the age of the patient.

Key words: Ageing, diabetes, Amadori-adducts, oxidative-stress.

Supported by FIS-04:1839/1955 & RGDM-03/212.

P280065

Transgenic UDP-Glucuronosyltransferase 1 (Tg-UGT1) Mice are Protected from Obesity-Induced Type 2 Diabetes

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Obesity-induced type 2 diabetes is a growing human health issue in Western societies. Fibrates used to treat diabetes are targeted by glucuronidation for elimination. To examine the contribution of human glucuronidation towards obesity-induced type 2 diabetes, wild type (WT) and Tg-UGT1 male mice were fed a normal diet or a 35% fat diet (HFD). Mass gain was monitored and diabetic status established. HFD WT mice were insensitive to insulin, retained elevated blood glucose levels, and displayed hyperinsulinemia. HFD Tg-UGT1 mice were protected from these markers of type 2 diabetes. To determine if human UGT1A expression correlated with protection, UGT1A protein levels were measured by Western blot. UGT1A proteins in the small intestine were down-regulated in HFD Tg-UGT1 mice. Time course studies established the point of UGT1A protein reduction in relation to disease onset and disease protection. While it is not clear why down-regulation of the human UGT1A proteins might protect against type 2 diabetes, the reduction may increase the potential for toxicity of drugs in diabetic patients. Supported by USPHS grant GM49135

Key words: Transgenic mice, UGT1A, diabetes, glucuronidation

P280066

Correlation of oxidative and antioxidative status with lipid profile in patients with insulin-dependent and noninsulin dependent Diabetes mellitus

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Diabetes mellitus (DM) is associated with many metabolic disturbances, including alterations in redox regulation. There is increasing data about free radicals involvement in the development of DM. The association between antioxidative defence (AD), systemic oxidative preasure and lipid profile was investigated in patients with insulin-dependent DM(IDDM) and noninsulin dependent DM(NIDDM). Both in diabetics and control subjects were determined: activities of copper zinc superoxide dismutase (CuZn SOD), catalase, glutathione peroxidase (GSH-Px) and glutathione amount in erythrocytes, plasma lipid peroxides (LP) level and serum triglycerides and cholesterol concentration. In IDDM patients, GSH-Px activity was higher than in control subjects. Besides, erythrocyte GSH-Px activity was significantly lower and CuZn SOD was higher in NIDDM compared to healthy subjects. Moreover, increased level of LP and cholesterol and triglycerides concentration was found in NIDDM, compared both with control and IDDM subjects. The results of the present study suggest that disturbance of the AD and lipid profile is important events in NIDDM etiology, subsequently leading to elevated oxidative damage.

P280067

Effect of Resistin on Glycogen Metabolism in Primary Cultured Rat Hepatocyte

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Obesity is a major risk factor for insulin resistance and type 2 diabetes. Since liver glycogen metabolism plays an essential role in maintaining glucose homestasis, this study was investigated the effect of resistin on hepatic glycogen metabolism. First, liver glycogen contents were determined in primary cultured hepatocytes treated with resistin. Compared with control, hepatocytes exposed to resistin showed a decrease in glycogen content in the presence of insulin and no obvious difference in basal glycogen contents in the absence of insulin. Then the expression of IR, GLUT2, GK, GS and GP, and activity of GK and GP were analyzed to investigate the possible molecular mechanism. The results showed that IR expression was decreased and GP activity was enhanced after treated with resistin. In final, no significant difference was observed in expression of GLUT2, GK, GS, GP and GK activity. Our data showed that resistin may cause disorder glycogen metabolism in primary cultured rat hepatocytes through blocking insulin action. These results strongly suggest that resistin is highly associated with insulin resistance and type 2 diabetes.

Key words: resistin; insulin; glycogen; hepatocyte; type 2 diabetes

P280068

Impairment of Parasympathetic-dependent and –independent insulin action in Zucker Diabetic Fatty rats

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Obesity is a condition often associated with insulin resistance, which is related with an impairment of the hepatic parasympathetic (HPN)-dependent insulin action. The Zucker diabetic fatty (ZDF) rat model further shows type 2 diabetes.

Thus, we tested the hypothesis that the HPN insulin action is decreased in ZDF, contributing to the diabetic condition. Insulin sensitivity (IS) was assessed by an euglycemic clamp in male 9-weeks-old ZDF (n=6) and lean Zucker rats (LZR, n=5), before (control) and after 3 mg/kg atropine-induced blockade of the HPN component. The difference between the control and the post-atropine IS represents the HPN-dependent contribution to total IS. Total IS was lower in ZDF (116.3±13.3 mg glucose/kg b.w) than in LZR (299.3±27.6 mg glucose/kg b.w, $p<0.0001$). The insulin resistance observed was due both to a decrease of the HPN-dependent component (129.0±17.5 for LZR to 71.3±9.0 mg glucose/kg b.w for ZDF, $p<0.05$) and of the HPN-independent component of insulin action (170.3±24.8 for LZR to 45.1±4.7 mg glucose/kg b.w for ZDF, $p<0.001$). In this study we observed that IS is decreased in the ZDF rats due to a dysfunction of both the HPN-dependent and independent components of insulin action.

P280069

The leptin and TNF-α expression in retroperitoneal and epididymal adipocytes of hypothyroid rats

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Since thyroid hormones enhance the basal metabolic rate, which is a predictor of the risk of development of obesity, it is generally held that altered thyroid function contributes to obesity. Two of the most intensely investigated proteins secreted by adipose tissue are leptin and tumor necrosis factor (TNF-α). Leptin has a major role in the regulation of appetite and energy balance, while TNF-α is a pro-inflammatory cytokine, with effects on lipid and glucose metabolism.

In this study, leptin and TNF-α expression were compared in rat retroperitoneal and epididymal adipocytes after 21 days-treatment with metimazole (anti-thyroid agents). Two adipose depots were dissected and routinely processed for leptin and TNF-α immunohistochemistry.

In control rats the majority of retroperitoneal adipocytes have higher leptin and similar TNF-α expression as compared with epididymal adipocytes. Hypothyroidism reduces leptin immunopositivity in retroperitoneal adipocytes, while in epididymal increases, especially around coalescing lipid bodies. TNF-α expression is completely abolished in both adipose depots.

Thus, differences in adipocyte leptin and TNF-α expression are marked in hypothyroidism.

P280070

Impaired AQP5 trafficking in parotid interlobular duct of rats with type 1 diabetes mellitus

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To clarify the mechanisms underlying diabetic xerostomia, we investigated subcellular localization of aquaporin-5 (AQP5) in parotid interlobular ducts of control and streptozotocin-induced diabetic rats stimulated or unstimulated by cevimeline. Immunohistochemical study indicated that AQP5, under unstimulated conditions, was colocalized with flotillin-2 and GM1 with a diffuse pattern in the cytoplasm of interlobular ducts in both control and diabetic rats. Ten minutes after intravenous injection of cevimeline, AQP5 was dramatically increased together with flotillin-2 and GM1 in the apical plasma membrane of parotid cells of control but not diabetic rats. Protein synthesis for AQP5 was decreased in parotid glands of diabetic rats, even though the transcription step was increased. Treatment of parotid tissues with cevimeline for 10 min induced an increase in the solubility of AQP5 by Triton X-100 in control but not diabetic rats. Administration of insulin to diabetic rats produced the cevimeline-induced trafficking of AQP5 as observed in control rats. The results show that administration of a muscarinic agonist results impaired AQP5 translocation in salivary gland of diabetic rats.

P280071

Acute hyperglycaemia impairs endothelial function in rat isolated skeletal muscle arteries

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In type 2 diabetes, vascular complications are preceded by endothelial dysfunction. The present study characterised endothelium-dependent vasodilatation (EDV) in skeletal muscle arteries, and investigated the effect of an acute hyperglycaemic insult on endothelial function. Changes in diameter of rat isolated gracilis arteries (80 mmHg intraluminal pressure, ≥40% myogenic tone) were measured via video microscopy. ACh (0.1 nM-10 μM)-induced vasodilatation comprised a predominant (apamin + charybdotoxin-sensitive) EDHF component, and a smaller (L-NAME-sensitive) NO component (n=5-10). High glucose (HG; 40 mM, 1 hr intraluminal; n=8), but not mannitol (n=6), significantly reduced the ACh pEC50 (7.6±0.2 cf. 8.1±0.2, $P<0.05$), however vessels remained L-NAME-sensitive. Dilation attributed to EDHF was inhibited by HG exposure. Baseline i.d. was significantly increased following HG or mannitol treatment, suggesting an osmotic effect on myogenic tone. Thus, acute hyperglycaemia impairs endothelial function in rat gracilis arteries, possibly by interfering with the EDHF vasodilator pathway.

P280072

Effects of Shenqi Compound on the improvement of insulin resistance rats induced by high fat diet

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AIM: To observe the effects and mechanisms of Shenqi Compound in insulin resistance rats induced by high fat (HF) diet. METHODS: Male Spraque-Dawley rats were divided into normal-diet group and HF-fed group during the first four weeks of experiments. HF rats were then treated with vehicle (HF), Metformin or Shenqi Compound for 28 days. Concentrations of fasting blood glucose (FBG), fasting plasma insulin (FINS) and leptin in serum were measured. Homeostasis model-Insulin resistance (HOMA-IR) index was calculated. The expressions of protein and mRNA of leptin in adipose tissue were detected by western-blot and RT-PCR. RESULTS: (1) Shenqi Compound could markedly reduce the HOMA-IR, serum FINS and leptin levels in rat model of insulin resistance. (2) Shenqi Compound treatment also suppressed mRNA and protein expression of leptin in adipose tissue from HF-induced insulin resistance rats. CONCLUSION: Shenqi Compound could attenuate the insulin resistance in rats caused by high fat diet which may be due to its action in suppressing the expressions of leptin in adipose tissue.

KEY WORD: Shenqi Compound; Insulin resistance; Leptin; high-fat diet

P280073

The experimental study on the myocardium expression of TGF-β1 and apoptosis in the diabetic rats.

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OBJECTIVE: to study the myocardium expression of TGF-β1 and apoptosis in diabetic rats. METHODS: The diabetes models were established by streptozotocin in rats. the expression of TGF-β1 in the cardiomyocytes. were detected as the index to evaluate the degree of fibrosis. The method of TUNEL was used for apoptosis. RESULTS: 1. The weights of diabetic rats were apparently lower than those before the diabetic model was built, and the increase of weights in diabetic rats within three month were less than those in normal group. 2. Compared with the control group, the concentration of blood sugar were continually elevated during the experiment. 3. The expression of TGF-β1 in the diabetic cardiac muscle was much more than the normal group ($p<0.01$). 4. The apoptosis of myocardium measured by the method of TUNEL were apparent in the diabetic groups than the normal one ($p<0.01$). but no significance was detected in the different courses of diabetic groups. CONCLUSION: TGF-β1 might be a significant factor in diabetic myocardium fibrosis and apoptosis might play an important role in the initial stage of diabetes in which leading the diabetic cardiomyopathy to heart failure.

Key words: Diabetic cardiomyopathy; TGF-β1, Apoptosis

P280074

Experimental gestational diabetes decreases noradrenaline release in the myometrium of the rat

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Aim: Diabetes mellitus (DM) develops in 4-9% of frequencies and causes a remarkable risk of neonatal morbidity and mortality. The aim of the present study was to investigate the effect of experimentally induced DM on noradrenaline release profile and on agonists-induced contraction of myometrial rings.

Methods: SPDR rats were treated with streptozotocin (60 mg/kg i.v.), the ex-

periments were carried out 10 days later. Uterine samples were loaded with [^{3}H] - noradrenaline and put into a superfusional chamber. After a washout period 3 - min fractions were collected and electric field stimulations were applied. Cumulative dose - response curves for sympathetic agonists (noradrenaline and terbutaline) were additionally generated. Both types of experiments were carried out as a function of gestational age.

Results: Electrically - induced liberation and noradrenaline content of the uterus of diabetic rats were significantly decreased compared to control values. The reactivity for sympathetic agonists were slightly affected.

Conclusion: DM deteriorates the function of adrenergic nerves while its effect on exogenous sympathomimetics is limited.

Key words: experimental diabetes, uterus, noradrenaline

P280075

Defective connexin 40 - associated gap junctions contribute to endothelial dysfunction in mesenteric arteries from insulin - resistant obese Zucker rats

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The study objective was to characterise the endothelial dysfunction in 3rd - order mesenteric arteries from 25 - week insulin - resistart obese Zucker rats (OZR). Endothelium - dependent relaxations to acetylcholine (ACh) were significantly smaller in pressurised mesenteric arteries (ID 100 - 150 um) from OZR compared to control lean Zucker rats (LZR). These relaxations were not altered by blockade of the NO pathway with nitroarginine methyl ester (100 uM) and ODQ (1 uM), but were abolished by blockade of endothelium - derived hyperpolarizing factor (EDHF) with Tram - 34 (1 uM) and apamin (1 uM). ACh responses in LZR and OZR were not altered by the CYP2C inhibitor sulfaphenazole (10 uM) or the gap junction inhibitor 43Gap26 (300 uM). In contrast, the connexin 40 inhibitor 40Gap27 (300 uM) significantly inhibited ACh responses in lean rats but not obese rats.

Connexin 40 protein and mRNA expression were markedly less in mesenteric homogenates from OZR than LZR. These results suggest that endothelial dysfunction in 3rd - order mesenteric vessels from OZR is attributable to reduced EDHF activity associated with a decrease in connexin 40 - associated gap junctions.

Key words: insulin resistance, EDHF, gap junction, connexin 40

P280076

siRNA - mediated gene silencing of potential Akt substrates reveals that GTPase activating protein TBC1D1 regulates glucose uptake in adipocytes.

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It is well established that Akt is required for insulin - stimulated glucose transport. To understand the mechanism by which Akt mediates the insulin's action, we applied proteomics approach to identify Akt substrates recognized by an antibody against the phospho - Akt substrate motif (RXRXXpS/T). In this study, several GTPase activating proteins (GAPs) including TSC2, TBC1D1, TBC1D4 and a new 220 kD RapGAP (RapGAP220) were identified as potential Akt substrates. Gene specific silencing of these GAPs with siRNA revealed that only TBC1D1 knockdown significantly increased glucose uptake by 3T3 - L1 adipocytes, both in the absence and presence of a low dose of insulin (1 nM). Interestingly, depletion of TBC1D1 also led to the increased expression of the GLUT1 glucose transporter in the adipocytes. Furthermore, point mutation of Akt phosphorylation motif in TBC1D1 (T590A) completely abolished insulin - stimulated phosphorylation of the Rab GAP, suggesting it is a novel Akt substrate. Taken together, our data suggest that TBC1D1 may be involved in controlling glucose transport in cultured adipocytes and therefore is a potential therapeutic target for type II diabetes.

P280077

Effect of a novel gonadotropin - releasing hormone (GnRH) antagonist LXT - 101 on growth of LNCaP human prostate carcinoma in vitro and in vivo

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LXT - 101 is a new GnRH antagonist which showed excellent character of chemical castration. In this study, the effect of LXT - 101 on growth of LNCaP human prostate carcinoma in vitro and in nude mice was investigated. Competitive binding assay showed that LXT - 101 could specific bind pituitary GnRH receptor with high affinity. Cell viability was markedly reduced by LXT - 101 as showed by MTT method, and LNCaP tumor growth was inhibited as shown by a significant decrease both in tumor volume and in tumor weight accompanied with serum testosterone reduced to castration level. Western blot assay showed a marked decrease in androgen receptor and a slight increase in GnRH receptor on LNCaP cell after LXT - 101 treatment in vitro, indicating a possible mechanism of direct inhibition of GnRH antagonist LXT - 101 on LNCaP cell growth. LXT - 101 can inhibit the proliferation of LNCaP prostate cancer in vitro and in vivo, and it might possibly be developed as an ideal candidate for treating prostate cancer.

Key words: GnRH antagonist, LXT - 101, prostate cancer, LNCaP.

Acknowledgement: This work was supported by the Chinese National Key Project of Technology (2002AA2Z3121).

P280078

EFFECT OF SILYMARIN IN THE PANCREATIC TRANSCRIPTION FACTORS RNAm EXPRESSION IN EXPERIMENTAL DIABETES MELLITUS AT EARLY STAGES OF THE TREATMENT.

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Diabetes Mellitus is a world health problem disease. There are not reports about any drug that recovers the β- pancreatic cell function. We have been reported that Silymarin produces a morphological and functional recovery of alloxan damaged rat pancreas. The aim of this work was to study the effect of Silymarin in the RNAm expression of insulin and in the pancreatic transcription factors Pdx1 and Nk6.1 (which play a key role in the insulin expression) at early stages of the treatment (3 to 21 days) with this drug in alloxan induced male diabetic rats. After 20 days of alloxan administration one group of diabetic rats were treated with Silymarin. We found in diabetic rats a gradual decrease in the RNAm expression of Pdx1, Nk6.1 and insulin within the time course of alloxan exposition. Silymarin treated diabetic rats (3 to 21 days) presented a decrease in the RNAm expression of Pdx1 and Nk6.1 whereas insulin RNAm expression was maintained during the period of the treatment. Silymarin decreased seric glucose levels of diabetic rats and increased seric insulin levels. These results suggest that Silymarin induces a regenerative effect in the pancreatic damage induced by alloxan in diabetic rats.

P280079

The effect of modulation magnetic field on Na - K ATPase in diaphragm of Streptozotocin - induced diabetic rat

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Biological effects of magnetic fields raise the question of whether imposed magnetic fields constitute a hazard in terms of physiological processes. Besides, the development of diagnostic and therapeutic applications of magnetic field draws attention to possible effects. In the other side, insulin - deficient diabetes impairs carbohydrate metabolism in a variety of tissues. Skeletal muscle may be susceptible to the diabetes - induced disturbance in glycolysis since Na^+ - K^+ ATPase in this tissue preferentially utilisez ATP generated by glycolysis. The aim of this study was to determine the effects of modulation magnetic field on the Na^+ - K^+ ATPase of diaphragm muscle preparations in both the healthy and diabetic rats.

Wistar type albino male rats were used. Rats were divided four groups. These are control (C, N=5), control + magnetic field (CMF, N=5), diabetes (D, N=5) and diabetes + magnetic field (DMF, N=5). Groups of diabetes were injected 45 mg/kg STZ solved in 0,1 M cold citrate buffer solution in teil vein. DMF and CMF was left in selenoid within a magnetic field of 50 Hz frequency and 5.0 mT strenght for 165 min. per day during one month. At the end of this time, the rats were decapited and dissected diaphragm muscle preparation. Measurement of Na^+ - K^+ ATPase activity: Assays were carried out in a final volume of 2.5 ml containing 0.3 mg tissue protein as the enzyme source. Enzyme activity expressed as n mol Pi. mg prot - . h - . Na^+ - K^+ ATPase enzyme group of diabetes at the rate of 43,3 % was decreased in diabetic group compared control groups. Na^+ - K^+ ATPase enzyme DMF group at the rate of 34,5 % was increased compared D group.

In conclusion, these results indicate that magnetic fields exposed on the diabetic rats prevented any further increase in hyperglycemia

Key words: Magnetic field, Na - K ATPase, diabetes, rats, skeletal muscle

P280080

THE mRNA EXPRESSION OF RENAL AQUAPORIN-2 IN RATS WITH DIABETES MELLITUS AND THE ROLE OF ASTRAGALUS MEMBRANACEUS

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Objective To investigate the changes of renal medullary aquaporin-2(AQP-2) in rats with diabetes mellitus induced by streptozotocin(STZ) and the role of astragalus membranaceus(AM).

Methods Forty male Spragne-Dawley rats were randomized into four groups matched for body weight.(1) Diabetes model group(2) Low dosage group: astragalus injection 5g ×kg-1 ×d-1(3) High dosage group: astragalus injection 10g ×kg-1 ×d-1, ip, use medicine for 21 days in succession(4) Control group. On the day 21, obtain the kidney medulla as soon as possible. The real time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) technique was used to determine the levels of AQP-2 mRNA expression on SDS-5700 machine.

Results The mRNA expression of AQP-2 was up regulated in the kidney of diabetic rats. High dosage AM treatment could alleviate the over expression of AQP-2($P<0.05$), but could not in low dosage($P>0.05$).

Conclusion AM exerts its therapeutical effects on diabetes mellitus may related to the significantly decreased expression of AQP-2 in the kidney medullary.

Key Words Aquaporin-2; Diabetes Mellitus; Astragalus Membranaceus

P280081

Investigation on MyD88 mRNA Expression of Pancreatic β Cells and Regulative Mechanism of Astragalus Membranaceus in Diabetic Rats

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Aim: to study expression of Myeloid differentiation factor (MyD88) of Pancreatic βcells in Streptozocin(STZ)-induced diabetic rats, And mechanism of Astragalus membranaceus(AM) regulation.

Methods: 40 male SD rats are divided into four groups:(1) type 1 dibetes mellitus(T1DM) model group;(2) low dosage group, AM injection 5g.kg-1.d-1;(3) high-dosage group, 10g.kg-1.d-1;(4) control group. ip, 21 days. The expression of MyD88 was determined by real-time RT-PCR, β-actin was used as endogenous control, Measure MyD88 expression level through ratio of expanding output quantity between MyD88 and β-actin. Use Dissociation curve and agarose gel to determine the peculiar quality.

Results: compared with control group, MyD88 expression of T1DM model group was increased distinctively($P<0.05$); compared with T1DM model group, that of AM high-dosage group is significantly decreased($P<0.05$).

Conclusion: MyD88 expression of pancreatic βcells in diabetic rats are obviously increased; AM injection can significantly decreases the expression for a long-term using.

Key words: Astragalus membranaceus MyD88 RT-PCR

P280082

SULODEXIDE STIMULATES NITRIC OXIDE SYNTHASE ACTIVITY IN THE KIDNEY OF TYPE 1 DIABETIC RATS

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Decrease levels of glycosaminoglycans (GAGs) have been observed in kidney and other organs, in human and animals models of diabetes. Long term administration of a glycosaminoglycan sulodexide (SUL) have been demonstrated a beneficial effect on morphological and functional renal abnormalities in diabetic rats. We assessed the effect of SUL (100 µg/ml) on nitric oxide synthase (NOS) activity in the rat kidney. Diabetes was induced in male Sprague-Dawley rats by i.v. administration of streptozotocin (STZ). Animals were randomly allocated in three groups (C = control, STZ and STZ + SUL = pretreated with SUL 15 mg/kg, s.c.), and after three months follow up were sacrificed and kidney microdissected. Basal and SUL-stimulated NOS activity was assayed by monitoring the conversion of radiolabelled L-arginine to L-citruline. Basal NOS activity was lower in STZ-diabetic rats than in control group, and this activity was restored by in vivo SUL treatment. In vitro, SUL increased NOS activity in control (40%), STZ (46%) and STZ + SUL groups (35%). Our results demonstrated a role for GAGs in regulation of kidney NOS activity in diabetic rats.

Key words: Glycosaminoglycans, nitric oxide, diabetes
(Supported by CDCH P09-11-5102-2003).

P280083

Experimental Study of the Effects of Puerarin on Diabetic Vascular Complications and Its Mechanisms

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Aims: To study the effects and mechanisms of puerarin on Diabetic vascular complications(DVC). Methods: 1) To establish the chronic diabetic rats models induced by streptozotocin(STZ), The FBS, FINs, NO, sICAM-1, ox-LDL, TNF-αand RAGE mRNA levels were measured at the eighth and twelfth week. To observe the pathological changes of endothelium of thoracic aorta and kidney. 2) To make diabetic nephropathy(DN) models with 1/2 nephrectomized rats were operated and induced by STZ. After administration of puerarin for six weeks. MAU, Ang II concentration and PKC activity of renal cortex, TGFβ1, LN and Co IV expressions in glomeruli were measured. 3) To investigate the influences of AGEs and puerarin on CAM's angiogenesis. 4) To analyze the proliferation of HUVEC in high glucose. 5) GMCs were cultured in high glucose or plus puerarin, protein expressions of c-fos, c-jun, Co IV, TGF-β and PKC activity were measured. Results: Puerarin could be beneficial to controlling the development of DVC and DN; preventing cultured HUVEC against lesion and inhibition of proliferation and efficiently meliorate abnormalities in cultured GMC caused by high glucose. Conclusions: Puerarin may be beneficial to preventing and curing DVC.

P280084

Crocetin Reduces Expression of Receptor for Advanced Glycation End Products (RAGE) in Endothelial Cells Induced by AGEs

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Objective To investigate effect of crocetin on receptor for advanced glycation end products(RAGE) expression in bovine endothelial cells(BEC) induced by advanced glycation end products(AGEs) and the possible mechanism involved.

Methods: BEC were preincubated with crocetin(1uM,0.1uM) 12h, then exposed to AGEs(100ug/ml). RAGE protein and mRNA expression were investigated by Western blotting and RT-PCR analysis, respectively. Extracellular superoxide ion and TBARS were assessed. Intracellular H_2O_2 was also detected using the probe 2,7-dichlorofluorescein (DCFH), Mitochondrial membrane potential (MMP) and mitochodrial Succinate dehydrogenase(MSD) were analyzed by the retention of rhodamine123 (Rh123) and MTT. Results: Compared with AGEs group, crocetin was able to significantly reduce RAGE protein and mRNA expression ($P<0.05$), decrease super anion, TBARS in super media($P<0.01$ or $P<0.05$) and H_2O_2 in cells ($P<0.05$). Simultaneously, Mitochondrial membrane potential(MMP) and mitochondrial Succinate dehydrogenase(MSD) improved.

Conclusion: These results demonstrated that crocetin could inhibit RAGE over-expression in AGEs-exposed BEC by suppressing ROS generation.

Key words: Crocetin; AGEs; RAGE:ROS

P280085

EFFECT OF SILYMARIN IN THE PROLIFERATION OF PANCREATIC β-CELLS IN EXPERIMENTAL DIABETES MELLITUS AT EARLY STAGES OF THE TREATMENT

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Diabetes Mellitus is a world health problem disease. There are not reports about any drug that recovers the β-pancreatic cell function. We have been reported that Silymarin produces a morphological and functional recovery of alloxan damaged rat pancreas. The aim of this study was to analyze the effect of Silymarin in the proliferation of pancreatic β-cells at early stages (3 to 21 days) of the treatment with this drug in alloxan induced male diabetic rats. Bromodeoxyuridine (BrdU) was administered to rats for label proliferating pancreatic βcells at the end of Silymarin treatment of diabetic rats. Immunohistochemical analyses was assessed in pancreatic tissue for insulin and BrdU immunoreactivity cells. We not found any immunoreactivity label in the pancreatic tissue of diabetic rats. Silymarin treated

diabetic rats showed immunoreactivity for insulin and BrdU at 14 and 21 days of treatment. Also Silymarin decreased seric glucose levels of diabetic rats and produced an increase in seric insulin levels. These results suggest that Silymarin induces proliferation of pancreatic β cells in alloxan induced diabetes mellitus of the rats.

Study partially supported by the grant 44614-Q CONACYT (México).

P280086

Polysaccharide from Ganoderma lucidum reduces expression of VCAM-1 and ICAM-1 in endothelial cells stimulated by advanced glycation end products

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AIM: TO examine the in vitro effect of polysaccharide from Ganoderma lucidum (GL-PS) on the expression of vascular cell adhesion molecular-1 (VCAM-1) and intercellular adhesion molecular-1 (ICAM-1) in human umbilical vein endothelial cells (HUVEC) stimulated by advanced glycation end products (AGEs) and explore its mechanism. METHODS: AGEs were prepared by incubating bovine serum albumin with glucose and.

HUVEC were isolated from umbilical cords and endothelial cell surface expression of VCAM-1 and ICAM-1 were determined by cellular enzyme-linked immunosorbent assay and flow cytometry. Intercellular reactive oxygen species (ROS) formation was measured using fluorescent probe and activation of nuclear factor-Kappa B (NF-κB) was detected by confocal microscope. RESULTS: AGEs upregulated the expression of VCAM-1 and ICAM-1 in HUVEC dose and time-dependently, GL-PS (0.1, 1 μg 2 ml-1) significantly reduced AGEs-induced VCAM-1 and ICAM-1 expression in HUVEC, further study showed GL-PS could inhibit the AGEs-induced ROS generation and NF-κB activation in HUVEC. CONCLUSION: It suggested that GL-PS would be a potential therapeutic agent for diabetic vascular complications.

Key words: Ganoderma lucidum, AGEs, diabetes

P280087

4-Hydroxyisoleucine improves glucose metabolism and insulin signal transduction in hepG-2 cells

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To study the therapeutic effect and molecular mechanism of 4-Hydroxyisoleucine on insulin resistance model of hepG-2 cells induced by high concentrations of glucose and insulin. HepG-2 cells were treated with high-glucose and high-insulin for 48h, then added with 4-Hydroxyisoleucine for 24h. Finally glucose uptake in different groups were determined. Meanwhile, the expressions of insulin receptor, insuline receptor substance-1, insuline receptor substance-2 and glucose transporter-2 were observed by RT-PCR. High concentrations of glucose and insulin decreased the uptake of glucose, increased the expression of IR, IRS-2 and GluT-2, but had on effect on the expression of IRS-1. Treatment of insulin-resistant HepG2 cells with 4-Hydroxyisoleucine improved glucose uptake and attenuated the expression of IR, IRS-2 and Glut-2, which did not reach control levels. These results suggested high concentrations of glucose and insulin induced insulin resistance in HepG2 cells, whereas 4-Hydroxyisoleucine improved glucose uptake and changed insulin signal transduction of cells by decreased the expression of IR, IRS-2 and Glut-2.

Key word: 4-Hydroxyisoleucine, Insulin resistance, IRS-2, Glut-2

P280088

The nutrition effect of glyde-glutamine dipeptide by enteral feeding on the recipient small intestinal metabolism and ultrastructure following allogenetic liver transplantation in rat

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12 Lewis rats were as the donors, 24 BN rats were as the recipients and divided randomly into the control group (ALA group) and the experimental group (GLN group). In each group, 6 BN rats were collected the samples as the normal control on the 3rd preoperation day (PRD3); the residual 6 rats in the ALA group received alanine 0.6 g/kg.d for 3 days before operation and 7 days after operation by perfusing stomach; the 6 rats in the GLN group were given glycle-glutamine 0.6g/kg.d in the same way. After 3 days fasting (free to water), they were hypodermic injected by CsA (2 mg/kg.d) for 7 days and collected samples on the 8th postoperative day (POD8) following liver transplantation under aseptic condition. The content of mucosal glutamine, protein and glutathione, and mucosal ultrastructure were detected for these 24 BN rats. The results of two groups on the POD8 become worse significantly compared with the results of the two groups on the PRD3; however, these results of the GLN group were remarkably better than those of the ALA group on the POD8. It meant that Gly-Gln improved small intestinal metabolism and ultrastructure following liver transplantation in rat.

Key words: glutamine, metabolism

P280089

CPU86017-SR, an isomer of CPU86017 (p-chlorobenzyltetrahydroberberine chloride) regresses hepatic steatosis in high-fat diet feeding rats.

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To investigate the effect of CPU86017-SR on hepatic steatosis induced by high fat-diet relevant to ET system in rats. Male SD rats (220±20g) were randomly divided into 3 groups: control, high-fat feeding induced hepatic steatosis model and treated with the SR. Hepatic slides with HE stain were performed to evaluate fatty infiltration.

The liver and body fat fatty weight index, serum AST, ALT and hepatic lipase (HL) in hepatic homogenates were measured. The mRNA levels of prepro-endothelin-1 (ppET-1) and endothelin converse enzyme (ECE) were also detected by RT-PCR. We found hepatic fatty infiltration was significant in the untreated and totally regressed by CPU86017-SR, with decreased liver/fat index and AST, ALT level and increased HL activity. The up-regulated mRNA expressions of ET-1 and ECE were markedly in untreated and brought down by CPU86017-SR. It is the first data to describe that the hepatic steatosis of metabolic syndrome is mediated by an activated ET system and regressed completely by CPU86017-SR.

Key Words: hepatic steatosis; CPU86017-SR; metabolic sysdrome; the ET system;

Supported by a key project No: 30230170 from NSFC & Jiangsu provincial project No BK2002120.

P280090

SS-31 Prevents Streptozotocin-Induced Pancreatic Islet Apoptosis in Mice

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Background: Streptozotocin (STZ) has been used to trigger apoptosis. SS-31 belongs to a series of cell permeable and mitochondria-targeted antioxidants. We have recently shown that SS-31 decreased intracellular ROS, increased mitochondria potential and prevented tBHP induced apoptosis. In this study, we present evidence that SS-31 prevents pancreatic islet destruction by STZ-induced apoptosis. Methods: Three groups of mice were studied after 3 weeks: (1) no STZ treatment; (2) STZ (40 mg/kg i.p.qd) for 5 days, and (3) same STZ treatment for 5 days with SS-31 (3 mg/kg i.p.qd) for 16 days. Pancreas was examined for apoptosis, using TUNEL assay and immunohistochemical staining for insulin-containing cells. Results: STZ caused a significant destruction of pancreatic islets, with significant lymphocytic infiltration. Immunohistochemical staining showed decreased insulin content compared to control samples. Co-treatment with SS-31 for 2 weeks inhibited apoptosis, reduced lymphocytic infiltration and preserved insulin content in islets.

Conclusion: STZ induced apoptosis and reduced insulin content in mouse pancreatic islets, an effect prevented by SS-31.

P280091

The interaction of 18α-diammonii glycyrrhizinatis and glibenclamide in alloxan-induced diabetic rats

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The aim of present study is to investigate the influence of 18α-diammonii glycyrrhizinatis (DG) on pharmacokinetics and pharmacodynamics of glibenclamide (Gli) in alloxan-induced diabetic rats. After treated with DG (25 mg·kg^{-1}·d^{-1}, i.p. ×5d) and Gli (1 mg·kg^{-1}·d^{-1}, i.g. ×5d), k_e of Gli was decreased while C_{max}, AUC_{0-14h} and $T_{1/2ke}$ were significantly increased by 18%, 59% and

63%; simultaneously, fasting plasma glucose was declined, plasma insulin and liver glycogen were increased vs Gli - treated group. The activities of CYP3A participating the metabolism of Gli were significantly decreased in rats treated with DG and DG+ Gli. Immunohistochemistry showed that the beneficial effect of Gli on the pathological morphology of pancreatic islets and βcell could be further improved by DG. Our results revealed DG lead to the enhancement of the hypoglycemic effect of Gli (by inhibiting the activity of CYP3A) which should be paid attention to in clinic; on the other hand DG protected islet βcell and liver damaged in diabetic which suggested that DG had the possibility to be used as an adjuvant drug of oral hypoglycemic agents in proper dose, especially to the diabetic patients accompanied with liver impairment.

Key words: DG; Gli; pharmacokinetics; CYP3A

P280092

Insulin secreting activity of a fraction from Argyrolobium roseum

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The present study was carried out to evaluate the antidiabetic activity of plants. Ethanolic extract of Argyrolobium roseum (Cambers Jaub & Spauch), a virgin herb found in the tropical and sub - temperate tracts of north - western India, exhibited antihyperglycemic effect in Glucose tolerance test (GTT) and Streptozotocin (STZ) treated Wistar rats models. The extract was further fractionated into pet ether, chloroform and butandic fractions. Butanolic fraction evoked a dose response stimulation of insulin secretion in the in vitro (RIN- 5F cells) and in vivo models when compared with glibenclamide. The significant antihyperglycemic effect in vivo and insulin secretion activity in vitro demonstrate the presence of natural antidiabetic and insulin secreting product(s) in Argyrolobium roseum. Detailed investigation for the isolation of pure molecules from butanolic fraction and the mechanism of action for each and any of the fraction is being carried out separately.

Key Words: Antidiabetic, Insulin secreting, Argyrolobium roseum; butanolic fraction

Acknowledgement: Authors are thankful to Sh. Dharm Raj, Ex- STA, RRL, Jammu for his technical assistance.

P280093

1α,25- D3 and TLR2 agonist Improve Insulin Sensitivity in MSG Obese Rat by regulation of regulatory T cells and Th1/Th2 immune responses

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The underlying cause of metabolic syndrome is a chronic inflammatory response characterized by enhancement of Th1 immune response that may be responsible for pathogenesis of insulin resistance. We wonder if shift of Th1/ Th2 balance toward to regulatory T (Treg) cells or Th2 responses improves insulin sensitivity in MSG obese rats. The insulin sensitivity was determined by body weight, the insulin sensitivity index, oral glucose tolerance test, insulin tolerance test, and hyperinsulinemic - euglycemic clamp. The expression and activity of TLRs and their signal pathways were determined by PCR or western blot. We found that 1α, 25- D3 (1α,25- D3), an immunomodulator, significantly improved the insulin sensitivity via increasing the Treg cell number leading to polarization of T cell development toward Treg direction. Interestingly, a TLR2 agonist peptidoglycan (PGN), but not TLR4 agonist, markedly improved the insulin sensitivity because PGN stimulated TLR2 leading to a Th2 immune response. In summary, 1α,25- D3 and PGN improve insulin sensitivity via elevation of Treg cells or shift of Th1/ Th2 balance toward Th2 immune response.

Key Words: metabolic syndrome, MSG obese rat, TLR2, Th1/ Th2/ Treg

P280094

EFFECT OF BOTULINUM TOXIN TYPE A ON HYPERALGESIA ALLOXAN AND STREPTOZOTOCIN INDUCED DIABETIC NEUROPATHY

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Recently we found that botulinum toxin type A (BTX- A) reduced pain hypersensitivity in rats with surgical neuropathy (Bach - Rojecky at al. J. Neural Transm 2005;112:215). Here we report that BTX- A has antinociceptive activity in diabetic neuropathy, too. Adult male Wistar rats were made diabetic by subcutaneous injection of alloxan or streptozotocin. After 5 days only animals with a tail - vein blood - glucose concentration of above 15 mmol/l were considered diabetic and included in the study. Paw- pressure and hot plate tests were first performed 3 weeks following betacitotoxic injection. Only the animals with significantly different mechanical thresholds compared to control group were considered neuropathic (hyperalgesic) and were than subjected to BTX - A treatment. On day 5 after BTX - A 5 and 7 U/kg treatment significant antinociceptive effect was observed i.e. diminished number of flinches and shakes of the formalin - injected paw. The lowest used dose (3 U/kg) was ineffective. With the paw pressure test results were practically the same. To our knowledge this is the first demonstration that a single peripheral injection of BTX might have antinociceptive effect in diabetic neuropathy.

Key words: botulinum toxin type A, neuropathic pain, antinociception, diabetes mellitus

Acknowledgement: Supported by Croatian Ministry of Education Science and Sport, and Deutscher Akademischer Austausch Dienst (DAAD)

P280095

Extraction of Herba Portulacae and Their Antidiabetic Activity in vitro

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Objective To prepare the agents derived from Herba portalacae (HP) and investigate their effect on antidiabetic activity in vitro. Methods Three HP fractions were extracted using water, petroleum ather and n - butanol. The in vitro reducing sugar function and its mechanism of HP extracts were observed by GOD- POD and GPO- PAP assay on 3T3 - L1 adipocyte induced by insulin, respectively. Results In vitro insulin - sensitizing activity (3T3 - L1 adipocyte) demonstrated that cultured glucose concentration of supernatant were decreased, whereas intracellular triglyceride concentration were increased significantly in water and n - butanol extract groups compared with that of control group. Conclusion The results suggested that HP extracts have good effect on the uptake and utilization of glucose.

Key words: Herba Portulacae, extract, antidiabetic activity, glucose, triglyceride

P29. Integration of Modern and Traditional Medicines

P290001

The traditional healer as part of the primary health care team in South Africa

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There is a global trend in health care towards the patient - centred approach and respect for patient autonomy, including free choice in health care options. One of these options in South Africa is the traditional healer. The present study was undertaken to investigate to what extent it is feasible to include the African traditional healer in the primary health care team. It was found that traditional healers are still firmly established health care providers in their respective communities. However, patients also value the efficacy of modern scientific medicine, and many are 'dual' health care consumers. Traditional practitioners are usually interested in cooperation with the Western health care worker, while the modern doctor tends to regard traditional practices as unscientific, largely unregulated, often harmful and sometimes fatal. Thus, while Government has committed itself to make use of this vast manpower potential and involve healers in the official health care system, it is concluded that true cooperation will only be possible through statutory rgulation of the traditional sector to ensure health care being delivered in a safe and competent manner.

P290002

Protective effect of BR - 16A against immobilization - induced oxidative stress: Possible GABAergic mechanism

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Physiological stressors are known to induce complex biochemical changes (MDA, SOD, GSH, catalase) and several behavioral responses (antinociception, locomotion). Objective of the present study was to explore the protective effect of BR

16A, polyherbal preparation against immobilization-induced oxidative stress and possible involvement of GABAergic mechanism. Laca mice were immobilized for two hours by taping all the four limbs to board after putting on their backs using zinc oxide hospital tape. Immediately after oxidative stress, behavioural observations were performed followed by biochemical estimations. BR16A (100, 150, 200 mg/kg, po) dose dependently protected the lipid peroxidation (percentage increase in MDA level) and improved the reduced glutathione level that was significant as compared to control, respectively. Further on combination studies of BR 16A with diazepam (0.5 mg/kg) caused further protection of lipid peroxidation. However reduced gluthathione was not influenced significantly. Protected effect of the BR16A was further potentiated by muscimol, a GABA agonist and blocked by bicuculline, a GABA antagonist. This suggests that GABAergic mechanism is involved in protective effect of BR16A.

P290003

The endothelial cell cytotoxicity, chromatographic profiles and chemical constituents of Siriraj Ayurved Herbal Recipe - Chantaleela.

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To study the endothelial cell cytotoxicity, chromatographic and chemical characteristic features of Siriraj Ayurved Herbal Recipe - Chantaleela using chromatographic profiles. Chantaleela was obtained 8 herbal types. For cytotoxicity assay, HUVEC were incubated with Chantaleela and each 8 herbal components for 24 h after which the cells were collected to measured cell proliferation using MTT and CV assay. For chromatographic characteristic study, Chantaleela and each 8 herbal components were examined by using HPLC and LC-MS. For chemical constituents, TLC was used to detect phend and steroid constituents. Chantaleela and each 8 herbal components did not affect on cell viability of HUVEC for 24 h incubation. HPLC and LCMS characteristic peaks in the UV spectrum were identified. The phenol constituent was found but there was not steroid constituent in recipe. The distinct characteristic features revealed in this study can serve as evidence for the identification of Siriraj Ayurved Herbal Recipe - Chantaleela. Moreover, Chantaleela did not affect on cell viability of HUVEC for concentration up to 1 mg/ml.

P290004

Comparison the effects of aqueous extract of Carum Carvi, Dexamethasone and stress on acute and chronic pains in mice

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Our previous investigation showed that Carum Carvi (CC) modulates pain in mice. The aim of this work was to examine the role of CC on acute and chronic pain and compares its effect with Dexamethasone (Dex) and stress (ST) using formalin test in mice.

In this study male albino mice (25-30 gr.) in 8 groups (n=56) were used. CC (100, 500 and 1000 mg/kg), Dex (0.5, 1 and 2 mg/kg) and vehicle were injected 30 min before test. Stress was applied by 1 min swimming in cold water (18-22°). Acute (5 min) and chronic pains (5-40 min) were assessed after injection of formalin 5% (25 µl) in right paw by using of standard scores.

Results indicated that CC, Dex and ST have analgesic effects in both on acute and chronic pains ($P < 0.01$) in comparison with control group. Further, the analgesic effect of higher dose of CC was significantly higher than Dex and ST.

Finding above showed that CC extract, Dex and ST have modulator effects on both acute and chronic pain in formalin test. Further research is required to determine the mechanisms by which CC extract has an inhibitory effect on pain sensation.

P290005

Effects of *Curcuma aeruginosa* **Roxb. methandic extract on rat uterine contraction.**

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Methanolic extract of C. aeruginosa Roxb. (Zingiberaceae) dried rhizomes was studied on isolated rat uterine contraction. Female Wistar rats (200-250 g) were primed with diethylstilbestrol (0.1 mg/kg, i.p.) 24 hours before the experiments. Animals were then sacrificed by cervical dislocation and the uterine horns were isolated. Uterine strips were dissected and suspended in Locke-Ringer filled organ bath. Contraction of the strip was recorded isometrically with a FT03 force transducer connected to a Grass Polygraph. Effects of the plant extract were investigated on agonists: oxytocin (OXY, 1 mU/ml), acetylcholine (ACh, 30 µM) and KCl (40 mM)-induced contractions in comparison with a Ca^{2+} channel blocker, verapamil. The extract (10-400 µg/ml) caused concentration-dependent and completely inhibition against OXY, ACh and KCl with the IC_{50} of 89.5, 198.1, 73.5 µg/ml (amplitude); and 68.6, 184.5 µg/ml (frequency) against OXY and ACh, respectively. IC_{50} of verapamil against OXY and KCl were 23.6 and 43.4 ng/ml (amplitude), respectively and 58 ng/ml (frequency) against OXY. Thus, it is suggested that the relaxant effect of the extract might due to the interference of influx of extracellular Ca^{2+}.

P290006

Study on anticancer activity of sulforaphane from broccoli

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To study the anticaricinogenic activity and mechanism of sulforaphane[1-isothiocyanato-4-(methysulfinyl)-butane] obtained in broccoli. in detail, literature at home and abroad are refered to. The result shows that sulforaphane is considered as the most powerful anticancer component from vegetables so far. The mechanism studies show that sulforaphane can induce phase II enzymes which can protect cells against the toxic and carcinogenic effects of electrophiles and oxidants, disturb the combination of carcinogen to DNA and decrease the formation of adducts such as N7-methyl guanine and O6-methyl guanine. Sulforaphane can also selectively inhibit the cytochrome P450 enzymes involved in carcinogen metabolic activation. Sulforaphane is beneficial in protecting against human carcinogenesis.

KEY WORDS: broccoli, sulforaphane, anticarcinogenic actvitiy

ACKNOLEDGEMENT: Thanks for the Committee of National Natural Science Foundation of China, by which the project is supported. (NO.30300284, NO. 30400591)

P290008

Effects of AMPS-II on IEC-6 cells function related to ODC activation and polyamine synthesis

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Aim: To determine whether AMPS-II, a complex polysaccharide from Atractylodes macrocephala, had effects on IEC-6 cells function and ornithine decarboxylase (ODC) activation and putrescine synthesis. Methods: Cells morphology were observed under microscope and electron microscope, cells migration was evaluated by counting the number of migrating cells after scratch damage, cells proliferation was measured with MTT assay, villin and ODC mRNA were analyzed by RT-PCR, villin protein was examined by immunocyotchemical analysis and ODC protein was assayed by ELISA, ODC activity was determined using a radiometric technique, the putrescine content was analyzed by HPLC. Results: After treatment with AMPS-II, the differentiation phenotype and the migration ability of cells were promoted. Meanwhile, villin mRNA levels, villin expression, ODC mRNA levels, ODC expression, ODC activity and putrescine content were increased. Nevertheless, the cells proliferation was unchanged after AMPS-II treatment. Conclusions: AMPS–II plays a role of induction in IEC-6 cells differentiation and migration, which is related to ODC and polyamines regulation mechanism.

Key words: AMPS-II; IEC-6 cells; cell function; ODC; putrescine

P290009

Herb-herb interactions in traditional Chinese medicine based on cytochrome P450

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To study the Herb-herb interactions in tradtional Chinese medicine based on cytochrome P450, the effect of aconitum coadministration with trichosanthes on the enzyme activity, protein expression and mRNA level of cytochrome P450 isoen-

zymes were firstly investigated. Aconitum coadministration with trichosanthes obviously inhibited the activities and protein expression of CYP1A2, CYP2E1 and CYP3A1/2, the mRNA level of CYP1A2 were also markedly inhibited treated with aconitum and trichosanthes. Aconitine, a highly toxic diterpenoid alkaloid in aconitum, next we studied the metabolism of aconitine and the effects of selective cytochrome P450 (CYP450) inhibitors on the metabolism of aconitine in rat liver microsome. Six metabolites of aconitine were characterized through CYP metabolism, which mediated primarily by CYP 3A1/2, with a probable secondary contribution of CYP 1A2. CYP2B1/2, 2E1 and 2D1 are likely to be not involved in aconitine metabolism. Herb-herb interaction of Aconitum coadministration with Trichosanthes may occur through the inhibitory effect of CYP3A and CYP1A2, which likely decrease the metabolism of co-administrated herbal extracts containing aconitine and cause toxic effects.

P290010

Anti-inflammatory Mechanism of Total Glucosides of Acanthopanax Giraldii

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We investigated the effect of total glycosides of Acanthopanax Giraldii (TGA) on T lymphocyte proliferation by mice splenic and thymic lymphocytes proliferation, the expression level of cyclooxygenase-2 mRNAs expressions and the production of prostangland in E2 (PGE2), nitric oxide (NO) and tumor necrosis factor-α (TNF-α) by mouse macrophages. The expression level of cyclooxygenase-2 (COX-2) mRNAs expressions in RAW264.7 macrophages and the production of PGE2, NO were decreased by TGA. These results suggest that TGA exhibit anti-inflammation effect through inhibition of NO, and COX-2 induced PGE2

Key words: Acanthopanax Giraldii; Total glycosides of Acanthopanax; Anti-inflammation;

P290011

Antiseizure effects of traditional Chinese medicine and its molecular mechanisms

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Objective: To investigate effect of antiseizure and molecular mechanism of Chinese medicine (TCM). Methods: A comprehensive study was done. Clinic effect was observed in 22 children with epilepsy for 12 months. Rat epilepsy models were randomly divided into 6 groups (n=10), NS10, TCM10, 20g/kg, phenobarbital 50, clonazepam 1mg/kg, i.p. q.d. respectively. Antiepileptic effect by 5 degrees was measured 7-14 days. Mechanism was studied by western blot, immunohistochemistry, radio-ligand receptor binding assay, and quantitative hemolysis 3H-TdR incorporation. Statistics was analyzed by SSPS. Results: Seizures-controlled rate was 64.6% in epileptic children. Seizure-free rate was 75% in seizure models. All was without obvious adverse effects. Capacity of GABAA receptor and expression of α1 subunit were increased to control, no change in NMDA system. The biological activity of macrophage, interleukin-2, proliferation of T lymphocyte, RBC-C3bRR and RBC-ICR were also enhanced. Conclusions: TCM is promising. Mechanisms might relate to increase GABAA/NMDA function and improve immune state. That is accord with "fu zheng gu ben" theory of TCM.

Key words: traditional Chinese medicine; epilepsy; GABA; immune

P290012

Experimental Research of Anti-cancer Effects and Related Mechanism of Traditional Chinese Medicine of Qian Kun Dan

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OBJECTIVE AND METHODS: to evaluate the anti-cancer effects of Traditional Chinese medicine, Qian Kun Dan (QKD), and to explore its related mechanism, by means of cDNA microarray, cell culture, flow cytometry, immunohistochemistry, drug-containing sera tests RESULTS: I 1. In vitro, it has repressive effects for H22, B16 and Lewis lung carcinoma, and inhibits the diversion of Lewis lung carcinoma. In vivo, it has repressive effects for SMMC-7721, MCF-7. 2. It enhances weight of mice with tumor, their life quality and cell immunological abilities, without toxicitical effects. 3. it can reverse the decline of life quality caused by 5-FU. II 1. It elevates the proportions of G0 and G1 cells, lowers the quantity of G2/M cells. 2. It down-regulates several oncogenes of SMMC-7721, the expression of signal transfer molecules, genes related to tumor growth, proliferation and IL-1; it up-regulates MAP2K6(Hs.118825), tumor suppressor gene such as NF-2, TNFSF9, TNFSF7. 3. It can promote the proliferation of lymphocyte, the level of anti-cancer cell factors such as IFN-g, TNF-a. CONCLUSIONS: QKD has anti-cancer effects. Its mechanism has very close relationship with regulation of cell cycle, target gene and immunity.

P290013

Effects of drug sera from rats per os Da Cheng Qi granules on intestinal intraepithelial lymphocytes of mice

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The aim was to study effects of drug sera from rats per os Da Cheng Qi granules (DCQ) on intestinal intraepithelial lymphocytes(IELs) of Balb/c mice. Methods: Single dose of DCQ (SDS) administered to rats, five components of Rhubarb were absorbed; drug sera from rats per os 6 doses of DCQ(MDS) according to time span of Chrysophanol t1/2(ke) 3.20±0.54h. SDS and MDS were respectively added to IELs with 82.76±2.61(%) of CD3+, 9.91±2.52(%) of CD4+, 72.48±3.57(%) of CD8+. Results: Drug sera of different time points after SDS with DCQ of different concentrations promoted IELs proliferation, IL-2 and IL-6 production/secretion(IL-2 level is 100 times higher than that of IL-6). MDS caused moderate IELs proliferation, increased intracellular calcium($[Ca^{2+}]_i$); MDS without dilution had better effects on IELs proliferation and IL-2 and IL-6 than that with dilution. Conclusion: DCQ can enhance immunologic effects of IELs by promotion of IELs proliferation, increase in $[Ca^{2+}]_i$ and IL-2.

Key words: drug sera; intestinal intraepithelial lymphocytes; Da Cheng Qi granules. Sponsored by Sci & Tech develop fund of Tianjin edu.com.

P290014

Experimental study on Saikosaponin-d against liver fibrosis in rats

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AIM: To investigate the protective effect of saikosaponin-d (SSd) on liver fibrosis induced by dimethylnitrosamine (DMN) in rats. METHODS: Eighteen SD rats were randomly divided into control group, model group and SSd-treated group. After 4 weeks, the liver function was determined and serum type IV collagen (IV-C) level was measured. Pathology changes with HE stain and Sirius Red stain were observed by light microscopy and expression of α-SMA and TGF-b1 in the liver tissue were measured by immunohistochemistry method. RESULTS: Compared with the model group, the serum ALT and fibrosis marker IV-C were declined significantly in SSd-treated group. Fibrosis degree of the liver was ameliorated and the areas of collagen fiber decreased obviously when treated with SSd. Additionally, immunohistochemistry results showed that SSd significantly inhibited α-SMA and TGF-b1 expressions in rat liver tissue. CONCLUSION: SSd exhibited antifibrogenic effects against DMN-induced liver injury, which may be due to it regulates the collagen, suppressing the activation of hepatic stellate cells.

Key words: Hepatic Fibrosis; Saikosaponin-d; Dimethylnitrosamine; Rat

P290015

Anti-Fatigue and Endurance-Enhancing Properties of CordyMax, A fermentation Product of Cordyceps sinensis.

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Natural Cordyceps sinensis and its standardized mycelial fermentation product, CordyMax (CM), are traditionally known as medicinal herbal products for invigoration, health preservation, anti-aging, and antifatigue by use of symptom-analysis. Animal studies showed that CM improved steady state bio-energy of mouse liver using ^{31}P NMR spectroscopy and promoted efficient use of limited O_2 supply to support body's physiological activities and greater tolerance to hypoxic acidosis. We examined the anti-fatigue and endurance-enhancing properties of CM using an incremental work rate protocol on a cycle ergometer and treadmill in a double-blind setting, assessing aerobic capacity and physical capability of healthy sedentary adults of older ages.

Our data showed that 6 weeks of CM increased anaerobic threshold, VO_2 max, O_2 pulse and maximal ventilation during exercise. It reduced HR, RER and lactic acid during endurance exercise, reduced fasting blood glucose and accelerated recovery from maximal exercise. In summary, Cordy Max therapy influences favorably aerobic capacity and C, pulmonary, and metabolic functions during endurance exercise, improving fatigue and endurance performance.

P290016

Analysis of Anti-platelet Aggregation Components of Ginger Oleoresins through Chicken Thrombocyte Membrane Immobilized Chromatography Model

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Objective: To analyze the effective anti-platelet aggregation components of Ginger Oleoresins (Ethanol extracts of dried Zingiber officinale Rosc.,) Methods: The Ginger Oleoresins were combined with the receptors, channels of thrombocyte membrane under analogical physical environments. Unattached substances were washed away. Attached compounds were eluted and analyze by HPLC and LC-MS. The activity was proved by pharmacological model. Results: There were five characteristic compounds: 6-gingerol, 8-gingerol, 6-shogaol, 10-shogaol and 12-dihydrogeginerol binding to the membranes of thrombocyte. Conclusion: Except 12-dihydrogeginerol, other four compounds were reported to have anti-platelet aggregation activities in previous studies. 8-gingerol and 6-shogad were stronger than 6-gingerd and 10-shogaol. Chicken thrombocyte membrane immobilized chromatography is a high efficient and simplified model which can be applied to screen anti-platelet aggregation compounds from Traditional Chinese Medicine.

Key words: chicken thrombocyte; Ginger Oleoresins;
Project supported by the National Natural Science Foundation of China (No. 30400596 & No. 304712216).

P290017

Studies on the Cellular Signal Transduction Mechanisms of the Detoxification of LiangGeSan

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We investigated the cellular signal transduction mechanism of the detoxification of LiangGeSan (LGS), a traditional Chinese medicinal prescription with multiple effects on many infectious diseases. It showed that in vitro, LGS-containing serum could decrease the up-regulation of the expression of CD14, p-p38 MAPK and NF-κB induced by lipopolysaccharide (LPS). In vivo, LGS could inhibit the up-regulation of CD14 expression and the down-regulation of Scaverger receptor expression in a dose-dependent manner. The up-regulation of the expression of p-p38 MAPK, NF-κB, IL-6, TNF-αinduced by LPS could also be inhibited by LGS. The damages of lung and liver of mice induced by LPS could also be alleviated by LGS. These results indcate that, the detoxification of LGS may relate to its regulation effects on the pathway of cellular signal transduction.

Key words: LiangGeSan; cellar signal transduction; detoxification.
Acknowledgments: This work was supported by grants from National Natural Science Foundation of China (30171155).

P290018

The Effects of Chinese Herbal Medicines Salviandic acid B, Tetramethylpyrazine and Astragaloside IV on H_2O_2-induced Endothelial Cell Apoptosis

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Salviandic acid B (SAB), Tetramethylpyrazine (TMP) and Astragaloside IV (AS-IV) are active ingredients of Chinese herbal medicines, Salvia miltiorrhiza, Ligusticum wallichi Franchat, and Astragalus membranaceus (Fisch) Bge respectively, which are often used for prevention and treatment of cardiovascular disorders such as atherosclerosis. It is now considered that apoptosis of endothelial cell (EC) is an initial step in the development of atherosclerosis. And unidirectional laminar shear stress was shown to be capable of attenuating H_2O_2-induced EC apoptosis. MTT assay, TUNEL assay, Annexin V/PI staining, and poly (ADP-ribose) polymerase (PARP) cleavage assay revealed that under static condition, either SAB or AS-IV can protect EC from cytotoxic and apoptotic effects induced by H_2O_2 in a dose-dependent manner. A synergistic protective effect was also observed when a combination of SAB and AS-IV was used. However, TMP had no detectable protective effect. The potential protective effect of SAB and AS-IV in addition to laminar shear stress will be investigated.

Keywords: Chinese herbal medicines; cardiovascular disorder; endothelial cell; apoptosis

P290019

Pharmacological investigation of pro-angiogenic effect of Angelica sinensis extract on HUVEC cell in vitro

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Angelica sinensis, known as Danggui in China, It has been used for improving circulation, treating anemia, female irregular menstruation and amenorrhoea. Angiogenesis plays an important role in a wide range of physiological processes such as wound healing, fetal development, and formation of corpusluteum. The extract of Angelica sinensis, was investigated for the effect on angiogenesis in vitro. The effects of the extract on proliferation, invasion, migration and tube formation of human umbilical vein endothelial cells (HUVEC) were evaluated. The extract was identified to stimulate the proliferation of HUVEC cells by XTT assay and microscopic cell counting; in addition, flow cytometry analysis indicated that the extract increased percentage of HUVEC cells on the DNA synthesis phase. The extract showed an enhanced invading and migrating effects on the HUVEC cells. The extract was also demonstrated to promote tube formation of HUVEC cells on Matrigel. The differential expression of vascular endothelial growth factor (VEGF) was analyzed by real-time PCR and immunostaining method. Our results suggest that the Angelica sinensis extract exhibit stimulatory effect on angiogenesis.

Key words: Angelica sinensis, angiogenesis, human umbilical vein endothelial cell

P290020

The Effects of Dangguibuxue Tang on the Erythrocyte Function in the Acute Hypoxic Mice

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The effects of Danguibuxue Tang (Chinese Angelica Decoction for Replenishing Blood; DBT) on the erythrocyte function was studied in the acute hypoxia mice. The mice were divided to five groups: Normal, Control, DBT (20g/kg.d, 10g/kg.d, 5g/kg.d). Water or DBT were fed for 10 days. Acute hypoxia model was made by putting the mice into hermetical specimen bottles for 10 minutes. Then the erythrocyte membrane fluidty, the erythrocyte deforusbility and the erythrocyte immunity was tested. The results showed that DBT 5g/kg.d could prevent the decreasing of the erythrocyte membrane fluidity, the erythrocyte deformability and the erythrocyte immunity function in the acute hypoxia mice. DBT 10g/kg.d could prevent the decreasing of erythrocyte membrane fluidity and erythrocyte deformability, too. Effects were not observed by the mice with DBT 20g/kg. The above study shows that certain dose DBT can improve the function of RBC, which is a possible theory for it can invigorate qi to promote blood in Traditional Chinese Medicine.

Key words: Dangguibuxue Tang, Erythrocyte Function
Acknowledgment: Thanks go to Professor Chen Wenwei for your help and guidance.

P290021

The Effect of Methanolic Extract of Hyoscyamus Niger L. on Seizure Induced by Picrotoxin In Mice

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Back ground: Hyoscyamus niger L. has some effects on nervous system. It has been suggested as anticonvulsant in Iranian traditional medicine. In this investigation, the effects of methanolic extract of Hyoscyamus niger L. on seizure induced by picrotoxin was studied in mice.

Methods: In this study seven groups of animals pretreated with different dose of methandic extract of Hyoscyamus niger (12.5, 25, 50, 100, 200, 300, 400 mg/kg) by intraperitoneal injection. After 20 minutes each animal received 12 mg/kg picrotoxin for induction of seizure. Latency of time for beginning of seizure, duration of seizure and mortality rate were determined in test and control groups.

Findings: The results showed that letancy of seizure was increased in groups that pretreated with different doses of extract (specially dose of 300 mg/kg) ($P < 0.01$). In addition, these doses specially does of 300 mg/kg delayed the death time in mice ($P < 0.01$).

Conclusion: The results showed that the does of 300 mg/kg was more effective in control of seizure induced by picrotoxin in mice and more experiments are needed in this field.

Key words: Hyoscymus Niger, Seizure, Picrotoxin

P290022

Effects of total rhizoma panacis japonica saponins on nerve growth factor expression in ischemic rat's brain

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Object To study the effect of total rhizoma panacis japonica saponins (tRPJS) on the expression of nerve growth factor(NGF) after focal cerebral ischemia. Methods Ischemia rat models were made using the method of thread inserting right middle cerebral artery occlusion. Immunohistochemical method was performed to observe the NGF expression as well as the effect of tRPJS on them after occlusion of middle cerebral artery for 72h in rats.

Results In the ischemic side of the model group, the number of NGF positive cells were lower as compared with those in the sham- operated side of the sham- operated groug($P < 0.05$). The number of NGF positive cells in the tRPJS group were significantly more than that of model group($P < 0.05$), similar to that of sham- operated groug Conclusion The results indicate tRPJS can improve the expression of NGF after cerebral ischemia. It may be one of mechanisms for tRPJS in the treatment of ischemic stroke.

Key words: total rhizoma panacis japonica saponins (tRPJS), nerve growth factor(NGF), cerebral ischemia

P290023

Enzymatic Formation of Prostamide $F_{2\alpha}$ from Anandamide Involves A Newly Identified Intermediate Metabolite, Prostamide H_2

Wu Yang*1, Jinsong Ni*, David F Woodward**, Diane D-S Tang-Liu* and Kah-Hing John Ling*

Prostaglandin $F_{2\alpha}$1-ethanolamide (Prostamide $F_{2\alpha}$) is a potent ocular hypotensive agent in animals and represents a new class of fatty acid amide compounds. Accumulated evidence indicated anandamide, an endogenous bioactive ligand for cannabinoid receptors, may serve as a common substrate to produce all prostamides including prostamide $F_{2\alpha}$. Following incubation of anandamide with cyclooxygenase 2(COX-2), the reaction mixture was profiled by HPLC and an intermediate metabolite was discovered and characterized as a cyclic endoperoxide ethanolamide using HPLC tandem mass spectrometry (HPLC-MS/MS). Formation of prostamide $F_{2\alpha}$ was also demonstrated when the intermediate metabolite was isolated and incubated with prostaglandin F synthase. These results suggest that the biosynthesis of prostamide $F_{2\alpha}$ proceeds in two consecutive steps, oxidation of anandamide to form an endoperoxide intermediate by COX-2, and reduction of the endoperoxide intermediate to form prostamide F2α by PGF synthase. This endoperoxide ethanolamide intermediate has been proposed as prostamide H_2.

P290024

Central pharmacological action of Chinese Materia Medica Cynanchum chinense **R.Br**

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Aim: To study the Central pharmacological action of the water and chloroform-extract compounds from Cynanchum chinense R. Br. Methods: The Independent activity test and the hypnotic synergism test by under threshold hypnotic dosage of pentobarbital were employed to evaluate the central pharmacological action of the extract-compounds, and the rotorod test for minimal neurotoxicity. All the extract-compounds were evaluated for anticonvulsant activity by maximal electroshock (MES) and subcutaneous metrazol (MET). Result: The two extract-compounds exhibited inhibition effect of the spontaneous motor activity in mice, and promoted the hypnotic effect of pentobarbital. The water-extract compounds exhibited significant protection in MET, but the chloroform-extract compounds don't produce protective effect in MET. The chloroform-extract compounds can protection mice in MES, but no protective effect did in the water-extract compounds. Also, the both extract-compounds show no neurotoxicity. Conclusion: The extract compounds from Cynanchum chinense R. Br show inhibition effect on CNS, and the water and chloroform-extract compounds show different anticonvulsant activity in different seizure model in mice.

Key words: Cynanchum chinense R. Br; anticonvulsant activity,

P290025

Investigations into mechanism of action of hepatoprotective effect of Sarcostemma brevistigma

S K Shah, G B Shah, D D Santani*, M B Shah* Department of Pharmacology, K. B. Institute of Pharmceutical Education and research, Gandhinagar * Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad. Study was designed to investigate the hepatoprotective effect of Sarcostemma brevistigma (family: Asclepediacece) in experimental animals.

The alcoholic extract (A) of stem and its different fractions visually petroleum ether (B), chloroform (C) and n-butanol (D) were studied at the dose of 300 mg/kg orally for their effect against paracetamol (2.5 gm/kg) induced liver damage. Liver function marker enzymes like SGOT, SGPT, ALP and BILIRUBIN activity were estimated. Oxidant and antioxidant parameters like SOD, Catalase, reduced Gsh and MDA were estimated. Liver tissue was subjected to histopathological study.

Paracetamol treated rats showed significant increase in liver function marker enzymes as compared to the control rats. Elevated levels of these enzymes were significantly reduced with the use of Ext A and C. Increase in Oxidant and decrease in antioxidant parameters was prevented by treatment with Ext A and C. Necrosis observed in liver tissue of paracetamol treated rats was also significantly prevented by Ext A and C. The effects were comparable with those produced by standard drug silymarin (50 mg/kg).

Sarcostemma brevistigma possessed significant hepatoprotective activity. Probable mechanism of action for the activity could be regeneration of hepatic parenchyma and antioxidant activity.

P290026

INVESTIGATION OF THE PROTECTIVE EFFECT OF GARLIC EXTRACT ON GLUCOSE-INDUCED CYTOTOXICITY IN PC12 CELLS: ROLE OF APOPTOSIS

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Hyperglycemia, which occurs under diabetic condition, induces serious diabetic complications such as neuropathy, nephropathy and retinopathy. Little is known about the direct toxic effect of high concentrations of glucose on neuronal cells. Therefore effects of high concentrations of glucose in PC12 cells as a suitable model of neuronal study were examined. The result showed 3-fold of the optimum glucose concentration for PC12 cells (13.5 mg/ml in culture medium) reduced cell viability significantly after 48 hours. For investigating possible protective effect of garlic in glucose toxicity in neuronal cells 10, 50 and 100 µg/ml of garlic extract added to culture medium. Interestingly, glucose induced toxicity was reversed by adding 50 µg/ml of garlic extract, providing possible implication of garlic extract in diabetic neuropathy. Moreover role of apoptosis in glucose-induced toxicity was studied. In western blot analysis, the ratio of Bax/Bcl-2 protein expression in high glucose treated cells was significantly increased compared to controls. Additionally higher glucose could produce DNA ladder pattern

in PC12 cells. These results taken together could provide more details on mechanism of glucose-induced toxicity in PC12 cells in which garlic extract may have protective effect.

Key Words: PC12, Glucose-induced toxicity, Garlic, Apoptosis

P290027

Action of Exendin(9-39) Amide on GLP-1(7-36) Amide and Exendin-4 Mediated Contractions of the Suncus Murinus **(House Musk Shrew) Isolated Ileum**

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In the present studies, we investigated the action of glucagon-like peptide (GLP-1) agonists GLP-1(7-36) amide and exendin-4 on the Suncus murinus isolated ileum. Segments of ileum were placed in an organ bath containing Kreb's solution. Agonists were added to the bath using a 2-6 min dosing schedule. GLP-1(7-36) amide and exendin-4 (0.1-100 nM) induced concentration-dependent contractions yielding pEC_{50} values of 8.6 ±0.3 and 8.3 ±0.3, respectively. The GLP-1 antagonist exendin(9-39) amide (0.3-3 nM) was inactive alone, but non-competitively antagonized the action of both agonists with apparent pK_B values of 9.8 and 9.7, respectively. In other experiments, tetrodotoxin (1 μM) and atropine (1 μM) significantly antagonized ($p < 0.01$) the contractile action of exendin-4 (10 nM), whereas hexamethonium (500 μM) had no action. In conclusion, the action of GLP-1 receptor agonists to contract the ileum probably involves the enteric nervous system and a release of acetylcholine to activate muscarinic receptors.

Keywords: GLP-1, exendin(9-39) amide, Suncus murinus, ileum
These studies were supported by a Direct Grant (CUHK 2005.1.042).

P290028

In vitro **and** in vivo **pharmacological studies of crude extract from** Pisonia alba **Span. leaves on tracheal smooth musde and cardiovascular system**

Supaporn Prasettho, Somsmorn Chittrakarn, Wandee Udomuksorn and Niracha Yanyiurn Department of Pharmacology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand Pisonia alba Span. or Seangchun traditional use as anti-inflammatory, dues to lack of pharmacological data. In vitro pharmacological study of crude extract was investigated using guinea-pig trachea and atria while in vivo using rat blood pressure. The crude extract exhibited profound bronchodilation effect when tested on carbachol-induced tracheal contraction. It also increased both force of contraction and heart rate when tested on guinea-pig atria preparation. The effects of crude extract may be resulted from some other active ingredients, not potassium or calcium in the plant. The crude extract increased both mean arterial blood pressure and heart rate on pentobarbital anesthetized rat. However, propranolol, prazosin, atropine and verapamil did not antagonize the effect of crude extract. It is therefore suggested that the activity of crude extract may not mediate via either beta-, alpha one-adrenoceptors and muscarinic receptor stimulation or, calcium channel blockade. The effects may be direct action on cardiovascular system.

Key word: Pisonia alba Span., bronchodilation effect
Acknowledgement: This study was financially supported by Faculty of Science, Prince of Songkla University

P290029

Pharmacodynamic study on Mongdian medicine Alatanwuwei Pilule on mice model with gastric ulcer

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To investigate and verify the preventive and therapeutic effect of Alatanwuwei Pilule (ALT) treating experimental gastric ulcer by administration by gavage and supply theoretical bases for its clinical application. Methods Designing different animal models with gastric ulcer to find out pharmacological effect of ALT by administration by gavage. Results ALT by high close (600 mg·kg^{-1}) and moderate dose (300 mg·kg^{-1}) can significantly protect stomach from the damage of gastric ulcer induced by cold-water stress, glacial acetic acid ignition, alcohol and pyloric ligation, and can neutralize gastric acid, lower the activity of pepsin, inhibit intestinal propellant speed and alleviate the pain induced by acetic acid in mice. conclusion ALT shows a marked preventive and therapeutic effect on gastric ulcer by strengthening the defense function of gastric mucosa.

Key words: Alatanwuwei Pilule (ALT); gastric ulcer; gastric acid; pepsin

P290030

Study on the facilitated effect of ethanol extracts of Asterias on gastric emptying in mice and the determination of the effective part

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To investigated the facilitate effect of starfish extract on gastric emptying in mice. The effective part of starfish and its primary mechanism have also been elucidated. Gastric emptying in mice is studied. The ethanol extract (0.48 g/kg), macroporous resin eluate of aqua (0.3 g/kg) and the further purification (100 mg/kg, 300 mg/kg) hasten gastric emptying in mice. Furthermore, they restrain the inhibition of dopamine on gastric emptying in mice, while they have no effect on the inhibition of atropine. The starfish extract has the facilitate effect on gastric emptying in mice. Its effective component may be acid substance. The component is the inhibition of dopamine receptor but has no exciting effect on parasympathetic nerve.

Key words: starfish; metoclopramide; atropine; dopamine; gastric emptying
Acknowledgement This study is supported by the project of Key-Laboratory for New Drug Screen of Liaoning Province.

P290031

The anti-inflammatory and diuretic effects about mongdian medicine san-wei-ji-li powder

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Objectives: San-wei-ji-li powder is Mongolian recipe and clinical common medicine of traditional mongolian medicine. It has dispersing damp-heat and diuretic function. It can treat difficult urination, fever, edema and anuresis. To observe anti-inflammatory and diuretic effects of san-wei-ji-li powder, the article has researched its pharmacodynamics. Methods: In order to explore its anti-inflammatory effects, several inflammatory models such as ear edema induced by dimethylbenzene and granuloma by tampon were done. To observe its diuretic effects, the article applied methords of weighing filter paper and urine collect through catheter. Results: San-wei-ji-li powder (1.8g/kg, 3.6g/kg) has distinct anti-inflammatory effect to ear edema induced by dimethylbenzene and granuloma by tampon. San-wei-ji-li powder (0.6g/kg, 1.2g/kg) has diuretic effects to water load in mice and rabbits. Conclusion: San-wei-ji-li powder has effects of clearing Heat, anti-inflammatory and diuretic effects.

Key words: San-wei-ji-li powder; anti-inflammatory effect; diuretic effect

P30. Pharmacology of Natural Products

P300001

Voltage-gated K channels located in smooth musde mediated the relaxation of human internal mammary artery and rat aorta induced by resveratrol

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Resveratrol (3,5,4-trihydroxystilbene) has recently been found to produce vasorelaxation in endothelium-dependent and endothelium-independent manner. The aim of this study is to define the mechanism(s) of endothelium-independent relaxation produced by resveratrol in the isolated human mammary artery (HMA) and rat aorta (RA) precontracted by phenylephrine. Endothelium was removed mechanically. Resveratrol induced concentration-dependent relaxation of HMA (EC_{50} = 42.8 micro M) and RA (EC_{50} = 8.7 micro M) rings. Highly selective blocker of ATP-sensitive K channels, glibenclamide as well as blocker of big Ca

- sensitive K channels, charybdotoxin did not block resveratrol - induced relaxation of HIMA and RA rings. 4 - aminopyridine and margatoxin, blockers of voltage - gated K (K_V) channels, abolished relaxation of HIMA and rat aorta, induced by resveratrol. In conclusion, we have shown that resveratrol relaxed HIMA and aorta rings by activation of K_V channels located in smooth muscle.

P300002

The effect of periplocin on gene expression profiles in murine cardiac microvascular endothelial cells by Cdna microarray.

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OBJECTIVE: To investigate in the mechanism of periplocin - a compound isolated from a Chinese herb: Cortex Periplocae, as a cardiovascular drug on cardiac microvascular endothelial cells (CMEC).

METHODS: CMEC were treated with periplocin or ouabain (5×10^{-5} mol/l) for 24hr, processed for the isolation of RNA, analyzed for differentially expressed mRNAs between periplocin and ouabain; periplocin and control by six Biostar? gene chips.

RESULTS: (1) Microarray analysis of the expression of 14112 murine genes in gene chips suggested that 1070 genes were significantly regulated by periplocin compare with control and 1333 genes compare with ouabain. (2) Periplocin led to strong upregulation of mRNA transcripts for ATP - binding, cell growth and maintenance, cell communication, protein kinase activity, nucleic acid binding and signal transduction. (3) Significant different pathway between periplocin and ouabain is oxidative phosphorylation, ATP synthesis, amino acid metabolism and apoptosis.

CONCLUSION: Suggesting that periplocin action on CMEC is mediated primarily through signal transduction and a receptor - associated regulation of gene transcription.

Key words: periplocin; gene expression; cardiac; CMEC

P300003

The in vivo and in vitro anti - oxidant activity of ghrelin: Attenuation of gastric ischemic injury in the rat

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Ghrelin, is produced by stomach cells, regulate food intake, gastric secretion and motility. However, its protective role in gastric I/R injury has not yet been investigated. The present study aims to test its in vivo effect on gastric I/R- induced lesion in rats and investigate in vitro its effect on ROS production by human PMNs. The study was carried out on 3 groups of rats: control, I/R, and I/R + ghrelin. 200ng/kg ghrelin was given i.v., 15 mins prior to I/R. Histological assessment and iNOS antibody immunostaining were done. TBARS, GSH, LDH and TNF - alpha were measured. In vitro studies were done on human PMNs cells for ROS generation by CL. Results showed that ghrelin attenuated gastric injury, it also decreased serum LDH and tissue content of TNFalpha. Decrease in TBARS and increase in GSH was observed. Ghrelin treatment attenuated iNOS protein expression upregulated by gastric ischemic injury. In vitro studies showed that ghrelin inhibited ROS production by human PMNs. In conclusion, these results provides evidence that ghrelin protects against gastric I/R injury, which is possibly accomplished through its anti - oxidant activity suggested by both in vivo and in vitro studies.

P300004

Arnica: New insights in the molecular mode of action of this traditional medicinal plant

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Preparations from Arnica montana flowers have a long lasting tradition for the external use to treat haematomas, contusions, sprains, rheumatic diseases and superficial inflammations of the skin. Recent studies have considerably enhanced our knowledge on the pharmacological activity and efficacy of this traditional medical plant. The most effective compounds, the sesquiterpene lactones (SLs), such as helenalin and dihydrohelenalin esters, inhibit the transcription factors NF - kappaB and NF - AT at micromolar concentrations thus targeting inflammatory processes at a very central point. Both transcription factors regulate the transcription of genes of many inflammatory mediators. Pharmacokinetic studies have shown that SLs being part of the extract penetrate from the respective preparations into the stratum corneum of the skin and permeate in deeper skin layers. First clinical pilot studies proved the efficacy in inflammatory diseases after external application. In all cases Arnica preparations were well tolerated. Accordingly, very recent results only suggest weak sensitizing properties. Therefore, the opinion in literature that SLs are strong contact allergens has to be revised.

P300005

Adoptive transfer of insulin - specific tolerogenic dendritic cells prevents diabetes in NOD mice

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Aim: To investigate the role of antigen specific regulatory DC in inducing peripheral tolerance for prevention diabetes. Methods: We examined the activity of DC for generating immune tolerance in NOD mice after insulin injection subcutaneously and ability to suppress diabetes transfer by diabetogenic effector cells in secondary NOD - SCID recipients. Results: We showed that subcutaneous administration of insulin once a week delays the onset and reduced the incidence of diabetes in NOD mice over 30 weeks. Surface expression of MHC II, CD86 on NOD - derived DC was decreased after insulin treatment, while CD11c remained unchanged. Moreover, protection against diabetes following injection of insulin was associated with IL - 4 and IL - 10 production. Furthermore, dendritic cells characterized by an immature phenotype from animals subcutaneously treated with insulin adoptively transfer protection against diabetes in NOD - SCID mice. Conclusion: Our findings demonstrate that subcutaneous insulin administration generates immune tolerance by dendritic cells which favoring Th2 regulatory responses and conferring protection from diabetes development.

Key words diabetes; dendritic cells; immune tolerance

P300006

BIOLOGICAL STUDY OF PARTIALLY PURIFIED EXTRACTS FROM THE LEAVES OF ALSEODAPHNE PERAKENSIS (AP)

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Alseodaphne perakensis (AP) belongs to the family of Lauraceae. It is a tree of moderate size that is widely distributed throughout Peninsular Malaysia. Although there has been no reported use of this plant in traditional folk medicine practice, but in field test, the leaves were found to be rich in alkaloids, Similar alkaloids from other plants has been reported posses anti - emetic and antinociceptive activities. Partially purified alkaloid compounds from AP were evaluated on Guinea pig ileum, (GPI) Rat vas deferentia (RVD) and Mouse vas deferentia(MVD). The crude extracts from AP was obtained using methanol followed by fractionation with methylene chloride to obtain the alkaloid extracts on GPI, RVD and MVD were evaluated in an organ bath using Krebs solution as the tissue medium. The alkaloid extract DCM 'A' from AP inhibited electrically induced twitches on GPI, RVD and MVD. It also antagonized contractions induced by histamine and acetylcholine on the unstimulated GPI, and phenylephrine on the unstimulated RVD. DCM 'B' induced a contraction on the unstimulated GPI; and the contraction was inhibited by mepyramine. It may be concluded from this study that the alkaloid fractions DCM 'A' and DCM 'B' extracted from the leaves of AP appear to possess morphine - like, anticholinergic, antihistaminergic and histaminergic properties.

P300007

A Comparative study of cerebrum cortex and hippocampus on BDNF protein using gisenoside - Rg1 against brain ischemia

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Objective: To comparatively study of cerebrum cortex and hippocampus on BDNF protein using gisenoside - Rg1 against brain ischemia. Methods: Adult male SD

rats were created animal models of cerebral infarction in the territory of middle cerebral artery in rat. Then, constitution were prepared with 12 μm frozen section and the sections were stained under the same condition using specific BDNF(1:500) antibody by the immunohistochemistry ABC method. Results: Gisenoside-Rg1 could increase BDNF protein content and positive neurons amount in hippocampus and hippocampus after brain ischemia. But gray worth of cerebrum cortex is lower than hippocampus (* P < 0.05; * * P < 0.01), and positive neurons amount of hippocampus is higher than hippocampus (* P < 0.05; * * P < 0.01). Conclusion: BDNF protein were to spur by Gisenoside-Rg1 in hippocampus and hippocampus after brain ischemia, but index of both parts is being significant difference.

P300008

Comparative study on in vitro anti-free radical effects of quercetin and its monoglycoside isoquercetin and diglycoside rutin

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Objective To study in vitro anti-free radical effects of three flavonoids: quercetin(Q) and its monoglycoside isoquercetin(I) and diglycoside rutin(R) so as to further investigate their structure-effect relationship. Methods Hydroxyl free radical was generated via Fenton reaction, superoxide anion using pyrogallol auto-oxidation method. Hepatocellular and RBC lipid peroxidation was caused by hydroxyl free radical. IC_{50} was calculated and used to compare the anti-free radical activity. Results In 4 different in vitro models, the above three flavonoids showed extremely potent free radical-scavenging activity. The effect intensity was as follows: R > I > Q in hydroxyl free radical and superoxide anion chemcial reaction systems; Q > I > R in hydroxyl free radical-caused hepatocellular and RBC lipid peroxidation biologic models. Conclusion The three flavonoids have potent anti-free radical effects in a dose- and glycosyl structure-dependent manner. With the decrease in glycosyl group the effects gradually increased in biologic system while the reversed results was observed in chemical system.

Key words: free radical, quercetin, isoquercetin, rutin

P300009

The effect of PAMd on EAAC1 mRNA expression of hippocampus neurons of cerebral ischemia in rat * *

lianjun Guo*, Tie Sun*, Xuling Xu*, Ling Qu*.

AIM: To study the effect of PAMD on EAAC1 mRNA expression in hippocampus after cerebral ischemi. METHODS: Focal cerebral ischemia model was induced by transient occlusion of the middle cerebral artery. After MCAO 2h, PAMd ($10 mg \cdot kg^{-1}$) was administered. The effect of PAMd on hippocampal neuronal glutamate transporter EAAC1 mRNA expression in ischemic rats by RT-PCR was observed. RESULTS: The rats showed significant neurological deficit in 3h after MCAO, 24h after ischemia, the score was was 2.17 ±0.42. with PAMd the score was was 1.18 ±0.30. there were significant difference compared with the ischemia group; In 24h after MCAO, the infarct volume was 25.9 ±2.9%, PAMd($10 mg \cdot kg^{-1}$) could reduce infarct volume to 22.1 ±3.8%; The EAAC1 mRNA expression of ischemic hippocampal neurons was increased in 24h after ischemia, and PAMd could reduce EAAC1 mRNA expression CONCLUSION: The results suggested PAMd could reduce Neurological evaluation, infarct volume and EAAC1 mRNA expression of the ichemic rats.

KEY WORDS: cerebral ischemia; glutamate transporter; EAAC1 mRNA; PAMd

* * The project supported by National Natural Science foundation of China. No. 30171082

P300010

The effect of PAMd on Calpain activities of cortex and hippocampus in the ischemia-reperfusion rats

Tie Sun*, lianjun Guo*, Qing LV*, Xuling Xu*. yes

AIM: to investigate the effects of PAMd on Calpain activities of cortex and hippocampus in the ischemicreperfusion rats. METHODS: Focal cerebral ischemia (2h)-reperfusion(24h) model was induced by transient occlusion of the middle cerebral artery. PAMd 20 mg/kg i.p. The activities of Calpain in hippocampus and cortex were determined by spectrophotography. RESULTS: After ischemia the Calpain activities of cortex increased significantly to (2.81 ±0.38) △A/mg (protein), and that of normal cortex was (1.68 ±0.21) △A/mg (protein). after PAMd treatmented the Calpain activities of cortex decreased to 1.98 ±0.34. The Calpain activities of hippocampus increased significantly to (2.96 ±0.41) △A/mg (protein) during I-R, but PAMd could decreased the Calpain activities to 2.08 ±0.34 CONCLUSION: The results proved PAMd could decrease the Calpain activities of cortex and hippocampus in I-R rats.

KEY WORDS: brain ischemia reperfusion; cortex; hippocampus; Calpain; PAMd

* * The project supported by National Natural Science foundation of China. No. 30171082

P300011

Effect of *Ziziphus mucronata* **leave and seed extracts on isolated human neutrophils**

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Ziziphus mucronata leaves, bark and roots are used for pain relief to respiratory complaints, skin infections, expectorants or emetics in cough and chest problems and to stop bleeding. The aim of the study was to determine the effect of different extracts of the leaves and bark of the plant on the superoxide production of human neutrophils. Plant extracts were prepared and samples collected at 5 min, 3 hours and 24 hours. The samples collected at 24 hours were used to prepare concentrations for dose response experiments. Human neutrophils were isolated and incubated with the extracts the superoxide production response was determined. Toxicity tests were performed using human neutrophils and *Vibrio fischerii* bacteria. Only extracts for leaves could significantly reduce superoxide production of human neutrophils. This effect could be due to direct superoxide scavenging effects and not due to any toxic effects to the human neutrophils. Water extracts were not toxic when using the methods described. Ethanol extracts showed toxicity to *Vibrio fischerii* bacteria but not to human neutrophils.

Key words: *Ziziphus mucronata*, Neutrophils, ATP extraction, *Vibrio fischerii*, Superoxide

P300012

SCLM, total saponins extracted from Chaihu-jia-longgu-muli-tang, reduces chronic mild stress induced apoptosis in the hippocampus in mice

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Objective: To investigate the neuroprotective action of SCLM, total saponins extracted from Chaihu-jia-longgumuli-tang, in the reduction of apoptosis in hippocampal neurons using an experimental chronic mild stress (CMS) model. Methods: Mice were subjected to CMS procedure for 21 days. SCLM or fluoxetine was administrated orally during the stress periods. TUNEL staining and immunohistochemical assay were used to detect apoptosis in hippocampus. Results: CMS increased the number of TUNEL-positive neurons and upregulated the expression of Bax and caspase-3 in hippocampus. While SCLM or fluoxetine significantly reduced apoptosis as well as the expression of Bax and caspase-3. Conclusions: The present results suggest that the antidepressant-like property of SCLM may be mediated via protection against stress-induced neuronal apoptosis in hippocampus. These findings provide an important information that the anti-apoptotic effect of herbal medicines therapy may be beneficial for the treatment of depression.

Key words: Chaihu-jia-longgu-muli-tang; chronic mild stress; hippocampus; apoptosis

Acknowledgment: This research was supported by the Jiangsu Natural Science Foundation (BK2005149), Jiangsu, China.

P300013

The Anticancer Effects of Thai Medicinal Plants Containing Antioxidant Phenolics on Breast Cancer Cells

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Objectives: 1. To evaluate the anticancer effects of Phyllanthus emblica L. and Terminalia chebula Retz. on breast cancer cell proliferation and vascular endothelial growth factor (VEGF) expression. 2. To identify the presence and antioxidant activity of phenolics in the extracts. Materials & Methods: Cell proliferation and VEGF expression in breast cancer cells pretreated with each extract were evaluated by MTT reduction assay and RT-PCR, respectively. Thin layer chromatography (TLC)-1,1-diphenyl-2-picrylhydrazyl (DPPH) analysis was used to identify the presence and antioxidant activity of phenolics in the extracts. Results: Incubation of breast cancer cells with the extracts reduced cell proliferation and VEGF expression. Gallic acid and tannic acid possessing antioxidant activity in the extracts were identified by TLC-DPPH analysis. Conclusions: The plant extracts exerted antiproliferative and antiangiogenic effects on breast cancer cells. The key components of the extracts responsible for biological activity may be gallic acid and tannic acid.

Key words: medicinal plants, phenolics, antioxidants, anticancer effects
Acknowledgement: Faculty of Medicine Siriraj Hospital, Thailand, is acknowledged for financial support.

P300014

Neuroprotective effects of Safflor yellow B and its primary mechanisms

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To investigate whether safflor yellow B (SYB) had a protective effect on cerebral ischemic injury and to determine its possible mechanisms. Male Wistar rats were used to make the model of middle cerebral artery occlusion (MCAO). The behavioral test was used to measure neurological deficit scores for evaluation of the ischemic damage of brain. The infarction area of brain was assessed in brain slices stained with 2% solution of 2,3,5 triphenyltetrazolium chloride (TTC). Spectrophotometric assay was used to determine MDA and NO contents, antioxidant enzymes and total nitric oxide synthease (T-NOS) activities in brain. SYB at doses of 6.0 and 3.0 mg/kg markedly decreased the neurological deficit scores and the infarction area in MCAO rats. SYB significantly reduced T-NOS activity, NO and MDA levels, and increased antioxidant enzymes activities in brain. These suggest that SYB is able to provide a neuroprotection against the cerebral ischemic injury through antioxidant mechanism and antagonizing the toxic effect of overdose NO.

Key words: safflor yellow B; neuroprotection; antioxidant eneymes; NO
Acknowledgement: The study was financially supported by Shandong Engineering Research Center for Nature Drug.

P300015

Effect of 17β-estradiol and Ginsenoside on Osteoporosis in Ovariectomized Rats

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The aim of our study was to compare the effect of anti-osteoporosis of 17β-estradiol (E_2) and Ginsenoside, such as total ginsenoside (tR) and its main ingredients (Rb_1 and Rg_1), in ovariectomized (OVX) rats. We measured the bone mineral densities (BMD) of lumbar vertebra and tibia, analyzed the tibia histological morphological data, measured activity of Alkaline phosphatase (ALP) and the concentration of intercellular cAMP in cultured osteoblast. Results showed that both tR and E_2 could increase significantly BMD of lumbar vertebra and tibia in OVX rats, but the effect of tR was stronger. We found that E_2 and Rg_1 could increase the concentration of intercellular cAMP, accelerate the division and proliferation of osteoblast; increase activity of ALP and promote mature of osteoblast, but Rb_1 could not. The present findings indicate that E_2 and tR have effect of anti-osteoporosis in OVX rats.

KEY WORDS: osteoporosis; ginsenoside; 17β-estradiol

P300016

Buddlejasaponin IV through the inhibition of iNOS and COX-2 expression in RAW264.7 macrophages

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Buddlejasaponin IV isolated from Pleurospermum kamtschaticum is an anti-inflammatory compound that inhibits NO, PGE2 and TNF-a production. Here, we studied the mode of action of this compound. Buddlejasaponin IV reduced lipopolysaccaride-induced levels of iNOS and COX-2 at the protein levels, and iNOS, COX-2, TNF-a, IL-1B and IL-6 mRNA expression in RAW264.7 macrophages in a concentrationdependent manner, as determined by Western blotting and RT-PCR, respectively. Buddlejasaponin IV inhibited the LPS-induced activation of nuclear factor-kB, a transcription factor necessary for pro-inflammatory mediators, iNOS, COX-2, TNF-a, IL-1B and IL-6 expression. This effect was accompanied by a parallel reduction in Ik-Ba degradation and phosphorylation, and by the nuclear translocation of the NF-kB p65 subunit. The effects of buddlejasaponin IV on acute phase inflammation were studied on serotonin- and carrageenannduced paw edema. Maximun inbitions of 26% and 41% were noted at a dose of 20 mg kg-1 for serotonin- and carrageenan-induced paw edema, respectively. The analgesic effect of buddlejasaponin IV was evaluated using acetic acid-induced writhing and hot plate tests.

P300017

Effects and mechanisms of Paeoniflorin, a bioactive glucoside from paeony root, on adjuvant arthritis in rats

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To determine the mechanisms of Paeoniflorin (PF) in the treatment of adjuvants arthritis (AA). AA in rats was induced in male Sprague-Dawley rats. PF (5, 10, 20 mg/kg/d) was orally administered to rats from day 14 to 20 after immunization. Interleukin-1 (IL-1) was determined by 3-(4,5-2dimethylthiazal-2yl) 2,5-diphenyltetrazoliumbromide (MTT) assay. Prostaglandin E2 (PGE2) was measured by radioimmunoassay. IL-6, vascular epidermal growth factor (VEGF), and granulocyte macrophage colony stimulating factor (GM-CSF) were measured by enzyme-linked immuno-absorbent assay (ELISA) assay. Expression of inhibitory αsubunits of G protein (Gi) and cyclo-oxygenase-2 (COX-2) were detected by Western blot analysis. The administration of PF (10, 20 mg/kg/day, ig, days 14-20) inhibited the inflammatory response and reduced the levels of IL-1, PGE2, IL-6, VEGF and GM-CSF in synovium homogenates of AA rats. Furthermore, PF not only reduced Gi expression at dose of 10 and 20 mg/kg but also decreased COX-2 expression at dose of 20 mg/kg in synovium homogenates of AA rats. PF suppresses rat AA by inhibiting abnormal proinflammatory mediators secretion and reducing Gi and COX-2 expression in synovium.

Key words: Adjuvant arthritis; Paeoniflorin; inhibitory αsubunits of G protein; cyclo-oxygenase-2

P300018

Study on anti-tumor effect of soponin from Asparagus officinalis L.

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To study anti-tumor effects of soponin from Asparagus officinalis L. in vivo and in vitro, and the effect on the synthesization of nucleic acid in HepG-2. We use the classic pharmacology method, MTT, LSCM. After dealing with soponin from Asparagus officinalis L. with the dosage of 25, 50, 100 mg/kg, it showed marked anti-tumor effect on S180 tumor mice ($P < 0.05$), the largest inhibitory rate is 58%; it also can markedly lengthen average survival time of H22 tumor mice, the largest lengthening rate is 70%. In vitro, it has cell toxicity effect on 7901, BGC-823 and HepG-2, and the inhibitory rate is related with the concentration and the incubation time, the cell growth curve and the spilt index were also inhibited. The further study showed it inhibited the synthesization of DNA

and RNA, the fluorescence intensity of DNA and RNA in therapy group is weaker than control group, which has dose-response relationship. In conclusion, soponin from Asparagus officinalis L. has anti-tumor effect.

KEY WORDS: Asparagus officinalis L., anti-tumor, DNA, RNA

ACKNOWLEDGEMENT: Thanks for the Committee of National Natural Science Foundation of China, by which the project is supported. (NO.30300592, NO. 30400352).

P300019

Anti-apoptotic effects of polypeptide from Chlamys farreri on murine thymocytes under UV-Irradiation

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We previously reported that polypeptide from Chlamys farreri (PCF), a purified octapeptide isolated from Chlamys farreri, had a potent antioxidant activity, and protected skin cells against ultraviolet (UV) radiation. In an effort to identify other immunostimulatory effects, we evaluated the effects of PCF in vitro against UV radiation by measuring its effects on the murine thymocytes. PCF was found to significantly increase the number of thymocytes exposed to UV radiation, and decreased the thymocytes apoptosis rate. In addition, PCF maintained the concentration of cellular free calcium, inhibited UV-induced decreasing of mitochondrial membrane potential, and was able to enhance the expression of Bcl-2 gene, meanwhile decreased the expressions of p53 and Bax. We demonstrated that PCF pretreatment markedly protected murine thymocytes from the lethal effects of UV radiation in a dose-dependent manner, at doses of 0.125%, 0.25%, and 0.5%. These findings indicate that PCF may be a useful radioprotective agent to modulate the function of murine thymocytes under UV radiation.

Key words: polypeptide from Chlamys farreri; UV radiation; apoptosis; thymocytes

P300020

The combination of extracts of Panax ginseng and Ginkgo biloba modifies abnormal cholinergic function in experimental AD

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To investigate the effects of the combined extracts of Panax ginseng and Ginkgo Biloba (NWK) on cholinergic function, Morris Water Maze task was used to evaluate cognitive function in three animal models, including natural aging rats, Aβ1-40-treated rats, and D-galactose-treated rats. The level of acetylcholine (ACh) was determined by an improved HPLC method using ECD combined with two immobilized enzyme reactors. Acetylcholinesterase (AChE) activity was estimated spectrophotometrically at 412 nm. The constitution of the combination for NWK was derived from orthogonal experiments using normal mice and D-galactose-treated rats. It was found that the level of ACh in brain tissue was significantly increased by treatment with NWK (62 and 31 mg/kg/day, ig for 60 days) in three animal models mentioned above. However, NWK decreased AChE activity significantly in both Aβ- and D-galactose-treated rats where AChE activity was increased, while enhanced it in naturally aged rats where AChE activity was decreased. These suggest that NWK can modify the abnormal cholinergic function, which depends on the functional state of neurons.

P300021

Establishment of the Pharmacological Basis of the Therapeutic Effects of Ligusticum chuanxiong, a Traditional Chinese Herb for Cardiovascular Diseases

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Ligusticum chuanxiong is a popular Chinese herb for cardiovascular diseases in China. However, its use is limited by lack of a scientific foundation. The present study aims to establish the pharmacological basis of the therapeutic effects of Ligusticum chuanxiong. Among 17 major constituents identified in Ligusticum chuanxiong, 6 were orally absorbable as predicted using a Caco-2 colonic cell model. Subsequent screening on vasorelaxation, the most widely examined effect of the herb, showed that absorbable constituents ligustilide, senkyunolide A and butylidenephthalide had relaxing effects comparable to the parent herb. The same three constituents also possessed similar anti-platelet aggregation and anti-thrombotic profiles to the parent herb. Ligusticum chuanxiong was found to have vasorelaxing, platelet-inhibitory and anti-thrombotic actions. These effects were most likely due to the combined contribution of the three major absorbable constituents ligustilide, senkyunolide A and butylidenephthalide. Key words: Ligusticum chuanxiong, vasorelaxation, platelet, thrombosis

Acknowledgement: The current study was supported by Innovation and Technology Commission, Hong Kong SAR (UIM034).

P300022

EFFECTS OF ESSENTIAL OIL FROM THE LEAVES OF CLAUSENA ANISATA HOOK. ON SMOOTH MUSCLE CONTRACTIONS.

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Preliminary study of the pharmacological action of essential oil from the leaves of Clausena anisata Hook. was carried out in different smooth muscle preparations. Cumulative doses of the essential oil (5×10^{-5} - 3.2×10^{-3} % v/v) stimulated the contractile response of all smooth muscle preparations study. The highest stimulation was found in isolated rat aorta (47.03 ±7.89 %). The others were guinea-pig ileum (39.40 ±4.61 %) rat fundus (26.19 ±5.31 %) guinea-pig trachea (15.78 ±2.33 %) and rabbit jejunum (4.99 ±0.50 %). These spasmodic effects were investigated through autonomic receptors. The result demonstrated that atropine was not able to attenuate the stimulation effect of the essential oil on the isolated rabbit jejunum and guinea-pig ileum while the inhibitory effects of atropine (1×10^{-7} and 1×10^{-6} M) were prominently found in the contraction induced by the essential oil on rat fundus. Sympathetic mechanism of the essential oil was confirmed in rat aorta since prazosin reduced the contractile response produced by the essential oil significantly. It could be concluded that Clausena anisata's essential oil possessed smooth muscle stimulation effect partly through sympathetic and parasympathetic receptors.

Key words: Clausena anisata.

P300023

Antitumor study of oral use of hydroxycamptothecin and in combination with other drugs

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AIM The antitumor effect of oral use of hydroxycamptothecin (HCPT) was studied in animal tumor models. The action mechanisms and the effect of HCPT in combination use with other drugs were studied too. METHODS The mouse tumors including sarcoma-180 (S-180) and solid hepatoma (Hep-S) were chosen. Flow cytometry method was employed to examine cancer cell apoptosis. The change of p53 and bcl-2 genes expression was evaluated by immunohistochemical staining technique. The effect of combined use of HCPT with adriamycin, teniposide (VM-26) and some Tradtional Chinese Medicines (TCM) was also investigated. RESULTS Oral administration of HCPT at 4-8 mg·kg^{-1} could inhibit growth of S-180 and Hep-S from 32% to 69%. Oral HCPT at 2-6 mg·kg^{-1} for 5 days induced apoptosis in S-180 cells. The expression of p53 and bcl-2 was obviously down-regulated. HCPT in combination administration with adriamycin, VM-26 etc enhanced antitumor effect markedly. CONCLUSION Oral use of HCPT produced marked antitumor action on animal tumors. HCPT could induce apoptosis in S-180 tumor cells and downregulate p53 and bcl-2 expression. In combined use of HCPT with adriamycin, VM-26 and some TCM the anticancer action was more obvious.

P300024

Effects of Astragali radix on renal function and its protein expression of IgA nephropathy in mice

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In a 12-week pharmacological study the aqueous extract of Astragali radix

(AEAR) was found to induce protective effect on renal function in IgA nephropathy (IgAN) mice induced by dextran treated with 10g/kg per day. Two-dimensional electrophoresis (2-DE) of the kidney tissues samples was carried out respectively. With the protein patterns of 2-DE, comparing with normal control group, about 334 kidney proteins were found significantly changed in the untreated group, and 10 proteins were uniquely expressed in untreated group. Comparing with untreated group, significant treated-related quantitative changes in AEAR treated group were found among different kidney proteins between normal control group and untreated group. About 50% of above 334 different proteins were regulated to near normal one in AEAR treated group. In above 10 unique proteins, 5 spots fully recovered to the un-expression state of normal control group, 4 spots observably decreased and neared the normal expression level, and 1 protein slightly increased in AEAR treated group comparing untreated group.

Key words: Astragali radix; IgA nephropathy; Two-dimensional electrophoresis

P300025

Relaxant mechanisms of an ethanol extract from rhizomes of *Kaempferia parviflora* **on isolated human cavernosum**

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How do crude ethanol extracts of *Kaempferia parviflora* (KP) relax the human cavernosum (HC)? Isolated human cavernosal strips, pre-contracted with phenylephrine (Phe) or with 80 mM KCl underwent relaxation when treated with KP. N-nitro-L-arginine caused a rightward shift of the relaxant curve to KP. Viagra, but not KP, potentiated the relaxant responses to glyceryl trinitrate on Phe contracted HC. Contraction of HC induced by 1 μM Phe was reduced by nifedpine, and KP induced a further reduction. In a Ca^{2+}-free Kreb's solution with EDTA, KP lowered the Phe induced contractile response. Nifedipine did not change the phasic contraction slope of HC to 1 μM Phe but Y 27632 or KP reduced the slope. The slope of phasic contraction of HC induced by 80 mM KCl was less steep in the presence of nifedipine or KP, but not with Y 27632. All drugs depressed the amplitude of tonic contraction. KP has relaxart activities on HC but not through phosphodiesterase 5. Possible mechanisms include (1) stimulating the release of nitric oxide, (2) inhibiting calcium entry via voltage- and store-operated calcium channel, (3) disturbing the mobilization of store-intracellular calcium, and (4) acting as a Rho-kinase inhibitor.

P300026

Periplocoside E, an effective compound from Periploca sepium Bge, inhibited T cell activation in vitro and in vivo

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Followed the bioactivity-guided isolation, the most potent immunosuppressive compound, periplocoside E (PSE) had been identified from Periploca sepium Bge, a traditional Chinese herb used for treating rheumatoid arthritis. We investigated the immunosuppressive effects of PSE in vitro and in vivo. The results showed that PSE suppressed a delayed type hypersensitivity reaction, and ovalbumin (OVA) induced antigen-specific immune responses in mice. Purified T cells from OVA-immunized mice with PSE treatment showed its low ability for activation by OVA plus normal APC stimulation in vitro. PSE dose-dependently inhibited anti-CD3 induced primary T cell proliferation, activation for IL-2R expression, and cytokine (IFN-gamma and IL-2) production also at the transcriptional level. PSE significantly inhibited the activation of ERK and JNK in T cells stimulated with anti-CD3. These results demonstrated that PSE is an immunosuppressor, which directly inhibit T cell activation in vitro and in vivo. This study provided evidence to the therapeutic effects of Periploca sepium Bge on T cell-mediated disorders.

Key words: Periplocoside E, immunosuppression, T cell activation
Acknowledgement: Grant: No. KSCX2-SW-202

P300027

WJ-53-6 Modified Collagen-Induced Arthritis in DBA/1 Mice via Inhibiting T cell Activation

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WJ-53-6 identified from Periploca sepium Bge, displays strong immunosuppressive activities in our previous studies. This study is to investigate whether WJ-53-6 has anti-arthritic potential in type II bovine collagen (CII)-induced arthritis (CIA). DBA/1 mice were immunized with CII to induce arthritis and administrated with WJ-53-6. The severity of arthritis was evaluated according to the clinical score and joint damage. The effects of WJ-53-6 on immune responses were determined by serum antibody levels, lymphocyte proliferation and cytokine assay. We demonstrated that WJ-53-6 treatment significantly reduced the incidence and severity of CIA. The beneficial effects of WJ-53-6 may be associated with reduction of serum anti-CII IgG, IgG2a, and IgG1 levels and inhibition of CII-specific lymphocyte proliferation, IFN-γ and IL-2 productions. These findings highlight that WJ-53-6 prevents CIA by suppressing T cell proliferation and activation, with a potential for treatment of rheumatoid arthritis.

Key words: Periploca sepium Bge, Arthritis, T cell activation
Acknowledgement: Grant: No. KSCX2-SW-202

P300028

Inhibition Effect of Hydroxysafflor Yellow A on Rat Cardiac myocyte Apoptosis Induced by Myocardial Ischemia

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Hydroxysafflor yellow A (HSYA) is the main ingredient of Carthamus tinctorius L.. To study the effect of HSYA to relieve rat cardiomyocyte apoptosis inducd by myocardial ischemia in cultured cardiomyocytes and in vivo test. Neonatal rat cardiomyocytes were subjected to 3h hypoxia and 2h reoxygenation. Apoptosis was observed with DNA ladder and fluorescence microscope. The protective effect of HSYA against apoptosis and MMV (mitochondria membrane voltage) decrease was studied by FCM (flow cytometry) with propidum iodide and Rhodamine 123 staining. Rat myocardial ischemia was induced by isoproterenol. Effect of HSYA against cell apoptosis was observed by transmission electron microscopy and TUNEL staining, its effect on apoptotic related gene (Bcl-2 and Bax) expression was observed by immunohistochemical and RT-PCR techniques. In cultured cardiomyocytes, the cell apoptosis rate was reduced and its MMV decline was alleviated by HSYA. Rat myocardial cell apoptosis was inhibited by HSYA. Bax gene expression was down-regulated while Bcl-2 was upregulated by HSYA. These results suggest that HSYA is effective to inhibit cardiac myocyte apoptosis.

Key words: HSYA; Myocardial ischemia; Apoptosis

P300029

NEW CUBAN NATURAL PRODUCT FROM STEM BARK OF *MANGIFERA INDICA* **L. (VIMANG). PHARMACOLOGICAL PROFILE AND THERAPEUTIC POTENTIALITY.**

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The aqueous extract from stem bark of Mangifera indica L (VIMANG) has been used in Cuba during several years in ethnomedical practices for the improvement of quality of life of patients with different pathologies. Phytochemical characterization of the extract has led to the isolation of different phenolic constituents, with the glucosylxanthone mangiferin as the majority component. The extract has demonstrated as the main pharmacological property its antioxidant activity. Others studies have shown that the extract also possesses others pharmacological activities, such as: anti-inflammatory, antiallergic, analgesic and immunomodulator, with very complex and multifactorial mechanisms of action involved. Different clinical studies have been developed, demonstrating the therapeutic effectiveness of Vimang as antioxidant supplement in pathologies where oxidative stress is related with their etiology.

Key words: Mangifera indica L, natural products, antioxidant, anti-inflammatory

P300031

Antitumor activities and immunoenhancement properties of Arca Granosa Linnaeus extracts in tumor-bearing mice

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This study investigated the antitumor activities and immunoenhancement properties of Arca Granosa Linnaeus extracts, named P1 (protein content: 67.3%). We tested the antitumor activities of P1 (100, 200, 400 mg/g) in tumor-bearing mice (Lewis lung carcinoma in C57BL/6 mice, sarcoma 180, hepatocarcinoma 22 (H22) and Ehrlich ascites carcinoma (EAC) in kunming mice, respectively). Also immunoenhancements of P1 were evaluated in C57BL/6 mice immunized with sheep red blood cells (SRBC 10%, ip), or with mitogens (10 ug, lipopolysaccharide, ip). Tumor weights of P1 groups decreased more than those in control group and the inhibition rates of P1 were 59.81 to 67.25% in Lewis lung carcinoma mice, 41.10 to 49.08% in S180 mice and 36.29 to 49.19% in H22 mice respectively. Also the life span in P1 groups was significantly prolonged in EAC bearing mice. And P1 enhanced IgM antibody and anti-SRBC IgM-specific antibody production. Thus P1 may be used as a potent antitumor extracts through its immunoenhancement properties.

KEY WORDS antitumor; immunoenhancement; Arca Granosa Linnaeus;

Acknowledgement: Project supported by Qingdao Science and Technology Bureau (05-1-HY-81 and 2005SKI-04)

P300032

The effect of Eucalyptus globulus oil on the expression of TLR4 in rat acute lung injury induced by lipopolysaccharide

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Objective: To study the distribution of toll-like receptor 4 (TLR4) in rats respiratory tract and the effect of lipopolysaccharide (LPS) and Eucalyptus globulus oil on the distribution of TLR4. Method: The Sprague-Dawley rats were intratracheally instilled with LPS (2 mg/kg per day) for two days to induce acute lung injury (ALI). At 72 hours, lung morphology was studied, TLR4 was detected by immunohistochemistry and the expression of NFkB in nuclei was measured by western-blot. Results: The immunohistochemistry result: TLR4 distributed widely in common rats respiratory tract, increased in the group of ALI, but decreased in the group of Eucalyptus globules oil (300 mg/kg). The lung morphology result: inflammation in lung morphology increased apparently in the group of ALI than the models, but decreased in the group of Eucalyptus globules oil. The western-blot result: The treatment of Eucalyptus globules oil couldn't inhibit the increase of NF-kB induced by LPS. Conclusion: The expression of TLR4 distributed widely in rats respiratory tract. The stimulation of LPS could reinforce the expression of TLR4. The Eucalyptus globules oil could reduce the increase of TLR4 induced by LPS in bronchioles.

P300033

The Effects Of Physcion On Intracellular Calcium Mobilization And TNF-α production Of Rat Peritoneal Macrophage

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Physcion is an effective ingredient in rhubard, a Chinese herb which has been used for treating inflammation. To investigate its immunopharmacological mechanism, we tested the effects of physcion on the production of tumor necrosis factor-α (TNF-α) and intracellular calcium concentration ($[Ca^{2+}]_i$) by the MTT assay and single cell Ca^{2+} imaging method respectively, using the rat peritoneal macrophage as model cell and the lipopolysaccharide (LPS) as the stimulator.

Physicon inhabited LPS-induced TNF-α secretion of the macrophage in a dose-dependent manner, however for the no-LPS-treated macrophage, it moderately increased its TNF-α secretion. As it is well established cytokines productions are related with the intracellular Ca^{2+} mobilization, we further tested the effects of physcion on the $[Ca^{2+}]_i$. The results showed that physicon inhabited the LPS-induced $[Ca^{2+}]_i$ increase significantly, and this was due to it blocked the Ca^{2+} influx as well as Ca^{2+} release from intracellular store. For the no-LPS-treated macrophage, physicon slightly caused its Ca^{2+} influx, and then increased the $[Ca^{2+}]_i$. These results suggested that physcion affected Ca^{2+} mobilization, therefore modulated the TNF-α production.

P300034

Evaluation of the toxicological properties of Origanum majorana oil

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The acute, subacute and chronic toxicity studies in laboratory animals showed that volatile oil derived from Origanum majorana is a well tolerated substance. The oral acute LD_{50} in mice and rats was 1,203 mg/kg and 1,666 mg/kg respectively. Repeated oral dosing was without effects upto 40 mg/kg in rats and mice, except a significant decrease in blood glucose level after 3 monthes from drug administration. Reproduction studies in rats showed no evidence of impaired fertility. Oral teratology study has been performed on pregnant rats at higher doses and revealed no evidence of teratogenic potential of the Origanum majorana oil. The tested preparation was devoid of mutagenc activity in mice at higher doses upto several times the recommended human doses. It could be concluded that Origanum majorana oil is a safe herbal remedy.

P300035

Study on the Mechanism of Anti-ageing by Natural Small Molecules from TCM

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The aim of this study is to construct two signaling pathway using the human diploid fibroblast cell as a model according to two pathway of p53-dependent and independent senescence by treatment of cells with H_2O_2 and the HDAC inhibitor, trichostatin A, TSA. We can establish a platform to screening the medicine efficiently, through the analysis which come from the change of the activity of β-galactosidase and the important proteins related to cell senescence, such as SIRT1, p53, p21, MDM2, etc. This platform screens out the medicine that can slow down the cell senescence by targeting the receptors and the enzymes, and can be used to detect the action mode of the small molecule natural product which extracts from several plants, such like protecting cells from oxidation, or ensuring the stabilization of the genetic matter, and can be used to estimate the degree that the medicine slows down the senescence.

Key words: Small Molecule Natural Product; Senescence; p53; SIRT1

P300036

Antiproliferation and Apoptosis by Silybin A and Silybin B, Two Novel Isomers of Silybin, in Human Leukemic K562 Cells

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In this study, we assessed the apoptotic induction effects of two novel isomers from silybin in human chronic myeloid leukemia (CML) K562 cells. MTT assay was used for assessment of cell proliferation. DNA damage, DNA agglomeration and DNA ladder were observed by comet assay, fluorescence staining and agarose gel electrophoresis, respectively. Western blot was employed for Bcl-2, Bcl-xL, Bax, p53, and c-abl detection. To investigate the transcription of extracellular signal regulated kinase (ERK1/2), RT-PCR was applied. Reactive oxygen species (ROS) and Ca^{2+} were tested by Flow Cytometric. As the results, treatments of the two isomers led to proliferation inhibition and significant apoptosis in K562 cells with down-regulation of Bcl-2, Bcl-x_L, c-abl, up-regulation of Bax, increase of activated caspase-3, -9, enhancement of phospho-p53 and inhibition of ERK1/2 transcription. Results also showed an increase of ROS and Ca^{2+} level in the treatments. Taken together, the two novel isomers of

silybin both have strong apoptotic induction effects in K562 cells, greater than silybin itself: implication for CML intervention.

Key words: silybin A, silybin B, apoptosis, leukemia

P300037

Inhibitory Mechanisms of Tetramethylpyrazine in Middle Cerebral Artery Occlusion-Induced Focal Cerebral Ischemia in Rats

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Tetramethylpyrazine (TMPZ) is an active ingredient isolated from Ligusticum wallichii Franchat, which has long been used in China for the treatment of vascular diseases. In the present study, TMPZ significantly attenuated middle cerebral artery occlusion (MCAO)-induced focal cerebral ischemia in rats. Administration of TMPZ at 10 and 20 mg/kg produced concentration-dependent reductions in infarctsize compared with that of control rats. The expressions of nitrotyrosine and iNOS were markedly inhibited by TMPZ (20 mg/kg) treatment. Furthermore, TMPZ (100 ~250 μM) concentration-dependently inhibited respiratory bursts in human neutrophils stimulated by fMLP (800 nM) and PMA (320 nM). TMPZ (100 ~250 μM) also significantly inhibited neutrophil migration stimulated by fMLP (800 nM) and LTB_4 (160 nM). Furthermore, TMPZ (100 and 200 μM) greatly reduced the ESR signal intensity of hydroxyl radical formation. In conclusion, we demonstrate a neuroprotective effect of TMPZ in MCAO-induced focal cerebral ischemia in vivo. TMPZ mediates a portion of the free radical-scavenging activity, and inhibits neutrophil activation, resulting in a reduction in the infarct volume in ischemia-reperfusion brain injury.

Key words: TMPZ, middle cerebral artery occlusion (MCAO), nitrotyrosine, inducible nitric oxide synthase, neutrophil activation, free radicals.

P300038

Comparative Efficiency of Durian Polysaccharide Gel Dressing Patches for Wound Healing in Pig and Dog Skins In Vivo

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This studies aimed to compare an efficiency of polysaccharide gel (PG) from rinds of Durio zibethinus Murr., as a dressing patch (PG fiber) in treatment of opened wounds in skin of pigs and dogs. Full-thickness excisional wounds, 2.0-2.45 cm. in diameter, were operated along both sides of dorsal midline area of animals. The wounds in each experiment was randomly divided into 2 groups. Group 1 was treated with povidone iodine (control) and group 2 was treated with PG fiber (treatment). Every 3 days, all wounds were cleaned and performed the same treatment, healing rates were observed until experiment ended. Histopathology was studied. The results demonstrated that in treatment groups of both species had signitficanltly smaller wound areas and faster healing than those of their control groups on day 12. Complete healing wounds in treatment and control groups were 100% and 50% by day 21 in dogs, and 80% and 69% by day 18 in pigs, respectively, revealed the effect of species difference. Histopathological study showed less granuloma formation in all PG fiber treated wounds. In conclusion, PG fiber dressing patch was more effective than povidone iodine in healing wounds in pig and dog skins.

Key words: wound healing, Durio zibethinus

P300039

Study on antineoplastic effect of ITCs from broccoli

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Isothiocyanates (ITCs) are the hydrolyzed products of Glucosinolates by the myrosinase enzyme in broccoli To study the anti-neoplastic effect of ITCs in vivo and in vitro, the classic pharmacological methods were used, such as MTT in vitro, S180 and H22 tumor mice model in vivo. The results showed that ITCs had remarkable cell toxicity effect on SGC-7901 HepG-2 and LS-174 cells with the dosage of 0.1 ~10 mg/kg ($P < 0.01$). The IC_{50} is 17.37, 12.18, 3.15 μg/mL separately. In vivo test, ITCs showed marked anticancer effect on S180 solid tumor mice at the dosage of 15 ~60 mg/kg ($P < 0.05$). The largest inhibitory rate is 51.4%. ITCs could also markedly lengthen average survival time of H22 tumor bearing mice at the dosage of 30 ~60 mg/kg ($P < 0.05$), and the largest lengthening rate is 69.74%. The inhibitory rate and the lengthening rate are related to the concentration of ITCs in vivo. In conclusion, ITCs from broccoli have antineoplastic effect. But the mechanism should be further studied.

KEY WORDS: broccoli, isothiocyanates (ITCs), antineoplastic effect

ACKNOLEDGEMENT: Thanks for the Committee of National Natural Science Foundation of China, by which the project is supported. (NO. 30300284, NO. 30400591)

P300040

Inhibitory effect on male mice procreation and chemical composition analysis of RVLEAE

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Objective: To probe into the effect of Rhynchosia volubilis Lour ethyl acetate extract (RVLEAE) on male mice procreation and analyses their chemical composition. Methods: 80 male mice were randomly and equally divided into four groups: Normal Saline control; positive control with 0.1% tripterygium wilfordii glycoside, 1% RVLEAE (I) and 4% RVLEAE (II). Every mouse is taked 0.1ml/10g for eleven consecutive weeks, once a day. natural mating went on one week. After 2 and 10 weeks. RVLEAE were separated with column chromatography, and chemical composition were identified with infrared chromatography and nuclear magnetic resonance. Results: The pregnancy rate of female mice were markedly decreased and the number and viability of spermatozoon of male mice slightly reduced in I and II group after 2 and 10 weeks. Main chemical composition were identified as saccharide, glycosides, alcohols, and phenols. Conclusions: RVLEAE, which glycosides interfere the maturation of spermatozoon in the epididymis cauda, can inhibit the procreation of male mice.

Key words: RVLEAE, inhibit the procreation, epididymis, fucose

P300041

EVALUATION OF THE LIPOPHILIC EXTRACT OF Cucurbita pepo **L. ON THE BENIGN PROSTATIC HYPERPLASIA.**

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We studied the effect of the lipophilic extract of seeds of Cucurbita pepo L. (ELMSC) in the pattern in vitro of the deferential conduit of rats and in the benign prostatic hyperplasia induced by testosterone propionate during 15 days. An increase of the concentration half inhibitoria of norepinefrina was observed (7,5 x 10^{-7} at 2 x 10^{-5}) of the deferential conduit in presence of 1 mg/mL of the ELMSC in the bathroom of isolated organ; to the doses of 400 and 200 mg/kg the extract caused a significant decrease of the growth prostatic. Our data indicate that the ELMSC to dose bigger than 200 mg/Kg inhibits the growth prostatic induced by the testosterone in the experimental pattern of prostatic hyperplasia in rats and it presents activity antagonistic alpha adrenergic in the pattern of isolated organ of deferential conduit of rat to the concentrations of 1 and 3 mg/mL.

Key words: Benign prostatic hyperplasia, Cucurbita pepo L.

P300042

Regulation of glucose transport in L8 muscle cells by Lagerstroemia speciosa leaves.

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The leaf of plant of Lagerstroemia speciosa L. (LS) is used as an antidiabetic herbal remedy in many countries. In an attempt to discover mechanisms of action of the LS watery extract (WE) that stimulate glucose uptake, a cell based radioactive assay of glucose uptake was performed using L8 cells. Glucose uptake into

L8 myotubes was observed in long-term treatment of WE in a dose-dependent manner. The WE stimulation was slightly inhibited by SB203580. The inhibitory effect of wortmannin on WE-stimulated glucose uptake was demonstrated suggesting the WE action on glucose transporters translocation. WE-induced glucose uptake was completely reversed by cycloheximide. In addition, increased amount of total glucose transporter 1 protein content was observed indicating that the new protein synthesis is necessary for elevated glucose transport. WE also potentiated insulin-stimulated glucose transport. These results suggest that WE action is mediated primarily via the synthesis of new transporters and involving insulin-dependent and independent pathways.

Key words: L8 myotube, glucose uptake, Lagerstroemia speciosa.

Acknowledgement: This work was supported by a grant from Prince of Songkla University.

P300043

Effect of Herba houttuyniae extract on lipopolysaccharide-induced lung inflammation in mice

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Objective: To study the effect of Herba houttuyniae extract on lipopolysaccharide (LPS)-induced lung inflammation in mice. Methods: stablish a mouse model of lung inflammation by nose injection with LPS (6 mg/ml, 10 mg/kg, 3d). Results: ① Total leucocytes in BALF: the model showed significantly more total leucocytes than the normal group did, while groups of both Herba houttuyniae extract 200 mg/kg and 400 mg/kg showed significantly less than the model group did; ② Lung pathological observation after being stained with HE: the model showed severer lung inflammation than the normal group and drug-given groups did. ③ Immuohistochemisty analysis: TLR_4 (Toll like receptor 4) was expressed both in bronchus and bronchiole in all groups. TLR_4 in the model had higher expression than that in the normal group; Herba houttuyniae extract could not reduce TLR_4 expression. Conclusions: ① Nose injection with LPS can establish the mouse model of lung inflammation. ② Herba houttuyniae extract may alleviate lung inflammation, and reduce the infiltration of inflammatory cells; the minimum acting dose is 200 mg/kg; ③ The anti-inflammation mechanism of herba houttuyniae extract can not conduct through TLR4 signal transduction pathway.

Key words: Herba houttuyniae extract; lipopolysaccharide; lung inflammation; toll-like receptor 4

P300044

Effect of Ganoderma lucidum extracts on cytochrome P450 content, CYP2E1 and CYP1A2 activity in BCG immune hepatic injury in rodents

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Objective: To investigate the effect of Ganoderma lucidum (GL) extracts on cytochrome P450 metabolic activity in immune hepatic injury in rodents. Methods: Liver injury was induced by Bacille Calmette Guerin (BCG, 125 mg/kg, i.v.) in rodents. GL-S (sterol, 20, 40, 80 mg/kg) was oral dosed in mice in vivo and GL-PS (polysaccharide, 50, 100, 400, 800 μg/ml) was co-incubated with rat microsome in vitro. Alanine aminotransferase (ALT) level and CYP450 content were determined by spectrophotography. CYP2E1 and CYP1A2 activity was assessed by the levels of probe drug chlorzoxazone and phenacetin in microsome using HPLC. Results: After stimulation of BCG, the serum ALT level was increased, but CYP450 content and CYP2E1 activity were decreased significantly ($p < 0.05$). Administration of GL-S partly reversed the effects of BCG on ALT level, CYP450 content, and CYP2E1 activity in vivo. Both CYP2E1 and CYP1A2 activity were decreased by GLPS in vitro. Conclusion: This result suggested that GL extracts improved the BCG-liver injury in vivo, and inhibited CYP2E1 and CYP1A2 activity in vitro, which might be contributed to toxic xenobiotic metabolism.

Key words: Ganoderma lucidum extracts, CYP450, immune liver injury

P300046

Study on Safflor Yellow and Hydroxysafflor Yellow A to alleviate rat myocardial ischemia and mitochondria damages

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Safflor Yellow (SY) is the main component of Carthamus tinctorius L. and Hydroxysafflor yellow A (HSYA) is the main ingredient of SY. The effects of HSYA to inhibit rat myocardial Mitochondria (Mt) damages and SY to alleviate rat myocardial ischemia triggered by isoprenaline (ISO) were observed. Its ventricular ATP and malonyldialdehyde (MDA) content were determined. Its plasma free fatty acid (FFA) level was assayed. Mt swelling, Mt membrane fluidity and Mt MDA after lipid peroxidation were determined. The result of in vivo test showed that ventricular MDA contents or plasma FFA of myocardial ischemic rat increased and its ventricular ATP decreased. There were some ischemic changes in electrocardiograph results (P all < 0.05). These injuries can all be alleviated by SYip (P all < 0.05). From above tests the Mt swelling, Mt membrane fluidity decrease and Mt MDA elevation were remitted apparently treated with HSYA (P all < 0.05). It was shown in the results that SY was effective to alleviate rat myocardial ischemia and HSYA was effective to inhibit rat myocardial Mt damages.

The project was sponsored by National Natural Science Foundation of China No.30171146.

Key words: SY; HSYA; Myocardial ischemia; Mt damages

P300047

Inhibitory inflammation effects of Chamaecyparis leaf extracts through a nitric oxide (NO) blocking pathway

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Inflammation is a host response to tissue injuries and is characterized by movement of leukocytes. Bacterial lipopolysaccharide (LPS)-induced NO production in macrophage has been used as a screen method for anti-inflammatory components. Extraction of Chamarcyparis leaf was used to investigate the possibility of antiinflammation activity.

Chamaecyparis leaf were extracted by water then eluted with methanol through a Sephadex LH-20 column. Seven different fractions were collected for study. Indomethacin (0.25 mM) was used as a positive control. RAW246.7 cells were stimulated in the presence of LPS (1 μg/ml) with or without the extracts. NO production was measured as nitrite (suing Griess reagent), iNOS protein and mRNA were also investigated using western blotting and RT-PCR.

In the concentration ranges that were devoid of cytotoxicty, Chamarcyparis leaf extracts fraction 4 produced a dose dependent inhibition in LPS-induced NO production. Protein expression of iNOS was also blocked by the extracts. This study shows the extracts of Chamaecyparis leaf effectively block LPS-induced NO production, is through blockage of expression of iNOS.

Key words: Inflammation, iNOS

P300048

Prevention of short UV wave-induced Caspase 3 activity by water extract of Chlorella in human skin fibroblast

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Short wave of UV light is known to possess higher energy than long wave to penetrate materials and cause damage to skin. Cell damage caused by UV radiation can lead to cell death and it is also believed that this damage is due to oxidative damage. Administration of Chlorella has been shown to play some biochemical functions. However, the real effects of extract of Chlorella on skin protection have not been studied. Aims of the study were to investigate whether the Chlorella extract can protect skin cells from UV damage and the underlying mechanism. Human skin fibroblast cells were treated with WEC257, Vitamin C, or Vitamin E. The cells were then exposed to UV (254 nm) for 30 min for 2 consecutive days. After the second UV exposure, cell proliferation was measured 1, 24, 48 and 72h later. Caspase 3 activity was assay 1h after second UV exposure. UV exposure caused cell death except in extract of Chlorella (WEC257) treated cells. Caspase 3 activity was lower in WEC257 treated cells than other groups after UV exposure. This study shows that treatment of WEC257 has cell-protection from UV radio-hazard, which may be due to decrease caspase 3 activity.

Key words: UV exposure, caspase 3 activity.

P300049

Smiglaside E enhances the efficacy of etoposide on B16F1 Cells via cell cycle arrest

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The effect of smiglaside E, a new phenylpropanoid glycoside, isolated from the rhizome of Smilax glabra on B16F1 cells response to etoposide was investigated in

vitro. In the present study, we demonstrated that sminglaside E combined with etoposide directly inhibited the proliferation of B16F1 cells in a dose-dependent manner. Then sminglaside E significantly enhanced etoposide-induce apoptosis, was measured by Annexin V/PI stain and caspase-3/7 activity assay. At the same time, the mitochondria transmembrane potential of B16F1 cells treated with etoposide plus sminglaside E, was also synergistically decreased than treated respectively. Furthermore, sminglaside E combined with etoposide dose-dependently increased the percentage of B16F1 cells in G2/M stage, meanwhile, the expression of phospho-cdc2 was downregulated and the expression of Bax and Bad were upregulated. These results suggested that sminglaside E significantly promoted the efficacy of etoposide on B16F1 cells through enhancing the apoptosis and inducing the arrest in G2 phase of B16F1 cells.

Key words: sminglaside E, etoposide, cell cycle, apoptosis

Acknowledgement: Supported by NNSF (Nos. 30300425 and 30500619).

P300050

Effect of pretreatment with *Mucuna pruriens* **seed extract on the pharmacological effects of** *Naja naja sputatrix* **(Malayan cobra) venom in rats**

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We examined the effect of pretreatment with *Mucuna pruriens* seed extract (MPE) on the pharmacological effects of *Naja naja sputatrix* venom in rats. Changes in the systemic blood pressure (BP), heart rate (HR), respiratory rate (RR) and gastrocnemius muscle contractions were monitored simultaneously for 5 h using anaesthetised rats (n = 9 per group), with and without pretreatment with MPE (21 mg/kg body weight, i.p., once weekly for 3 weeks), following a challenge by the venom (0.45 mg/kg, i.v.). Pretreatment with MPE significantly ($p < 0.01$) attenuated the depression effect of the venom on the BP, HR and RR of rats. At the end of 5 h, the BP, HR and RR were 7.1 ± 7.1 mmHg, 47 ± 47 bpm and 10.0 ± 10.0/min in the control rats, and 74.6 ± 12.8 mmHg, 283 ± 52 bpm and 88.3 ± 13.3/min, respectively, in the treated rats. However, there was no significant difference between the muscle twitch tension of control and treated rats (25.0 ± 10.1% and 47.2 ± 9.7% of pre-dose tension, respectively, at 5 h). In conclusion, pretreatment of rats with MPE can protect against the respiratory and cardiovascular depressant effects of *Naja naja sputatrix* venom in rats. This protective effect may be immunologically mediated.

P300051

Vasodilatation induced by the aqueous extract of Juliana adstringens on rats' aorta.

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Juliana adstringens (JA) is a plant native to central and southern Mexico, its name in Nahuatl is cuachalalatl. The cortex and roots had been used in traditional medicine as antiseptic in skin damage, to harder the gum of the mouth, and for gastric ulcer. A methandic extract of the stem bark of JA shows an inhibitory effect of gastric ulcers in rat (1). We studied isometric recordings in organ baths of aortic rings from male rat, with and without endothelium, exposed to JA aqueous extract (20%) from the cortex of the plant stem. JA showed a dose dependent contraction, on aortic rings with and without endothelium. The concentration-response curve to norepinephrine (NE) was shifted to the right in presence of JA. The addition of JA, induced relaxation on NE precontracted aortic rings with endothelium. JA inhibited the relaxation induced by Ach (10μM) on NE precontracted aortic rings with endothelium. Our results suggest that the relaxation induced by JA on NE precontracted aortic rings, could be mediated by nitric oxide and the contraction induced by JA is independent of the endothelium.

P300052

Promising role of a plant extract (TChi-2) in the post-treatment of LPS-induced acute lung injury in the rat

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Acute respiratory distress syndrome (ARDS) is a devastating clinical problem. It is caused by excessive secretion of proinflammatory and inflammatory mediators, resulting in diffuse alveolar damage, disruption of alveolar epithelium, and capillary injury. The aim of this study was to assess possible role of a purified plant extract (TChi-2) in treatment of lipopolysaccharide (LPS)-induced acute lung injury in urethane anesthetized male Sprague-Dawley rats. 24 hrs after its application, LPS (10 mg/kg, iv) significantly decreased white blood cells, elevated plasma tumor necrosis factor-α, and thickened interalveolar septa in lung. These changes were prevented by TChi-2 (15 mg/kg, iv or 30 mg/kg, ip), administered one and six hr after LPS-challenge. These treatments also caused significant attenuation of LPS-induced increase in plasma NO, and inhibition of LPS-induced iNOS expression, phosphorylation of IκB (an inhibitor of NF-κB), and activation of NF-κB in lung. These results suggest a promising role of TChi-2 in treating LPS-induced acute lung injury (supported by National Science Council, NIH HL27763 and HL47574, Tzu Chi Foundation, & So. Ill. Univ).

Key words: natural product, LPS, ARDS

P300053

Effect of acidic oligosaccharide sugar chain (AOSC) on H_2O_2-induced apoptosis in SH-SY5Y cells and its related mechanism

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Objective: In this paper, we investigated the action mechanism of AOSC on the apoptosis in SH-SY5Y cells induced by H_2O_2. Methods: We observed the effects of AOSC on the neurotoxicity and apoptosis induced by H_2O_2. Then the effects of AOSC on the concentration of $[Ca^{2+}]i$, the mitochondrial membrane potential (MMP) and the expression of P53, Bcl-2 and Caspase-3 were determined by flow cytometry and immunofluorescence stain. Results: We found that AOSC inhibited the elevation of malondialdehyde. AOSC inhibited the apoptosis mediated by H_2O_2 by suppressing the overload of $[Ca^{2+}]i$ concentration and the decrease of MMP. Furthermore, AOSC down-regulated the expression of P53 and Caspase-3 and up-regulated the expression of Bcl-2, indicative of the underlying mechanism of AOSC on the apoptosis induced by H_2O_2. Conclusion: Therefore, our results suggested that AOSC might be a potentially anti-oxidative.

Key words: AOSC, H_2O_2, Apoptosis, Mechanism

Acknowledgements: This work was financially supported by the high-tech '863' project (2001AA624140) and by a National Basic Research Program Grant (2003CB716400).

P300054

Protection of amyloid beta protein (25-35)-induced neurotoxicity by methanol extract of Smilacis chinae rhizome in cultured rat cortical neurons

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The present study aims to investigate the effect of the methanol extract of Smilacis chinae rhizome (SCR) from Smilax china L. (Liliaceae) on amyloid beta protein (Ab) (25-35), a synthetic 25-35 amyloid peptide, -induced neurotoxicity in cultured rat cerebral cortical neurons. Ab (25-35) (10 uM) produced a reduction of cell viability, which was significantly reduced by MK-801, an N-methyl-D-aspartate (NMDA) receptor antagonist, verapamil, an L-type Ca^{2+} channel blocker, and NG-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor. SCR, over a concentration range of 10-50 ug/ml, inhibited 10 uM Ab (25-35)-induced neuronal cell death, which was measured by an MTT assay and Hoechst 33342 staining. SCR (50 ug/ml) inhibited 10 uM Ab (25-35)-induced elevation of cytosolic calcium concentration ($[Ca^{2+}]c$), which was measured by a fluorescent dye, Fluo-4 AM. Pretreatment of SCR (10 and 50 ug/ml) also inhibited glutamate release into medium induced by 10 uM Ab (25-35), which was measured by HPLC, generation of reactive oxygen species and activation of caspase-3. These results suggest that SCR prevents Ab (25-35)-induced neuronal cell damage in vitro.

P300055

Effect of extract from two kinds of Chinese Medicinal herbs on the learning and memory ability of mice model induced by scopolamine

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To investigate the effect of root extraction of polygonum multiflora m (TSG) and fruit extraction of cornus officinalis (SSY - B2) on mice model induced by scopolamine. Methods: Mice were randomly divided into normal group, model group, TSG groups (0.03g/kg/d, 0.1g/kg/d, 0.3g/kg/d respectively), SSY - B2 groups (1.78g/kg/d, 5g/kg/d, 15g/kg/d respectively), and Piracitam group (0.7g/kg/d), and d. Drugs were intragastrically administrated for continual 5 days. On Day 5, except for normal group, mice of other groups were administrated with scopolamine by intraperitoneal injection (1mg/kg). 20 min later, all mice were subjected to Morris water maze test. The animals were killed 5 days later and brains were taken to assay M - cholinergic receptor binding. Results: Compared with model group, swimming time searching for target was less significantly in normal group, Piracitam group, TSG (0.03g/kg) and SSY - B2 (5g/kg/d, 15g/kg/d). TSG and SSY - B2 increased M - cholinergic receptor binding of model mice in brain. Conclusion: TSG and SSY - B2 can effectively improve the learning and memory ability of mice induced by scopolamine, possible related to the improvement of M - cholinergic receptor binding in brain.

P300056

VASORELAXANT EFFECT OF A BUTANOLIC FRACTION OF GYNURA PROCUMBENS

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The present study was conducted to evaluate the vasorelaxant activity of a butanolic fraction (BU) obtained from the leaves of Gynura procumbens and to elucidate the underlying mechanisms involved. In isolated rat thoracic aorta preparations, BU (10^{-6} - 10^{-1} g/ml) caused a concentration - dependent relaxation in endothelium - intact or - denuded aortic rings precontracted with phenylephrine (PE, 10^{-6} M) or KCl (80 mM). The BU fraction also inhibited the PE (10^{-9} - 3×10^{-5} M) - or KCl (10 - 80 mM) - induced contractions in a concentration - dependent manner in aortic rings with and without endothelium. Furthermore, the Ca^{2+} - induced vasocontractions were antagonised by BU both in a medium containing no Ca^{2+} but high K^{+} (60 mM), as well as in a medium that contains PE but without Ca^{2+} or K^{+}. However, contractions induced by noradrenaline (10^{-6} M) or caffeine (45 mM) were not affected by BU. These results demonstrate that the vasorelaxant properties of BU may act by inhibiting the influx of Ca^{2+} through receptor - operated and/or voltage - dependent calcium channels.

Key words: Gynura procumbens Vasorelaxation Calcium channels
Funding: MOSTE, Malaysia (Project No. 06 - 04 - 01 - 0115) and University of Malaya Vote F.

P300057

Green tea polyphend (-) - Epigallocatechin - 3 - gallate Inhibits Rat Vascular Smooth Muscle Cell Adhesion and Migration on laminin

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Smooth Muscle Cells (SMCs) play an important role in the development of atherosclerosis and restenosis after angioplasty and coronary bypass grafting. (-) - Epigallocatechin - 3 - gallate (EGCG) has been shown to have antiproliferative activity on SMCs through the inhibition of PDGFR activation. However, little attention has been paid on its effect on SMC adhesion. In the present study, the effect of EGCG on rat aortic SMC (A10 SMC) adhesion and migration was investigated. We demonstrated that A10 SMC adhesion to collagen and laminin was inhibited by EGCG but not by (+) - catechin. Our results showed that EGCG not only binds directly to laminin but also affects SMC's binding affinity. Further analysis showed that beta1 integrin expression on SMCs and SMC adhesion to immobilized integrin beta1 antibody were both reduced by EGCG treatment. In parellel, EGCG treatment also inhibited spontaneous and PDGF - induced SMC migration toward laminin. Taken together, we provided here the first evidence that EGCG can affect SMC adhesion and migration on laminin, possibly acting through binding to laminin and reducing the interaction between beta1 integrin and laminin.

Key words: adhesion, smooth muscle cell, EGCG

P300058

The effect of Zingiber extract on Creatinine and Blood Urea Nitrogen (BUN) of mice

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Zingiber officinale is a spice that has been used from two thousand years ago as a medicine in several Asian countries. To investigate the effect of Zingiber extract on Creatinine and blood urea nitrogen (BUN) and there for estimate Renal function. A extract of ginger was used every 48 hours/20 days, IP to male mice. The blood was used to investigate Blood urea nitrogen (BUN), Creatinine, Uric Acid. Low dose of ginger (10 mg/kg) shown significant difference in lowering BUN levels when compared with control animals. Middle dose of ginger (20 mg/kg) administered IP shown significant difference in lowering BUN levels when compared with control animals. High dose of ginger (40 mg/kg) were significantly effective in lowering serum BUN. No significant changes in serum Creatinine levels were observed upon administration of either the low or high dose of ginger. BUN/Creatinine Ratio show significant changes in all doses of ginger when compared with control animals. This indicated that with regards to the results ginger could be useful for changing blood urea nitrogen and Creatinine to gain normal body balance.

P300059

Vasodlation by Samhwangsasim - tang, a herb medicine, is associated with inhibition of Rho kinase

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Samhwangsasim - tang (SST) is a widely used herb medicine with vasodlatory actions. We hypothesized that SST modulates vascular contraction through inhibition of Rho kinase. SST inhibited vascular contraction in an endothelium - independent manner. Methylene blue, an inhibitor of guanylyl cyclase, did not affect the inhibitory action of SST. SST decreased vascular tension induced by 55 mM KCl, 1.0 μM phenylephrine, or 8 mM NaF, but not by 1.0 μM phorbol dibutyrate. SST also decreased the level of phosphorylation of MLC_{20} and MYPT1 induced by 8 mM NaF. These data suggests that SST has a vasodlatory action through inhibition of Rho kinase.

Key words: Samhwangsasim - tang, contraction, Rho kinase, herb medicine
This study was supported by a grant of the Oriental Medicine R&D Project, Ministry of Health & Welfare, Republic of Korea (B05 - 0042 - A.M0815 - 05N1 - 00030B).

P300060

Antinociceptive effect of an alcod - free extract obtained from skin of a vinifera grape (Vitis labrusca).

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Polyphenols possess a multitude of biological activities, including antihypertensive, vasodilation, antioxidation and inhibition of platelet aggregation, that are dependent on nitric oxide release, a compound that modulate nociceptive reaction, therefore a antinociceptive effect of a grape skin extract (GSE), rich in polyphenols (Soares de Moura et al., Antihypertensive, vasodlator and antioxidant effects of a vinifera grape - skin extract. J. Pharm Pharmacology.;54:1515 - 20;2002) was investigated in rodents. The antinociceptive effect of GSE was evaluated in mice (hot plate and writhing tests) and rats (formalin test), pretreated with saline, 7 - nitroindazol, yohimbine, scopolamine, naloxone and glybenclamide. A comparative study was also performed with dipyrone. GSE (ip or orally) induced a dose - dependent antinociceptive effect in all tests. The antinociceptive effect of GSE was not dependent on activation of NOs, α_2, muscarinic or opioid receptors, but was significantly reduced by glybenclamide. The antinociceptive effect of GSE was significantly higher that dipyrone. Our results suggest that the antinociceptive effect of GSE is probably related to activation of KATP dependent channels.

P300061

(-) - epicatechin - 3 - gallate, a green tea polyphend, is a potent agent against UVB - induced damage

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(-)-epicatechin-3-gallate (ECG) is a polyphenolic compound similar to (-)-epigallocatechin-3-gallate (EGCG) that is abundant in green tea. Numerous workers have proposed that EGCG protects epidermal cells against UVB-induced damage. However, little has been known whether ECG protects keratinocytes against UVBinduced damage. In this study, we found that ECG dose-dependently attenuated UVB-induced keratinocyte death as determined by cell viability assay. The mechanisms of action of ECG were further vertified. As assayed by flow cytometry and colorimetry, UVB-induced H_2O_2 generation in keratinocytes was inhibited by ECG, suggesting that ECG can act as a free radical scavenger while keratinocytes were photodamaged. The scavenging effect of ECG was confirmed by that ECG treatment attenuates H_2O_2-induced cell damage. In the parallel experiment, UVB- and H_2O_2-induced the activation of extracellular signal-regulated kinase (ERK) and c-jun-NH2 terminal kinase (JNK) in keratinocytes could be inhibited by ECG. Taken together, we provided here the action mechanisms that ECG protects keratinocytes from UVB-induced photodamage.

P300062

Involvement of proteasome inhibition on anticancer activities of Rhabdastrellic acid A

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Rhabdastrellic acid A is an isomalabaricane-type compound isolated from the genus Rhabdastrella of marine sponges. It has been well known that apoptosis induction and cell cycle arrest are typical biological effects observed in cancer cells after proteasome inhibition. Here, we reported that Rhabdastrellic acid A strongly reduced the proliferation rate of several human tumor cell lines in vitro. Meanwhile, Rhabdastrellic acid A arrested human leukemia HL60 cell line at G_2/M phase of cell cycle and induced apoptosis in a dose and time-dependent manner. The inhibitory effects on proteasomal chymotrypsin-like and trypsin-like activities were determined in vitro and in HL60 cells using specific fluorogenic peptides. Furthermore, the turnover of the cyclin-dependent kinase inhibitor $p21^{waf/cip1}$, a sign of deregulation of cell cycle progression and apoptosis induction by classical proteasome inhibitors, was disrupted. In addition, the ploy-ubiquitin protein was accumulated in presence of Rhabdastrellic acid A. Our results indicated that the inhibitory effect of Rhabdastrellic acid A on proteasome activities be involved in cell proliferation, cell cycle blockage and apoptosis induction in HL60 cells.

Key words: Rhabdastrellic acid A; cell cycle; apoptosis; proteasome

P300063

NMR and Pharmacologic Studies on the New Melanin from Bacillus thuringiensis

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The pure water soluble bacterial melanin was examined for its drug interacting characteristics by 400 MHz NMR spectroscopy. In D_2O buffer 1-3 mM melanin showed characteristic signals at 0.7-2.5 ppm attributed to aliphatic groups and dense overlapping signals at 2.5-4.5 ppm arising from heteroaliphatic groups. When ephedrine or atropine 3 mM was combined with the melanin, the spectral characteristics of the pigment or the aromatic or N-methyl group signals of the drugs were not altered. This finding is in contrast to that reported for L-dopa melanin. The pigment lacks significant interaction with the drugs, perhaps due to low content of paramagnetic centers. Pharmacologic evaluation of the melanin (0.3 mg/ml) on the isolated frog skeletal muscle, rat aorta, vas deferens, guinea pig ileum, tracheal smooth muscle and heart, did not interfere with the activities of agonists. On the ileum, however, the melanin produced small contraction. Thus physical and physiological properties of two types of melanins differ. More investigations on the bacterial melanin are needed.

Key words: Bacterial melanin, drug-melanin interactions, Bacillus thuringiensis

P300064

Cardiotoxic actions of Struthanthus venetus on guinea pig.

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The herb Struthanthus venetus (Sv), is used in traditional medicine to treat cough. Methandic extract of Sv leaves induces in rat a decrease in blood pressure. The aim of this work was to study the action of methanolic extract of Sv on guinea pig heart. All experiments were performed on Hartley male guinea pig (300-400 g). Electrocardiographic records (EKG) were done from the standard limb leads DI, DII, DIII, AVR, AVL and AVF before and after 50 mg Sv extract I.P. EKG was recorded at different minutes or days after Sv, EKG showed ST segment elevation, T wave inverted and Q waves in some leads. At the end of experiments the heart was obtained and prepared for histological study. This showed small size of the ventricles, pale infarct, edema of interventricular septum, necrosis of heart muscle fibers, karyorrhexis, hyperchromatic nuclei, microvacuoles within muscle fibers, collection of nuclei muscle fibers, and empty fibrovascular stroma. Our results show that Sv has cardiotoxic actions on guinea pig heart.

P300065

Effect of Shen-wu capsule and tetrahydroxystilbene glucoside on aged rats

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Objective: To observe the effect of Chinese medicine Shen-wu capsule and its effective component tetrahydroxy stilbene glucoside (TSG) on aged rats. Methods: SD rats were studied: aged 1, 3, 6, 18, and 24 months. The 24-month-old were divided into 5 groups: control, Shen-wu capsule (0.8 and 1.6g/kg/d), TSG (0.03 and 0.06g/kg/d), intragastrically 3 months. All rats were done behavior test (water maze). Hippocampal ultrastructure was observed. Results: 6-month-old rats had best learning and memory ability. The observation of hippocampal synapses showed that they were most numerous at 6-months-old, and clearly diminished at 24 month-old. Shenwu capsule and TSG can improve the learning and memory ability in aged rats. Conclusion: Learning and memory ability of rats shows increasing improvement from birth, reaching a peak at 6-month-old, declining to that of a 1-month-old rat at 24-month-old. The changes are related to hippocampal synaptic development. Shen-wu capsule and TSG can improve the learning and memory ability in aged rats.

P300066

In vivo and vitro antiviral activity of hyperoside extracted from Oenanthe javanica

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The present study usd the models of human hepatoma Hep G2.2.15 cell culture system and duck hepatitis B virus (DHBV) infected duck. Result: the 50% toxic concerntration (TC_{50}) of hyperoside was 3.90g/L, the maximum nontoxic concerntration (TC_0) was 2.00g/L. In nontoxic concerntraions, hyeroside significantly inhibited HBSAg and HBeAg in 2.2.15 cells after 8 days of treatment ($P < 0.01$, $P < 0.05$). Furthermore, at the maximum nontoxic concentration, hyperoside had an inhibition rate 64.4% on HBV-DNA of 2.2.15 cells on day 8. In the DHBV infection model, the DHBV-DNA levels decreased significantly in the treatment 0.05 g/kg and 0.10 g/kg dosage groups of hyperoside ($P < 0.01$). The inhibition of the peak of viremia was maximum at a dose of 0.10 g/kg and reached 60.79% on day 10 and 69.78% on day 13, respectively. Conclusion: These results suggested that hyperoside is a strong inhibitor of HBsAg and HBeAg secretion in 2.2.15 cells and DHBV-DNA levels in the HBV-infected duck model.

P300067

Isolation of cell cycle G_2/M arrest related differentially expressed genes induced by diallyl disulfide in human leukemia cell HL-60

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Diallyl disulfide (DADS), an oil-soluble allyl sulfur compound found in garlic,

was found to inhibit the growth of various tumors. Our previous studies showed that DADS induced the arrest of HL-60 cells in the G_2/M. The aim of our study was to isolate G_2/M arrest related genes in HL-60 treated with DADS using suppression subtractive hybridization (SSH). To construct SSH library of HL-60 using the mRNA from HL-60 cell treated by DADS and the HL-60 cell as tester and driver, respectively. Positive clones in the library were selected randomly, the sequences of cDNA fragments were analyzed and compared with that in GenBank. The SSH library contained about 220 positive clones. Random analysis of 57 clones with PCR demonstrated that 51 clones contained inserted fragments. The 51 clones were sequenced and BLAST analysis was conducted, 6 clones are shown to be novel ESTs, and were registered in GenBank. 6 novel gene fragments were isolated by the SSH, and it provided the basis for further cloning their full-length and studying their functions.

Diallyl disulfide (DADS); Suppression subtractive hybridization; G_2/M arrest related genes.

This work was supported by the National Natural Science Foundation of China (30270684).

P300068

Comparative study of two hemp genotypes grown under the same conditions - interaction of plant infusions with chlorpromazine in rats

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The aim of this work was to study the interaction of infusions of industrial hemp of genotype "Novosadska" (NK) and Indian hemp genotype "VIR SK" (VK) grown on the experimental field in Backi Petrovac (Serbia and Montenegro) with chlorpromazine that influence body temperature in rats, compared with control animals that drank water. The infusions were prepared daily by pouring 2.2 g of crushed leaves, twigs and flower clusters of hemp with 1 l of boiling water. The infusions were prepared daily by pouring 2.2 g of crushed leaves, twigs and flower clusters of hemp with 1 l of boiling water, whereby animals drank VK or NK infusions instead of water for 20 days. Infusion VK caused an increase in basal temperature of rats with respect to control, whereas NK infusion showed no any effect. None of the infusions showed significant interaction with chlorpromazine in its hypothermic action.

Key words: Hemp, chlorpromazine.

The authors acknowledge financial support of the Ministry of Science and Environmental Protection of the Republic of Serbia.

P300069

Wound healing activity of Achyranthes aspera.

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Achyranthes aspera, (Apamarga) a stiff herb, found in India is a much valued indigenous medicine. No scientific research has been carried out on its wound healing activity. The wound healing activity of the plant was studied using excision wound model. Gr.I served as control (vehicle treated), Gr.II, 5% ointment of Achyranthes aspera, GrIII with Himax (standard) applied topically daily (0-28 day) from day of post wounding. The pc of wound closure, gross, histopathological? study? carried out on day 7, 14, 21 and 28. Complete closure of wound (100%) observed on day 21, in control (81.02%) and standard (91.70%). Histological studies of granulation tissue revealed increased fibroblasts with thick bundles of collagen on day 21 in the Achyranthes aspera. In the control group, healing did not take place till 28th day post wounding. Significant reduction in scar area, faster epithelization, no bacterial colony observed in the treated group. Further study is in progress in the incision wound model to determine the tensile strength of the healing tissue. Thus the study provides pharmacological evidence on the folkloric use of Achyranthes aspera for its wound healing property.

Key words: Wound healing, collagen, granulation.

Acknowledgment: The first author is thankful to LSRB, Ministry of Defence, Govt. of India, New Delhi for providing financial assistance.

P300070

Effect of active ingredient of Salvia Miltiorrhiza on morphine induced conditioned place preference in mice

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Aim To investigate the effect of active site of lipid soluble Salvia Miltiorrhiza on morphine induced conditioned place preference in mice and identify the active site of Salvia Miltiorrhiza preliminary. Methods Morphine or NS was injected (sc) on alternate days to induce the obvious place preference in mice for 6 days. Mice were administered (ip) the different doses of active site of lipid soluble Salvia Miltiorrhiza and the major element of active site of lipid soluble Salvia Miltiorrhiza was identified by RP-HPLC. Results After treatment with active site of lipid soluble Salvia Miltiorrhiza (40 mg·kg^{-1}, ip), the time staying in morphine-paired white compartment were significantly reduced. The major element of active site of lipid soluble Salvia Miltiorrhiza was identifed with cryptotanshinone by RP-HPLC. Conclusion Cryptotanshinone could restrain the acquisition of morphine induced conditioned place preference in mice to a certain extent, and itself did not display psychic dependence in the experiment.

Key words: Cryptotanshinone; Salvia Miltiorrhiza; Morphine dependent

P300071

Protective Effects of Sasanquasaponin on Injury of Endothelial Cells Induced by Anoxia and Reoxygenation in vitro

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The present study attempted to observe the effects of sasanquasaponin (SQS) on anoxia and reoxygenation (A/R) injury and investigate its possible mechanism. Human umbilical vein endothelial cells (HUVECs) were exposed to normoxia or A/R in the absence or presence of SQS (10.0, 1.0, 0.1 μmol·L^{-1}). Activity of lactate dehydrogenase (LDH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and the content of malondialdehyde (MDA) were determined. Additionally, neutrophil adhesion to HUVECs was assayed colorimetrically. The results showed that SQS decreased the LDH activity and MDA contents, inhibited the neutrophil adhesion to HUVECs, whereas increased the mitochondrial SOD and GSH-Px activity in a concentration-dependent manner. It is suggested that SQS could protect HUVECs against A/R injury, and the protective mechanisms appear to be related to anti-lipoperoxidation and anti-adhesion.

Key words: Sasanquasaponin; Ischemia and reperfusion; Endothelial cells; Adhesion

Acknowledgement: This work was supported by a grant from the Natural Science Foundation of Jiangxi Province (№: 0140030), P.R. China.

P300072

Delayed protection of tetramethlpyrazine on rat cardiomyocytes subjected to anoxia reoxygenation injury

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Aim: To investigate the delayed protection of tetramethlpyrazine (TMPZ) preconditioning on rat cardiomyocytes subjected to anoxia-reoxygenation (A/R) injury and its dose-, time-effect relationship. Methods:

The primary cultured neonatal rat cardiomyocytes were preconditioned using TMPZ 25, 50, 100, 200, 400, 800 umol/L for 3 hours and subjected to A/R injury after 12, 24, 48 hours respectively. Viability and ultrastructure of myocytes, the activity of LDH in medium were measured to determine the protective effects against A/R injury.

Results: Increased cell viability and decreased LDH release were observed in cardiomyocytes treated with TMPZ. The cellular structures were extremely well preserved with TMPZ. The cardioprotective effects developed within 12 h, maximized at 24h and decreased at 36 h in the optimum concentration 100 umol/L.

Conclusion: TMPZ has a potent delayed cardioprotection and offered more capacity to tolerate the A/R damage at 100 umol/L and about 24 h after preconditioning.

Key word: Tetramethlpyrazine; anoxia-reoxygenation; delayed protection; cardiomyocyte

P300073

Effect of Resveratrol on differentiation of cardiomyoblast

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Resveratrol (trans-3,5,4'-trihydroxystilbene), a polyphendic compound found in the skins of red grapes, an medicine used to treat cardiovascular diseases. Our studies indicated that Reservatrol may place an important role on differentiation of cardiomyoblast. The cardiomyoblast cell line, H9c2, was exposed to 10uM to 100uM Resveratrol for 1 to 4 days. The cell proliferation and cellular damage were assessed by XTT and LDH respectively. The change of cellular differentiation morphology was observed under microscope. Cell cycle analysis was performed by flow cytometry analysis on PI'1 stained H9c2. The results indicated that short term treatment of Resveratrol (2 days) exhibited inhibitory effect on H9c2 proliferation via reversible cell cycle arrest. Treatment with Resveratrol up to 4-5 days induced an obvious differentiation of cardiomyblast into myocyte based on morphological examination. Activation of G1-S checkpoint arrest as well as differentiation of H9c2 was further confirmed by expression markers including MHC, PCNA and Cyclin B. Our results have implication on the role of Reservatrol on cell cycle arrest and differentiation on cardiomyoblast.

Key word; Resveratrol, cardiomyoblast, differentiation

P300074

Inhibitory Effect of Ficus erecta, **Jeju native plant, on the osteoporotic factors** in vitro.

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Osteoporosis is recognized as one of the major hormonal deficiency diseases, especially in menopausal women and the elderly. When estrogen is reduced in the body, local factors such as IL-1β and IL-6, which are known to be related with bone resorption, are increased and promote osteoclastogenesis. In this study, we investigated the anti-osteoporotic activities of Ficus erecta in vitro. MG-63 cells were stimulated with IL-1β (10ng/ml) to induce osteoporotic factors (IL-6 and COX-2) and RAW264.7 cells were stimulated with RANKL (100ng/ml) to induce differentiation into osteoclast. As results, hexane and EtOAc fractions of F. erecta fractions decreased the mRNA expression of IL-6 and the mRNA expression and protein level of COX-2 in a dose-dependent manner. Also, hexane and EtOAc fractions decreased the differentiation into osteoclast of RAW264.7 cell. Theses results suggest that F. erecta may have significant effects on osteoporotic factors and anti-osteoporotic potential.

Key word: Ficus erecta, osteoporosis, IL-6, RANKL.

P300075

Protective effects of Viscum coloratum flavonoids against cardiovascular disease

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The flavonoids extracted from Chinese mistletoe (Viscum album coloratum), has been found to have therapeutic benefit for the treatment of cardiovascular disease recently. The present studies were undertaken to determine the protective effects of flavonoids against cardiovascular disease in vivo. We recorded the survival time lacking of oxygen, and also collected the concentrations of serum lactate dehydrogenase (LDH), malondialdehyde (MDA) and superoxide dismutase (SOD) in myocardial ischemia model. The occurrence time of ventricular prematu re (VP) and incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) had also been observed in rat model of arrhythmia induced by aconitine. The results showed that the survival time lacking of oxygen was prolonged by Viscum coloratum flaonoids (VCF), and the concentrations of LDH, MDA and SOD were altered, too. The occurrence time of VP was delayed significantly by VCF, but the incidence of VT and VF showed a decrease tendency only. It was concluded that VCF increased tolerance to hypoxia, and had protective effects against myocardial ischemia and arrhythmia.

Key words: Viscum coloratum; flavonoid; ischemia; arrhythmia

P300076

Cytotoxic activities of DPE on human cervical adenocarcinoma and ovarian cancer cells by induction of apoptosis

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Indian Mockstrawberrg Herb (IMH), the herb of Duchesnea indica (Andr.) Focke and Duchesnea chrysantha (Zollinger & Moritzi) Miquel, is commonly used to treat cancer in China for centuries. The objective of our study was to demonstrated its anti-cytotoxicity on cancer cells in vitro and elucidate the underlying mechanism. We evaluated the cytotoxic activities of Duchesnea phenolic extract (DPE) using MTT assay, morphological observation, DNA fragmentation by electrophoresis and flow cytometric analysis. The results showed that DPE at 20-160ug/ml for 72h dose-dependently suppressed the proliferation of Hela, skov-3, HEC-1B and BGC-823 ($p < 0.05$). The induction of chromatin condensation appearance, DNA fragmentation, accumulation of sub-G1 phase and S cell cycle arrest in DPE treated Hela and skov-3 cells evidenced that the cytotoxicity is through activation of apoptosis. Taken together, our study suggests that DPE could inhibit proliferation of cancer cell lines via blocking cell cycle in S phase, inducing apoptosis.

Key word: Duchesnea indica (Andr.) Focke; Apoptosis; Cell cycle; Cytotoxic

P300077

Protective Effects of Tetramethlpyrazine Preconditioning Mediated by Up-regulation HSP70 Expression on Isolated Rat Hearts

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Aim: To study the preconditioning effects and mechanisms of tetramethlpyrazine (TMPZ) on isolated rat heart subjected to anoxia-reoxygenation (A/R) injury. Method: Isolated rat hearts were perfused in Langendorff mode, with TMPZ 0.1, 0.2, 0.4 mmol L^{-1} for 15 min, then subjected to A/R injury. Heart rate, coronary flow (CF), left ventricular pressure and its first derivative were recorded. The activities of LDH, CPK in CF solutions, expression of HSP70 of myocardium, the area of myocardial infarction were measured. Results: On the heart subjected to A/R injury, TMPZ 0.1, 0.2, 0.4 mmol L^{-1} preconditioning could make heart functions improved, the activities of LDH and CPK, the area of myocardial infarction decreased, moreover, up-regulated HSP70 expression in a concentration-dependent manner. Conclusion: TMPZ can induce the cardioprotective effects of pharmacological ischemic preconditioning and the mechanisms may be relative with up-regulation of HSP70 expression.

Key word: Tetramethlpyrazine, Ischemic preconditioning, HSP70, Isolated rat heart

P300078

EFFECTS OF HONEY ADMINISTRATION ON LOCOMOTOR ACTIVITY AND SLEEP-WAKE CYCLE IN RATS

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Honey is a natural product of bees, Apis mellifera adansonii (Hymenoptera: Apidae), recognized for medicinal properties since antiquity. Honey has been employed in folk medicine as sedative-anxiolytic, nerve tonic, analgesics among other uses.

We investigated the effects of honey in rats after electrodes implantation for electroencephalogram and electromyogram recordings. Spraque-Dawley rats received intraperitoneal injection of vehicle, and different doses of honey (0.5 or 1.0 g/kg, body weight). Injections were given at dark onset. Sleep-wake activity and locomotor activity were recorded during subsequent 12-h dark and 12-h light period.

Honey significantly decreased wakefulness for 6 h starting during the first hour after dark onset dosedependently.

NREM sleep was concomitantly increased while REM sleep was not greatly affected especially during the first 4-h time interval but it was increased significantly during the second 4 h time interval at a dose of 1.0 g/kg. Honey also decreased locomotor activity during this period.

In conclusion, it is suggested that honey can significantly improve sleep by promoting NREM sleep and REM sleep.

Key words: Honey; NREM; REM; Wake; Locomotion

P300079

Modulation Effects of Ginsenoside Rb1 and Rg1 on Voltage-dependent Calcium Currents in Opioid-dependent Locus Coeruleus Neurons

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Whole-cell calcium currents (ICa) recorded in acutely isolated locus coeruleus (LC) neurons in vehicle-treated and morphine-dependent rats showed that, the high voltage-activated (HVA) ICa, especially the N- and P/Q-type ICa were increased in morphine-dependent LC neurons after withdrawal. Ginsenoside Rb1 and Rg1, two active ingredients from Panax Ginseng, both inhibited the HVA ICa in vehicle-treated and morphine withdrawal LC neurons, and, L-type ICa were more sensitive to Rb1, while N- and P/Q-type ICa were more sensitive to Rg1. However, both Rb1 and Rg1 had no effect on whole-cell Ba2+ currents recordd from the L-, N-, P/Q or R-type calcium channels transiently expressed in HEK293 cells. Further studies showed that pertussis toxin, an inhibitor of Go/Gi protein, virtually eliminated the inhibition effects of Rb1 and Rg1 on HVA ICa in LC neurons, which indicated that both Rb1 and Rg1 might inhibit the HVA ICa through an unknown receptor linked to a pertussis toxin-sensitive G protein.

Key words: Ginsenosides; opicid dependence; voltage-dependent calcium channels.

Acknowledgments: This work was supported by grants from National Natural Science Foundation of China (30100240, 30572362).

P300080

In vitro and in vivo anti-inflammatory effects of alpha-lindenic acid from Actinidia polygama fruits

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The anti-inflammatory effects of alpha-lindenic acid (ALA) obtained from Actinidia polygama fruits were examined. In vivo anti-inflammatory effects of ALA were investigated using carrageenan-induced hind paw edema and acetic acid-induced vascular permeability models. 5 mg/kg of ALA significantly reduced the hind paw edema (70%) and vascular permeability (34%). To investigate the mechanism of the anti-inflammatory action of ALA, we examined the effects of ALA on lipopolysaccharide (LPS)-induced responses in RAW264.7 murine macrophage cell line. Exposure of LPS-stimulated cells to ALA inhibited nitrite and PGE2 productions, and corresponding protein and mRNA expression levels of iNOS and COX-2 enzyme were markedly reduced in a concentration dependent fashion. Furthermore, ALA caused to reduce in p65 protein in the nucleus and phosphorylations of ERK1/2, JNK and p38 MAP kinases. Taken together, these results suggest that the antiinflammatory properties of ALA might be ascribed to inhibition of iNOS and COX-2 expression through the down-regulation of nuclear factor-kB binding activity.

Key words: alpha-linolenic acid; lind paw edema; iNOS, COX-2.

This work was funded by Kyunghee University.

P300081

Toxicological effect of aqueous extract of Swietenia macrophylla (mahogany); Sub-acute toxicity

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Swietenia macrophylla (Meliaceae) has been used as folk medicine in Asia and Amazonian area for treatment of hypertension, diabetes, malaria and skin diseases. The aim of this study was to investigate the toxicological effect of aqueous extract of Swietenia macrophylla using sub-acute toxicity model. Four groups of adult female SD rats were used; one served as control, other groups were administered orally by gavage 0.2, 2 and 5 g/kg as a single dose/day of the aqueous extract for seven consecutive days. The food consuming rate and weight of rats were monitored in 1st, 3rd and 7th day during the experiment. The weight of organs (liver, kidneys, spleen, heart and lung) was determined and compared to the control group at day seven. The hepatocytes were isolated and the viability test of hepatocytes was conducted. As a result, no effect was noticed on food consuming, body weight of rats and no significant differences were noticed between treated rats and control for weight of organs and viability test of hepatocytes of rats. In conclusion, results from sub-acute toxicity revealed the safety of the aqueous extract of Swietenia macrophylla.

Key words: Swietenia macrophylla, hepatocytes, sub-acute toxicity.

P300082

Natural product complex CFX suppress the tumor growth and metastasis by regulating Th1/Th2 Polarization

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Polarization of Th1/Th2 responses is important in anti-tumor immunity. CFX is a significant immune modulator that promotes a shift of Th1/Th2 balance toward Th1 response. We wonder if CFX has anti-tumor effects and the underlying mechanisms. We used an experimental cancer lung metastasis of melanoma B16F10 cells and a tumor growth model of Lewis lung carcinoma. Mice received daily CFX orally for indicated time. Biochemical and immundlogical changes associated with the anti-tumor effects were investigated using flow cytometry, RT-PCR, and ELISA. CFX significantly inhibited tumor growth and metastasis in a dose-dependent manner. CFX markedly induced expression of Th1 cytokines and TLR4, decreased Th2 cytokines and regulatory T cytokines (IL-10 and TGF-β) expression in tumor tissues. Proliferation of CD8+ T lymphocytes was monitored in the blood, and decreasing Tregs recruitments in tumor tissues. These results indcate that CFX can induce stimulation of significant anti-tumor responses by promoting a shift of Th1/Th2 balance toward Th1 dominant response. Activation of TLR signaling may be responsible for CFX-stimulated antitumor immunity.

Key words: tumor growth, metastasis, Th1/Th2, TLR4

P300083

In vitro and in vivo antiplasmodial activity of stembarks extracts of Garcinia parvifolia Miq

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In vitro and in vivo antiplasmodial activity of stembarks extracts of Garcinia parvifolia Miq (Guttiferae), a medicinal plant traditionally used to treat malaria in Indonesia, have been conducted. Extracts of the plant i.e. n-hexane, ethylacetate and n-buthanol were tested on two Plasmodium falciparum strain, FCR-3 chloroquine-resistant and 3D7 chloroquine sensitive strains. Concentration inhibition 50% of the parasite growth (IC_{50}) ranged from 4.0 to 8.0 ug/ml according to the extract. For the most active extract, n-hexane extract (IC_{50} 4.11 ug/ml), its in vivo antiplasmodial activity was evaluated by 4-days supressive test on infected mice by P. berghei.

The effective dose reducing 50% of parasitemia of the n-hexane extract was 54.16 mg/kgBW per day.

P300084

Natural product CFX prevents and reverses cardiovascular hypertrophy and fibrosis by modulation of Th1/Th2 response in pressure-overloaded rats

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Cardiovascular remodeling is a critical prognosis factor for cardiovascular diseases. We wonder if natural product CFX, an immunomodulator, prevents and reverses cardiovascular hypertrophy and fibrosis in Wistar rat model of suprarenal aortic constriction. Cardiovascular hypertrophy and fibrosis were evaluated by histological and pathdlogy iconography. Cardiac function was determined by hemodynamic monitor. Expression of cytokines, Toll-like receptors in heart and vessels was determined by PCR, ELISA, or confocal microscopy. We found that hypertension-induced cardiovascular fibrosis and hypertrophy were associated with an increase in Th2/Treg cytokine production. CFX significantly ameliorated cardiac fibrosis and hypertrophy without affecting blood pressure. CFX enhanced the expression of IL-1 and IFN-γ and reduced expression of matrix metalloproteinase 2, IL-4, IL-5, and TGF-β. Our results suggest that anti-hypertrophy and fibrosis effects of CFX are due to its recruiting TLR4+OX62L+ dendritic cells to cardiovascular tissue, leading to a Th1 dominant microenvironment in the local tissue.

Key Words: CFX, Th1/Th2 response, cardiac hypertrophy and fibrosis; hypertension

P300085

Natural product complex CFX promoted maturation of dendritic cells and polarization of Th1 response via activation of TLR4 and dectin-1

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Natural complex CFX is able to attenuate pulmonary fibrosis, cardiovascular fibrosis and tumor metastasis via regulation of immune responses in pre-clinical studies. We wonder if CFX acts as an immunomodulator to activate dendritic cells (DCs) and direct polarization of Th1/Th2 and regulatory T responses. CFX-induced DCs maturation, production of cytokines and proliferation of T cells were analyzed by flow cytometry. The polarizing capacity of CFX-treated DCs was examined by allogenic-mixed lymphocyte reaction. We found that CFX activated DCs by increase in expression of CD11c and major histocompatibility complex II via activation of Tdl like receptor 4 and β-glucan receptor dectin-1. Also, CFX enhanced the percentage of CD11c+CD11b and CD54+ DCs and induced a high-level of interleukin-12 in DCs. CFX-maturated DCs significantly promoted the T cell proliferation and favored Th1 cell polarization. These results suggest that CFX, as a nonspicific ligand of TLR4 and dectin-1, regulates DCs and induces polarization of Th1 response, which provides a primary mechanism for CFX application in fibrotic disease and cancer therapy.

Key Words: CFX, Dendritc cells, immuno-regulator, Th1 response

P300086

Anti-oxidative effect of Terminalia Chebula Retz

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Terminalia Chebula Retz is a native plant in Southeast Asia. It was reported that Terminalia Chebula Retz has a variety of biological activity, including anticancer, antidiabetic, antimutagenic, antibacterial, antifungal, antiviral activicity, etc. However, there was few report on the antioxidant activity of Terminalia Chebula Retz. To study antioxidant effect of Terminalia Chebula Retz, inhibitation rates of liver microsome lipid peroxidation by Terminalia Chebula Retz ethanol extract in $FeSO_4$/Cys oxidative system were measured. Inhibitation rates of hemolysis caused H_2O_2 and self-oxidation were measured. DPPH free radical scavenging capacity was assayed. Our results showed that inhabitation rates of MDA (Malondialdehyde) generation was 55.12% by 12.5ug/ml Terminalia Chebula Retz ethanol extract in $FeSO_4$/Cys oxidative system and inhibit rates of haemolysis caused by H_2O_2 and self-oxidation were 50.05% and 56.62%, that DPPH free radical was scavenged by Terminalia Chebula Retz ethand extract, the scavenging rate of DPPH free radical was 49.52% by 3 ug/ml Terminalia Chebula Retz ethanol extract. It was concluded that Terminalia Chebula Retz has anti-oxidative effect.

Key words: Terminalia Chebula Retz, anti-oxidative effect, haemolysis, DPPH

P300087

The inhibitory effect of ginger juice on mouse duodenal motility in vitro is not fully explained by the presence of 6-gingerol.

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OBJECTIVE: To compare the effects of freshly prepared ginger (Zingiber Officinale) juice (GJ) with those of the major active constituent [6]-gingerol (6G) on the duodenum. METHODS: In vitro isometric recording was used to record spontaneous contractile activity from mouse (C57/BL6J) duodenum mounted longitudinally in 10 ml tissue baths. The 6G concentration in GJ was measured by HPLC. Statistical analysis was by ANOVA. RESULTS: 6G (10^{-7}M-$2x10^{-4}$M) reduced the amplitude of spontaneous contractions (by ~75% at $2x10^{-4}$M) in a concentration related manner with significance ($P < 0.01$) at 10^{-5}M (~20% reduction). GJ reduced amplitude in a dose-related manner with significance ($P < 0.05$-0.001) achieved between 50μl (~20%) and 400μl (~90%). The concentration of 6G resulting from application of 400μl GJ was < 10^{-7}M i.e. below the concentration of 6G which evoked an effect. CONCLUSION: The effect of GJ is not explained by the presence of 6G alone and suggests that either other constituents are responsible or potentiate the effects of 6G. The motility effects of GJ may contribute to its reported anti-nausea effects.

KEY WORDS: [6]-gingerol, ginger, motility, nausea.

P300088

Medicinal Plants of the Tunal, district of Lalaquiz, Huancabamba, Piura, Peru

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Medicinal plants are frequently used by residents of Tunal, located in the northwest region of Peru. These plants were harvested by means of a non-structured anonymous interview and underwent an ethnopharmacology study in order to identify them scientifically. Specimen samples were deposited at Universidad Nacional de Piura. A total of 34 samples of medicinal plants were obtained which belong to 27 families. Among others we have the following: Anacardiaceae, Bixaceae, Crassulaceae, Lamiaceae, Solanaceae. They are prepared by means of processes such as infusion, cooking, a fresh sample and accompanied by substances like honey, sugar, "marvelous curative", among others. From the identified medicinal plants, those of significant scientific information, are Bryophyllum pinnatum, Melissa officinalis, followed by Jatropha curcas. Finally, it exists another group that have scarce or null scientific information according to the bibliographical revision, therefore, they should be studied more thoroughly in the field phytochemical and pharmacological. These latter species are Loxopterigium huasango, Cordia lutea, Tinantia erecta, Bejaria aestuans, Vigna adenantha, and Pithecellobium multiflorum.

P300090

Creatine protects against HIV-1 protein Tat-induced increases in neuronal cell death and disruption of mitochondrial function

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Tat, an HIV-1 protein, disrupts mitochondrial function, increases apoptotic neuronal cell death, and may contribute to the pathogenesis of HIV-1 dementia. Here, we tested the hypothesis that creatine protects against Tat-induced neuronal death and mitochondrial dysfunction. With primary cultures of mouse cortical neurons, 100 nM Tat1-72 increased neuron cell death. Creatine (3 mM), co-applied with Tat, completely blocked Tat-induced increases in neuronal cell death. Tat reduced JC-1 dye ratio by 36% indicating mitochondrial membrane hypopolarization and creatine significantly protected against these reductions in mitochondrial membrane potential. Using of calcein-AM with cobalt chloride we found that Tat, but not Tat co-applied with creatine, increased mitochondrial permeability transition pore opening by 11%. Treatment of neurons with Tat decreased cellular levels of ATP from 11.5 to 7.9 nmol/mg protein and co-application of creatine with Tat maintained ATP near control levels. Creatine, a readily accessible dietary supplement, protects against Tat-induced neurotoxicity and may help lessen neurological complications observed with HIV-1 infection. (Supported by NCRR grant P20 RR17699-01)

P300091

Analgesic, anti-inflammatory and venotonic effects of Cissus quadrangularis Linn.

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Cissus quadrangularis is used for the treatment of hemorrhoid. Effects associated with hemorrhoid, analgesic, antiinflammatory and venotonic effects of C. quadrangularis (CQ) were assessed. For analgesia, acetic acid-induced writhing response and formalin test were used. Ethyl phenylpropiolate-induced ear edema, carrageenin- and AA-induced paw edema were used for testing of antiinflammatory activity. Venotonic effect was tested using human umbilical vein. CQ provoked significant reduction of the number of writhes and reduced liking time in both phases of the formalin test. The results suggest peripheral and central analgesic activity of CQ. CQ elicited inhibitory effect on edema formation of rats ear and on paw edema formation in rats induced by both AA and carrageenin. It is likely that CQ is a dual inhibitor of arachidonic acid metabolism. CQ exerted venotonic effect on isolated human umbilical vein. The results confirmed the traditional use of C. quadrangularis for treatment of pain and inflammation associated with hemorrhoid as well as reducing the size of hemorrhoids.

Keywords: Cissus quadrangularis, analgesic, antiinflammaory, venotonic

P300092

Role of Membrane Ion Transporters in the Apigenin-Induced Melanogenesis in B16 Melanoma Cells

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In this study we investigated the effect of apigenin, a plant-derived flavonoid, on melanogenesis and its mechanism of action in B16 murine melanoma cells. Apigenin at the concentrations inducing no significant alteration of cell viability, increased melanin synthesis in a dose-dependent manner. Interestingly, apigenin increased intracellular level of reactive oxygen species (ROS) in a dose-related fashion. Treatment with antioxidants (ascorbate and tocopherol) significantly inhibited the apigenin-induced ROS increase and melanin synthesis. In addition, apigenin reduced intracellular K^+ and Cl^- concentrations in a dose-dependent manner.

The apigenin-induced K^+ and Cl^- efflux was significantly suppressed by either Cl^--deficient medium, or known inhibitors of K^+, Cl^--cotransport (KCC), calyculin-A and $BaCl_2$. These KCC inhibitors also significantly blunted the apigenin-induced ROS generation and melanogenesis. Collectively, these results suggest that apigenin induced melanogenesis through the KCC-mediated ROS generation. These results further suggest that apigenin may be valuable for the therapeutic management of skin hypopigmentation disorders, such as vitiligo.

P300093

The Effects of Natural Products on the Metabolism of Amyloid Precursor Protein

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Alzheimer's disease (AD) is characterized pathologically by the presence of intracellular neurofibrillary tangles and deposition of beta-amyloid (Abeta) peptides of 40-42 residues, which are generated by processing of amyloid precursor protein (APP). It is urgent to develop effective therapies to treat AD, since our society rapidly accelerate aging. Abeta has been believed to be neurotoxic and now is also considered to have effects on the mechanism of memory formation. In this study, the effects of geldanamycin or radicicol, HSPs inhibitors were analyzed on the metabolism of APP and gamma-secretase complex. PKC inhibitor, rottlerin and antimicrobial peptides from insect were also accessed. Abeta ELISA study revealed that a natural product effects on the endogenous Abeta 42 secretion. Geldanamycin, rottlerin, and antimicrobial peptides showed regulatory effects on Abeta 42 secretion. We suggest that PKC, HSP 90, or Src tyrosine kinase effects on APP metabolism.

(This work was supported by grants from the Korea Research Foundation (E00194) and the Regional Research Centers Program of the Korean Ministry of Education & Human Resources Development through the Center for Healthcare Technology Development.)

Key words: Alzheimer's disease, amyloid precursor protein, Abeta peptides, gamma-secretase complex

P300094

Cellular Responses to the Enediyne Antibiotics Lidamycin Can Be Independent of P53 in a Dose Specific Way

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Enediyne lidamycin (LDM) showed strong killing activity towards tumor cells. We aimed to investigate whether cellular responses to LDM were p53 dependent. Various Human colon cancer cells with different p53 status were employed, like HCT116, LOVO, SW480, SW620 etc. Cell viabilities were detected by FACS and MTT.

Chromatin was observed using DNA staining. P53 expression was detected by western blot. Unlike conventional chemotherapeutic agents 5-FU and MMC, LDM induced significant p53 dependent cell death at as low as 10 nmol/L after 24 hours, and lost this dependence when reached 1 umol/L (killed as high as 90% cells). 10 nmol/L LDM could induce p53 expression in a time reliant way. Caspase inhibitor VAD-fmk inhibited only the effects of lower LDM, showing no effect on higher dosage. Exogenous p53 expression in HCT p53(-/-) cells sensitized the effect of LDM of lower dosage. LDM of Lower dosage induced typically apoptotic chromatin changes while higher dosage induced atypical dotted chromatin condensation. We conclude that LDM may induce cell death in both p53 dependent and independent pathway.

(supported by National Natural Science Foundation of China (No 30300424 & 30572204)

Key Words: Lidamycin, p53

P300095

Chelidonine blocks hKv1.5 channel current and human atrial ultra-rapid delayed rectifier K^+ currents

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Chelidonine, a major component of Chelidonium majus var. asiaticum, is known to possess various biological effects. We examined the effects of chelidonine on a rapidly activating delayed rectifier K^+ channel (hKv1.5) cloned from human heart and stably expressed in Itk- cells, and on the ultra-rapid delayed rectifier (I_{Kur}) in human atrial myocytes. Chelidonine inhibited hKv1.5 current in a concentration-, use-, time- and voltage-dependent manner with an IC^{50} of 11.5 ± 3.1 μM at +60 mV without affecting the HERG current expressed in HEK-293 cells. Chelidonine also inhibited I_{Kur} in human atrial myocytes. Additionally, chelidonine also prolonged the action potential durations in rabbit atrial myocytes in a frequency-dependent manner. In conclusion, chelidonine inhibits hKv1.5 channels primarily in an open state and the native hKv1.5 channels in a concentration-, use-, voltage-, state- and time-dependent manner.

(This work was supported by the Regional Research Centers Program of the Korean Ministry of Education & Human Resources Development through the Center for Healthcare Technology Development.)

Key words: Chelidonine; Antiarrhythmics; hKv1.5 channel; the ultra-rapid delayed rectifier K^+ current

P300096

Effect of Rutoside on pancreatic acina cell apoptosis in rats with acute pancreatitis and its mechanism

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Objective To study the effect of Rutoside (Ru) on pancreatic acina cell apoptosis in rats with acute pancreatitis (AP). Methods The AP model in rats was induced by retrograde injection of 3.0% sodium taurocholate into biliopancreatic duct. Ru (15, 30, 60 mg/kg/h) was administered by intravenous infusion for 6 hours immediately after the induction of AP. The histopathological changes of pancreas were observed under light microscope and electronic microscopy. The TUNEL method was used to detect apoptosis of pancreatic acinar cell. The expression of Fas and FasL protein was detected by immunohistochemical method. Results Ru (15, 30, 60 mg/kg) improved the histopathological changes of pancreas significantly. The apotosis index of pancreatic acinar cells and the expression of Fas in Ru (15, 30, 60 mg/kg) groups were significantly higher than that in the AP model group. But in AP model group, the expression of FasL was higher than in Ru-treated groups. Conclusions The protectal effect of Ru on AP may be concerned with the induction of apoptosis in injured pancreatic acinar cells. And the Fas/FasL system may contribute to the process.

Key words: Rutoside; Acute Pancreatitis; Apoptosis; Fas/FasL

P300097

Anti-inflammatory and Analgesic activities of the water extract of Malvastrum coromandelianum (L.) Garcke

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Malvastrum coromandelianum (L.) Garcke, family Malvaceae, was evaluated in inflammatory, algesic and pyretic models. Anti-inflammatory test of the water extract of M. coromandelianum (MC) was done by using carrageenin-induced hind paw edema in rats. For analgesic effect, formalin was injected into the hind paw of the mouse. Antipyretic activity using yeast-induced hyperthermia in rats also investigated. The water extract of MC given orally exhibited anti-inflammatory effect. The anti-inflammatory mechanism may be due to an inhibition of the synthesis or release of prostaglandins. The extract also showed analgesic effect on both the early and the late phases of the formalin test. The mechanism of anal-

gesic activity may involve with its action on the central nervous system and peripheral tissue. The water extract of MC at the dose ranging from 400 to 1,600 mg/kg did not show antipyretic effect in yeast-induced hyperthermic rats.

Key words: Malvastrum coromandelianum (L.) Garcke, carrageenin, formalin

P300098

The Effect of Gingerol on Endotoxemia Model Caused by LPS and D-GalN

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Objective: To investigate the effect of Gingerol (extracts of dried Zingiber officinale Rosc.,) on endotoxemia model caused by lipopolysaccharide (LPS) and D-galactosamine (D-GalN). Methods: The model was established on mice by injecting LPS (20 mg/kg) and D-GalN (800 mg/kg) intraperitoneally, 32 mice were randomly divided into four groups: Gingerol (75 mg/kg), Control (Cane glucolipid, 1 mg/kg), Chuanhuning (1 mg/kg) and Endotoxemia model group. The drugs had been given to the mice half an hour before injection, we recorded the survival time during three days. Results: The survival time in the mice pretreated with the Gingerol (1170.3 ±35.8 min) was significantly longer than the endotoxemia model (730.5 ±22.6 min) ($p < 0.01$) and Chuanhuning group (751.0 ±18.9 min) ($p < 0.01$). Conclusion: These results indicated that Gingerol has good effect of endotoxemia model caused by LPS and D-GalN, the treatment is even better than Chuanhuning.

Key word: Gingerol; Endotoxemia; LPS; D-GalN;
Project supported by the National Natural Science Foundation of China (No. 304712216).

P300099

Effect of Pharmacological Preconditioning of Total Flavone of Abelmoschl Manihot L. Medic (TFA) on Cerebral Ischemia-Reperfusion Injury in Rat

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Objective To study the effect of pharmacological preconditioning of TFA (TFR-PP) on cerebral ischemia reperfusion injury in rat. METHODS The cerebral ischemia-reperfusion injury was induced by rat middle cerebral artery occlusion, TFR-PP groups were subject to three cycles of 5 min intravenous injection of TFA periods interspersed with 5 minutes break. The nervous deficit was scored, the infarct size of cerebrum and the contents of malonaldehyde (MDA), nitric oxide (NO), PGE2 and the activities of lactate dehydrogenase (LDH) and nitric oxide synthetase (NOS) in serum were measured. RESULTS 20, 40, 80, 160 mg·kg^{-1} TFA dosedependently reduced the score of nervous deficit and the infarct size of cerebrum, significantly decreased the contents of MDA, PGE2 and the LDH activity, and markedly increased NO content and NOS activity.

CONCLUSION TFR-PP has significant protective effect on rat cerebral ischemical-reperfusion injury via increasing of NO production.

KEY WORDS: Pharmacological preconditioning, Total Flavone of Abelmoschl Manihot L. Medic, Cerebral Ischemical-Reperfusion

P300100

The Effects of Total Flavone of Abelmischl Manihot L. Medic (TFA) against Cerebral Ischemia-Reperfusion Injury

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Objective To observe the protective effect and its mechanisms of TFA on cerebral ischemia-reperfusion injury. Methods Rabbits were scheduled to undergo cerebral ischemia-reperfusion by ligatting both common carotid arteries and dripping sodium nitropusside. Prior to ischemia-reperfusion, TFA was perfused intravenously. The LDH activity and contents of MDA and ATP in the cerebrum were evaluated. Rat spinal cord electric current induced by Gly was measured by patch clamp method. Results TFA 12,24,48 mg·kg^{-1} significantly inhibited the decreases of LDH and ATP and the increase of MDA. TFA 0.1,0.2 mg·ml^{-1} possessed concentrationdependent inhibitory effects on rat spinal cord electric current induced by Gly Conclusion TFA had protective effects against cerebral ischemia and reperfusion injury via attenuating cerebral lipid peroxidation, improving utilization of ATP, and antagonizing the electric current induced by Gly.

KEY WORDS: Total Flavone of Abelmischl Manihot L. Medic, Cerebral Ischemia-Reperfusion, ATP, Patch clamp

P300101

Ligustrazine attenuates acute myocardial injury after thermal trauma

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Objective To investigate the effect of Ligustrazine on burn-induced myocardial injury and its mechanism. Methods Rats were given third-degree burns over 30% total body surface area and lactated Ringer solution for resuscitation. Myocardial injury was assessed at 6 h post-burn by using serum lactatate dehydrogenase (LDH) and Creatine kinase (CK), myocardial water content, as well as histological and ultrastructure alterations of myocardium. ATP and TNF- in myocardium were also examined. Results Burn trauma results in the increases of serum LDH and CK, elevated myocardial water content, made marked myocardial histological and ultrastructure lesions, decreased myocardium ATP, and increased myocardium TNF-. Ligustrazine 10.0 mg/kg significantly inhibited these alterations Conclusion Ligustrazine has significant protective effect on burn-induced myocardial injury via inhibiting the release of TNF- and improving utilization of ATP.

Key Words: Ligustrazine, Burn, Myocardium, Protective effect

P300102

Protective effect and mechanism of berberine on fatty livers of rats

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Protective effect and possible mechanism of berberine on fatty livers was investigated in this study. A fatty liver model was established by feeding rats with 10% fructose drinking solutions plus high fat diet. After four weeks, all the rats were sacrificed. Liver function, antioxidative function, blood and liver lipid, fasting blood glucose (FBG), fasting plasma insulin (FINS), HOMA insulin resistance index (HOMA-IR), and the liver histology were assayed. The results showed that glutamic pyruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT), blood total cholesterol (TC) and triglyceride (TG), liver TG, malondialdehyde (MDA), FBG, FINS and HOMA-IR increased significantly in the model group, while superoxide dismutase (SOD) decreased significantly, and the liver histology showed moderate to severe steatosis. Compared with the model group, the level of TG, MDA, liver TG and FINS, HOMA-IR in the berberine groups were significantly lower, while SOD increased remarkably. The liver histology changes were milder. It is suggestive that berberine might decrease lipid peroxidation and reduce fatty sediment in liver through improving insulin resistence and lipid metabolism.

berberine; fatty liver

P300103

Inhibitory effects of active principles from Ligusticum chuanxiong on the proliferation of rat hepatic stellate cells

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Platelet-derived growth factor (PDGF) is a very potent mitogen in hepatic fibrogenesis. The aims of the present study were to investigate the effects of Ligusticum chuanxiong (LC) active principles, Z,Z'-6,8',7,3'-diligustilide (LC1) and levistolide A (LC2), on the proliferation-related biomarkers in rat hepatic stellate cells (HSCs) stimulated with PDGF. DNA synthesis, cell cycle related proteins and apoptosis markers were determined. The results revealed that LC1 and LC2 (1-40 uM) concentration-dependently decreased the PDGF-induced cell proliferation and a-smooth muscle actin in HSCs. The inhibitory activity was associated with induction of cell cycle redistribution and apoptosis, activation of caspase-3, up-regulation of p21 and p27, and down-regulation of cyclins D1, D2, E, A and B1. In addition, JNK phosphorylation was increased by LC1 and LC2, while both showed no cytotoxicity to primary hepatocytes. Our results indicated that LC1 and LC2 were effective inhibitors for activated HSC growth and might be potential anti-fibrotic drugs for hepatic fibrosis.

Keywords: hepatic fibrosis, hepatic stellate cells, Ligusticum chuanxiong.

P300104

Antimalarial activity of indigenous South African medicinal plants

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Traditional South African medicinal plants used to treat fever and flu-like symptoms associated with malaria, include Pelargonium, Agathosma and Hermania. Solvent extracts of 21 Pelargonium, 17 Agathosma and 12 Hermania species were tested for antimalarial activity against a chloroquine-resistant Plasmodium falciparum strain using the [^{3}H]-hypoxanthine incorporation assay. Toxicity profiles were determined using the tetrazolium cell proliferation assay. Both the Pelargonium and Agathosma species had promising activity, with P. panduriforme, P. citronellum, P. radens and P. quercifolium being the most active (IC_{50} range: 1.34 ±0.29 to 2.66 ±0.36 µg/ml), while A. pungens, A. ovata and A. roodebergensis displayed promising activity (IC_{50} range: 3.61 ±0.27 to 5.18 ±0.15 µg/ml). Of the 17 Hermania species, only H. trifurca had activity below 20 µg/ml. Due to the more favourable toxicity profile of P. panduriforme, P. citronellum and P. quercifolium, HPLC analyses were performed and all three species were shown to accumulate high levels of flavones.

Key words: malaria, traditional medicine

We acknowledge the University of the Witwatersrand and the National Research Foundation (IKS).

P300106

Mangifera indica L. extract (Vimang) protects gainst 2-deoxyribose damage induced by Fe(III) plus ascorbate.

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Vimang is an aqueous extract of selected species of Mangifera indica L, used in Cuba as a nutritional antioxidant supplement. Many models of oxidative stress have been used to elucidate the antioxidant mechanisms of this extract. To further characterize the mechanismof Vimang action, its effect on the degradation of 2-deoxyribose induced by Fe(III)-EDTA plus ascorbate or plus hypoxanthine/xanthine oxidase was studied. Vimang was shown to be a potent inhibitor of 2-deoxyribose degradation mediated by Fe(III)-EDTA plus ascorbate or superoxide radicals. Vimang at concentrations higher than 50 micromangiferin equivalent, was equally effective in preventing degradation of both 15 mm and 1.5 mm 2-deoxyribose. At a fixed Fe(III) concentration, increasing the concentration of ligands (either EDTA or citrate) caused a significant reduction in the protective effects of Vimang. When ascorbate was replaced by superoxide anion radical (by hypoxanthine and xanthine oxidase) the protective eficiency of Vimang was also inversely related to EDTA concentration. The results strongly indicate that Vimang acts as an antioxidant by complexing iron ions, rendering them inactive or poorly active in the Fenton reaction.

P300107

GENOTOXIC POTENTIAL OF MANGIFERA INDICA L. EXTRACT (VIMANG), A NEW CUBAN PRODUCT WITH ANTIOXIDANT PROPERTIES.

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Vimang is a Cuban aqueous extract obtained from Mango trees with antioxidant effects. The genotoxic potential of Vimang was investigated using Ames, Comet and micronucleus assays. Histidine requiring mutants of Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100 and TA102 strains and in vitro micronucleus assay in primary human lymphocytes with and without metabolic activation were performed.

DNA damage was evaluated on blood peripheral lymphocytes of NMRI mice treated 2 days with intraperitoneal doses (50-150 mg/kg). Results showed Vimang (200-5000 ug/plate) did not increase the frequency of reverse mutations in Ames test. Vimang did not induce single strand breaks or alkali-labile sites on blood peripheral lymphocytes of treated animals compared with controls. Micronucleus studies showed Vimang induces cytotoxic activity (cell viability and PCE/NCE ratio), but neither increased the frequency of micronucleated binucleate cells in culture of human lymphocytes nor in mice bone marrow cells. Positive control included induced the expected changes. Vimang showed evidences of cytotoxicity but did not induce genotoxic effects in these experimental conditions.

P300109

Methylglyoxal Impaires Insulin Signaling and Causes Insulin Resistance

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Methylglyoxal (MG), a metabolite of sugar, has been linked to the development of insulin resistance. In this study, we investigated the association of high MG level and expression of insulin signaling genes in rats. Fructose feeding of Sprague Dawley rats for 9 weeks led to insulin resistance with elevated plasma insulin and triglyceride, hypertension, and reduced glucose uptake. MG levels were dramatically increased in serum and insulin sensitive tissues including skeletal muscle, liver and adipose tissues. Expression levels of insulin receptor substrate-1 and PI_3-kinase were reduced in skeletal muscle from fructose-fed rats or in cultured skeletal muscle cells treated with 10 uM MG, which were reversed by metformin (an anti-diabetic agent) or N-acetyl-cysteine (NAC, a scavenger of MG). Metformin and NAC also reduced the fructose-increased MG elevation and improved insulin resistance symptoms. Thus, our study indicated that endogenous accumulation of MG induced by fructose was associated with the impairment of insulin signaling and therefore the development of insulin resistance.

Key words: methylglyoxal, fructose, insulin resistance, insulin signaling

(Supported by HSFS & CIHR)

P300110

Suppression of Matrix Metalloproteinase-9 Expression by Andrographolide in Human Monocytic THP-1 Cells via Inhibition of NF-kB Activation

George Hsiao*, Duen-Suey Chou, Yung-Chen Chou, Joen-Rong Sheu. Pharmacology

In the present study, we investigated the effects and mechanisms of andrographolide, which extracted from Chinese herb Andrographis paniculata, on human monocytic MMPs activation. Andrographdide exerted aconcentration-dependent inhibition of MMP-9 activation induced by tumor necrosis factor-a (TNF-a) in THP-1 cells. In addition, andrographolide did not show inhibitory effect on the enzymatic activity of MMP-9.

Andrographolide also inhibited the TIMP-1 levels by the ELISA analysis. According to Western blot method, it concentration-dependently inhibited the expression of MMP-9 protein. By using reverse transcription polymerase chain reaction method, we found that andrographolide could suppress the expression of MMP-9 messenger RNA. Furthermore, we also found that it could concentration-dependently inhibit the degradation of inhibitor-kB-a and p65 transactivation as detected by EMSA. On the other hand, andrographdide did not significantly affect the phosphorylated activation of extracellular signal-regulated kinases. In conclusion, we demonstrate that andrographolide with inhibitory effect on MMP-9 expression, and its main mechanism might through NF-kB signal pathway.

P300111

Baicalein Reverses the Methamphetamine-Induced Striatal Dopaminergic Neurotoxicity in Mice

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The potential for neuroprotection by Baicalein (5,6,7-trihydroxyflavone), from the root of Scutellaria baicalensis Georgi, against methamphetamine (METH) induced neurotoxicity was studied. Each mouse was treated by repeated intraperitoneal administration, at 2 hr intervals, of either METH. (4 × 5 mg/kg), saline, baicalein (1 mg/kg) or baicalein pretreatment followed by METH. In the striatum of mouse, the tissue level of dopamine (DA) was monitored at day 3 and nitric oxide (NO) was assayed at 1 hr, 24 hrs and 3 days after the above treatment. The results showed that striatal DA was significantly depleted by METH, elevated by baicalein, pretreatment with which prevented the METH induced depletion. Nitric oxide at 1 hr post-treatment was depressed by METH, elevated by baicalein, but remained suppressed with baicalein + METH. At 24 hrs NO concentration was unaffected by METH but was significantly elevated by both baicalein and baicalein + METH. At 3 days post-treatment NO was elevated by METH, baicalein and further markedly elevated by baicalein + METH. These results suggest a potential neuroprotective role for baicalein with the possible involvement of NO.

Key words: Baicalein; Methamphetamine.

P300112

Anti-giardial activity of constituents from Boesenbergia pandurata

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Songkla University, Hat Yai, Songkhla, 90112, THAILAND.

Previously we reported that chloroform extract from fresh rhizomes of Boesenbergia pandurata had anti-giardial activity. We have now isolated eight known compounds, Alpinetin, Helichrysetin, Hydroxypanduratin A, Panduratin A, Pinocembrin, Pinostrobin, 2',4',6'-Trihydroxydihydrochalcone and Uvangoletin and one new compound, Panduratin C, from this rhizome and tested for their anti-giardial activity. Each compound and a standard drug, metronidazole, were incubated with Giardia intestinalis trophozoites in an anaerobic conditions for 24 h. The appearance and numbers of trophozoites were scored from 1 to 4 with 1 showing the most inhibition of growth and 4 showing no inhibition, and the minimum inhibitory concentrations (MIC) was determined. Three compounds, Helichrysetin, Hydroxypanduratin A, and Panduratin A exhibited significant inhibitory effects (MIC 125ug/ml). The MIC of metronidazole was 2.5ug/ml. This study shows that compounds from B. pandurata have potential for use as therapeutic agents against G. intestinalis.

Key words: Giardia, Boesenbergia pandurata, traveler's diarrhea

Acknowledgement: We would like to thank the Thai Government Budget for awarding the research grant.

P300113

The therapy from combined iron like a new tool to figth against the iron deficiency

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Iron deficiency is the most prevalent problem of the human healthy all over the world, which affect two billion persons, nearly 50% of them suffer anemia. Trofín is an antianemic and restorative product obtained from bovine blood that contains heme iron. Today, the iron salt, containing not heme iron are the conventional therapy to figth against iron deficiency. The aims of this work was to show technological workability of the obtention of tablets and oral suspension, containing both dry Trofín and Ferrous fumarate and the results about predinical studies. We obtained three different tablet formulations and two different oral suspensions. In order to evaluate both acute and repeated dosis toxicity test during 28 days, was suministrated the oral suspension with 50% of heme iron and 50% of nor heme iron to Sprague Dawley rats. In both assays 100% of rats survived. At the end of two assays animal corporal weight increased. In microscopical hystological preparations of both stomach and duodenum for the second study was observed adverses reactions reported by iron salts. The obtained results showed that products with combined iron could be new tools to figth against iron deficiency.

P300114

EXTRACTS OF SAW PALMETTO HAVE INDIRECTLY ACTING SYMPATOMIMETIC EFFECTS IN THE RAT PROSTATE GLAND

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Saw palmetto is widely used in the treatment of benign prostatic hyperplasia. It is thought to act by antiandrogenic actions but more recently it has been shown to inhibit alpha1-adrenoceptor binding. This study investigated whether commercially available saw palmetto extracts affect the contractility of rat isolated prostate glands using functional isolated organ bath techniques. Extracts were tested in the presence and absence of a variety of pharmacological tools to evaluate mechanisms of action. Isolated preparations of rat vas deferens and bladder were used for comparison. Unexpectedly, saw palmetto extracts caused contractions of the rat prostate gland which could be attenuated by prazosin, phentolamine, nifedipine, guanethidine, cocaine and desipramine but not by any of the other pharmacological tools. Similar contractile effects were observed in rat isolated vas deferens preparations but not in rat isolated bladder preparations. It is concluded that in the rat prostate gland saw palmetto extract causes indirect alpha1-adrenoceptor mediated contractions via the release of noradrenaline from sympathetic neurons.

Keywords: prostate, saw palmetto, contractility

P300115

Analysis of Antioxidant Phenolics in Thai Medicinal Plants-modulated Cellular Antioxidant Enzymes

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Objectives: 1. To identify the presence and antioxidant activity of phenolics in the extracts of Alpinia galanga, Curcuma aromatica, Curcuma comosa and Kaempfera galanga 2. To study the effects of the extracts on antioxidant enzymes in melanoma and fibroblast cells.

Materials & Methods: Rhizomes of all plants were extracted by 90% ethanol. Thin layer chromatography-1,1-diphenyl-2-picrylhydrazyl (TLC-DPPH) was used to identify the presence and antioxidant activity of phenolics in the extracts. The contents of antioxidant enzymes in melanoma cells (G361) and fibroblasts (SiF49) pretreated with the extracts were assessed by spectrophotometric methods.

Results: TLC analysis showed the presence and antioxidant activity of phenolics in all extracts. There was an increase in antioxidant enzyme contents in G361 and SiF49 cells.

Conclusions: All extracts tested increased antioxidant enzyme contents in G361 and SiF49 cells. The antioxidant phenolics in the extracts might account for modulating cellular antioxidant enzymes.

Key words: phenolics, antioxidant enzymes, antioxidant activity

Acknowledgement: Faculty of Medicine Siriraj Hospital, Thailand, is acknowledged for financial support.

P300116

GASTRIC ANTISECRETORY AND ANTI-ULCER EFFECTS OF MEZONEURON BENTHAMIANUM BAIL (CAESALPINIACEAE)

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The aqueous extract of Mezoneuron benthamianum (MB) was investigated for its potential to protect gastric mucosa against the ulcers induced by absolute ethanol, 0.6N HCl, 50mg/kg indomethacin and 5mg/kg histamine. MB pretreatment at doses of 400, 800 and 1600 mg/kg produced a significant ($P < 0.001$) and dosedependent protection against the ulcerogenic effects in rats by the different agents used. The degree of protection by the highest dose of MB (93.98, 86.05 and 90.00%) was significantly ($P < 0.001$) greater than that obtained with 50mg/kg cimetidine (56.89, 46.51and 70.83%) but comparable to that obtained with 50µg/kg misoprostol (89.47, 87.21and 87.50%) respectively in ethanol, HCl and indomethacin models. In the histamine model, however, comparable degree of protection (83.25 and 87.50%) was obtained with the highest dose of MB and cimetidine respectively.

In the HCl model MB (400-1600 mg/kg) increased the gastric mucus in rats.

In conclusion MB possesses both antisecretory and cytoprotective activities.

Mezoneuron benthamianum, antiulcer, cytoprotective.

P300117

ANTIINFLAMMATORY ACTIVITY OF DRYMARIA CORDATA (LINN) WILLD AQUEOUS EXTRACT

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The anti-inflammatory activity of the aqueous extract of Drymaria cordata (DC) was evaluated using the carrageenan, egg albumin, xylene oedema models and pleurity test. DC (100-800mg/kg) administered 1hr before induction of swelling in rat paw, by carrageenan and egg albumin injection, produced a significant ($P < 0.05$) dose dependent inhibitory effect. This was highest at the dose of 400mg/kg for carrageenan (73.66%) and egg albumin (63.69%) models. DC (800mg/kg) dose dependently inhibited (61.39%) ear oedema development by xylene. This effect was greater than for 10mg/kg indomethacin (55.45%). DC (400mg/kg) like indomethacin (10mg/kg) reduced the volume of pleural exudates (53.7%) and number of migrated leukocytes (44.0%) in the carrageenan induced pleurisy test. It can be concluded that the aqueous extract of DC possesses antiinflammatory activity possibly mediated by the inhibition of one or a combination of mediators like histamine, serotonin, kinins and prostaglandins.

Drymaria cordata, anti-inflammatory activity, oedema.

P300118

A Novel Property of Ginkgo biloba Extract: Alteration on the Binding of the Radiopharmaceutical on Blood Elements

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In nuclear medicine radiopharmaceuticals are injected into the blood stream and they may bind to the blood proteins. This binding can be influenced by drugs and can be related with the modfication of the biodistribution of a radiopharmaceutical. We studied the influence of Gingko biloba extract (40 mg/ml, 400 mg/ml) on the binding of sodium pertechnetate (99 mTcO4Na) on blood elements. Blood of Wistar rats was incubated with Ginkgo biloba extract or saline solution and 99 mTcO4Na was added. Blood was centrifuged; plasma (P) and blood cells (BC) were separated and precipitated with trichloroacetic acid (TCA) and ammonium sulphate (AS).

Soluble (SF) and insoluble fractions (IF) of P and BC were isolated and counted. The results showed that the percent of radioactivity (%ATI) of 99 mTcO4Na in the IF-P and IF-BC, which were precipitated with TCA and AS, decreased or it increased for both concentrations of Gingko biloba used. The extract can modfy the binding of 99 mTcO4Na on blood proteins and/or the different components of the precipitations are altered differently by the drug due chemical characteristics their.

Ginkgo biloba, Blood elements, radiopharmaceutical, Technetium-99m

P300119

Protective effects of Danbiqing granules on rabbits during ischemia and reperfusion injury

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AIM: The traditional Chinese medicine - Danbiqing granules are made of rhubarb、Dan-shen root, dahurian patrinia herb, Chinese pulsatilla root and Glycyrrhiza. They can remove blood stasis and eliminate heat or toxicity. Clinical practices have demonstrated that they can be used to treat acute cholangitis and abdominal infections. This study reports the protective effects and mechanism of Danbiqing granules on ischemia/reperfusion injury. METHODS: Rabbits were divided into five groups randomly: the sham group, model group, and pretreatment groups of Danbiqing granules (0.3g/kg; 1.2g/kg; 3.6g/kg). the superior mesenteric arteries were clamped for 60 minutes and reperfused for 6 hours. Blood samples at different times were collected. MDA, SOD, NO, LPS were measured. After reperfusion, rabbits were sacrificed, the tissues were collected, MPO was determined. RESULTS: Danbiqing granules could decrease MDA, MPO LPS, NO level induced by I/R injury, increase SOD activity and attenuate tissue injury. CONCLUSIONS: Danbiqing granules could protect body from intestinal I/R injury by modulating the circle of various intermediates and effector. KEY WORDS: Danbiqing granules I/R injury

P300121

Ginkgo biloba Extract, Isorhamnetin, Kaempferol, and Quercetin are In Vitro Inhibitors of the Procarcinogen-Bioactivating Human Cytochrome P450 1B1

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The present study investigated the effect of Ginkgo biloba extract and some of its individual constituents on the catalytic activity of human recombinant cytochrome P450 1B1 (CYP1B1). Ginkgo biloba extract containing known abundance of terpene trilactones and flavonol glycosides inhibited CYP1B1 (apparent Ki value = 2 +/- 0.3 ug/ml; mean +/- SE), as determined by the 7-ethoxyresorufin O-dealkylation assay. When assessed at the levels present in the Ginkgo biloba extract, bilobalide, ginkgolides A, B, C, and J, quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, and isorhamnetin-3-O-rutinoside did not affect CYP1B1 catalytic activity. The aglycones of isorhamnetin, kaempferol, and quercetin inhibited CYP1B1, with apparent Ki values of 3 +/- 0.1 nM, 14 +/- 3 nM, and 23 +/- 2 nM, respectively. Ginkgo biloba extract also reduced the extent of benzo[a]pyrene hydroxylation catalyzed by CYP1B1. In summary, Ginkgo biloba extract, isorhamnetin, kaempferol, and quercetin are in vitro inhibitors of human CYP1B1 catalytic activity.

P300123

Bunodosoma caissarum **effects on perfused rat kidney and arteriolar mesenteric bed**

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The sea anemone Bunodosoma caissarum is endemic in Brazilian southern coast. The aim of this work was to study the alterations produced by Bunodosoma caissarum venom (BcV) in the isolated rat kidney and its effects on arteriolar mesenteric bed. Isolated kidneys from Wistar rats (240-300g) were perfused with Krebs-Henseleit solution containing 6% of bovine serum albumin for 120 min. BcV (3μg/mL; n = 6) was added to system 30 min after the beginning of each experiment (internal control). The mesenteric bed was perfused with Krebs solution by a constant flow and variable perfusion pressure was measured by 80 min. The data were analyzed by Student's t-test ($p < 0,05$). In rat kidney perfused, the BcV caused an increase in perfusion pressure ($_{30}$PP = 94,77 ±0,93; $_{60}$PP = 119,1 ±5,04 mmHg), renal vascular resistance ($_{30}$RVR = 4,03 ±0,034; $_{60}$RVR = 5,03 ±0,23 mmHg/mL/g/min), urinary flow ($_{30}$FU = 0,2 ±0,005; $_{90}$FU = 0,31 ±0,003 mL/g/min) and glomerular filtration rate ($_{30}$GFR = 0,84 ±0,13; $_{120}$GFR = 1,34 ±0,03 mL/g/min). The infusion of BcV not affected the basal perfusion pressure of isolated arteriolar mesenteric bed. BcV affects renal function and without effects on arteriolar mesenteric bed.

Support: CNPq

P300124

CHARACTERIZATION OF A NOVEL HERBAL SEDATIVE ECBRC-AG USING TELEMETRIC EEG RECORDING AND MICROARRAY.

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Insomnia is characterized as difficulty in falling asleep; dfficulty staying asleep or experiencing non-refreshing sleep, and effects up to half of the adult population. The present project thus aimed at identifying a novel herbal derived sedative for use as an alternative treatment for insomnia. Telemetric EEG recording in Wistar rats demonstrated ECBRC-AG to have similar efficacy to existing sedative zolpidem in inducing sleep. Also, unlike currently available sedatives, the novel sedative was able to improve sleep quality in the later phases of sleep as identified by improved sleep wave architecture in rats. Molecular analysis using microarray demonstrated ECBRC-AG to have modulatory effects on a number of rat hypothalamic neuroreceptors' expression, including serotonin, histamine and hypocretin systems. Subsequent intracellular calcium recordings of rat hypothalamic cells demonstrated modulation of intracellular calcium may be a major target of ECBRC-AG.

Studies are underway to further characterise the mechanism of action of ECBRC-AG with the aim of identifying a safe and effective treatment for insomnia.

Insomnia, Telemetry, Herbal, EEG.

(Innovation and Technology Fund of the Hong Kong SAR).

P300125

Proapoptotic effects of selected indole phytoalexins in cancer cells.

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Objective: In the present work, we tested selected cruciferous indole phytoalexins for their antiproliferative and proapototic effects on cancer cell lines.

Methods: MTT cytotoxicity assay, cell cycle analysis, apoptosis detection by flow cytometry or DNA fragmentation.

Results: Our data indicate the highest activity of 1-methoxybrasinine (MB). The IC_{50} was 10 and 5 mmol/L in Jurkat and HL-60 leukemic cells, respectively. However, significant antiproliferative effect of all phytoalexines was also determined at concentration 0.5 mmol/L in both cell lines. In MB-treated cells we found significant increase in the fraction of cells with a sub-G_0/G_1 DNA content, which is considered to be a marker of cell death by apoptosis. Apoptosis was also confirmed by the annexin V staining and DNA fragmentation.

Conclusions: MB exerted potent antiproliferative activity probably due to cell cycle arrest and apoptosis induction. Further studies are necessary to elucidate its mechanism of action, nevertheless, this compound might have a potential to enter pre-clinical trials as a new anticancer drug.

Key words: phytoalexins - cancer - apoptosis

This study was supported by VEGA grants 1/1176/04 and 1/3365/06

P300126

Antiproliferative and antiangiogenic effects of selected chalcones.

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Objective: In the present work, we tested four newly synthesized chalcones (Ch1 - Ch4) for their antiproliferative and antiangiogenic effects.

Methods: MTT cytotoxicity assay, cell cycle analysis, apoptosis detection by flow cytometry or DNA fragmentation, endothelial cell migration (ECM), inhibition of capillary tube formation (CTF) by human umbilical vein endothelial cells

Results: We found the highest cytotoxic effect of Ch1. Incubation of Jurkat and HeLa cells with Ch1 at 1 mmol/L for 72h caused 87 and 45% reduction in cell survival, resp. Furthermore, it caused initial G_2/M arrest in both cell lines followed by an increase in the proapoptotic sub-G_0/G_1 fraction. Apoptosis was also confirmed by both methods.

From chalcones tested only Ch1 possess significant antiangiogenic effect. It completely inhibited CTF in concentrations 10^{-7} - 10^{-8} mol/L. Moreover, Ch1 in the same concentrations blocked also ECM..

Conclusions: The present study demonstrates antiproliferative and antiangiogenic properties of selected chalcones. Ch1 turned out to be the most effective agent of all.

Key words: chalcones - antiproliferative - antiangiogenic

This study was supported by VEGA grants 1/1176/04 and 1/3365/06

P300127

Kaempferol, a compound from Chinese medicine, potentiated relaxation in porcine coronary arteries via cAMP pathway and activation of potassium channel

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Objective: Kaempferol is found in Chinese medicine for the management of cardiovascular disorders. This study aimed to elucidate the vascular effects of kaempferol. Method: Isometric tension was recorded in isolated porcine coronary arteries using organ bath technique. Whole cell patch clamp was used to examine the action of kaempferol on membrane channel activity in human umbilical vein endothelial cells (HUVEC). Result: Kaempferol (3 mM) enhanced relaxation to bradykinin (0.01 nM- 1 mM), and this potentiating effect was abolished by SQ 22536 (an adenylyl cyclase inhibitor, 200 mM), Rp-8-Br-cAMPs (a cAMP blocker, 40 mM) and KT 5720 (a protein kinase A inhibitor, 0.4 mM). It also activated potassium channel in HUVEC. This action was inhibited by iberiotoxin or charybdotoxin (big conductance calcium-activated potassium (BKca) channel blockers, 0.1 mM).

Conclusion: Our results suggested that kaempferol exerted its vascular effects via activation of cAMP pathway and BKca channel.

Key words: cAMP, kaempferol; potassium channel.

Acknowledgement: The study was supported by the Institute of Molecular Technology for Drug Discovery and Synthesis, an Area of Excellence scheme under the UGC of HKSAR, China.

P300128

Combined inhibition of invasive behavior of metastatic human breast cancer cells by G. lucidum and green tea.

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The objective of the present study was to evaluate the combined effects of dietary supplements consisting of Ganoderma lucidum (GL) and green tea (GT) extracts on human breast cancer cells MDA-MB-231. The effect on growth was evaluated by the inhibition of cell proliferation (anchorage-dependent growth) and colony formation (anchorage-independent growth), whereas the effect on invasive behavior was evaluated by the inhibition of cell adhesion to vitronectin, cell migration and cell invasion through matrigel. GL as well as GT inhibited proliferation and colony formation of MDA-MB-231 cells in a dose-dependent manner, and these effects were profoundly enhanced by the combination of GL/GT. In addition, the combination of GL/GT demonstrated synergism against invasive behavior of breast cancer cells. The inhibition of cell invasiveness (adhesion, migration, invasion) is mediated through the urokinase-plasminogen activator (uPA), since GT, GL as well as GT/GL suppressed secretion of uPA. In summary, combination of G. lucidum and green tea extracts could be considered in the prevention/therapy of breast cancer.

Keywords: G. lucidum, tea, cancer.

Acknowledgment: This work was supported by Pharmanex LLC.

P300129

MOLECULAR-TARGETED ANTITUMOR NATURAL PRODUCTS: DISCOVERY OF HIF-1 INHIBITORS FOR BREAST CANCER

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The transcription factor hypoxia-inducible factor-1 (HIF-1) is a key regulator of tumor cell adaptation and survival under hypoxic conditions. Extracts of plants and marine organisms were evaluated using a T47D cell based reporter assay for inhibitors of hypoxia-induced HIF-1 activation. Extracts of the marine red alga Laurencia intricata and the aquatic plant Saururus cernuus yielded the structurally novel diterpene laurenditerpenol (IC_{50} of 400 nM) and the dinedignans known as manassantins (Manassantin B IC_{50} of 3 nM), respectively. Both series of compounds inhibit the hypoxic induction of the angiogenic factor VEGF protein in T47D cells. More than 40 naturally occurring lignans and other phenolic-based natural products were isolated and evaluated. Several structural and stereochemical features are essential for potent HIF-1 inhibitory activity.

Marine sponges also contain HIF-1 inhibitors. HIF-1 inhibitors may specifically target molecular/cellular processes specific to tumors.

Key Words: HIF-1, hypoxia, molecular target, natural products

Supported by NIH/NCI CA-98787-01; DOD PC040931; NOAA NURP/NUST NA16RU1496

P300130

Natural terpenoids inhibit the prdiferation and invasiveness of human breast cancer cells

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The objective of the present study was to evaluate the effect of Ganoderma lucidum and its isolated triterpenes on the growth and invasive behavior of breast cancer cells. The cell proliferation was evaluated by MTT assay and anchorage-independent growth by colony formation. Invasive behavior was assessed by cell adhesion, migration and cell invasion assays. Signaling pathways were evaluated by western blot, reporter gene and DNA-binding assays. Our results demonstrate that G. lucidum extract (GLE) and ganoderic acids (GA-H> GA-F> GA-A) suppressed proliferation as well as colony formation of MDA-MB-231 cells. This effect correlates with the downregulation of expression of cyclin D1 and Cdk4. GLE and ganoderic acids also inhibited invasive behavior through the suppression of Akt phosphorylation, resulting in the inhibition of transcription factors AP-1 and NF-kB, leading to the suppression of secretion of urokinase-plasminogen activator (uPA) from breast cancer cells. In conclusion, G. lucidum and its triterpenes could be considered in the prevention/therapy of breast cancer.

Key words: Breast cancer, G. lucidum, ganoderic acid, cell signaling

P300131

alpha-glucosidase inhibitory activity of the methanolic extract from Tournefortia hartwegiana: An antihyperglycemic agent.

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Tournefortia hartwegiana is a Mexican medicinal plant that is used for the treatment of diabetes, diarrhea and kidney pain. In a previous investigation, the methanolic extract of Tournefortia hartwegiana (METh) showed significant hypoglycemic and antidiabetic properties on normoglycemic and alloxanized rats. METh (310 mg/Kg) effect on alpha-glucosidase activity, METh intragastric administration was conducted to determine oral glucose tolerance test (OGTT), using different substrates. The increase in plasma glucose level was significantly suppressed by the extract after substrates administration. On the other hand,

METh inhibited alpha - glucosidase activity, in a concentration - dependent manner (IC50 of 3.43 mg/ mL) invitro. These results suggest that METh might exert its antidiabetic effect by suppressing carbohydrate absorption from intestine, and thereby reducing the postprandial increase of blood glucose. Finally, the bio - guided fractionation of these extracts led to the isolation of beta - sitosterol, stigmasterol, lupeol, ursolic acid, oleanolic acid, saccharose and myo - inositol, using various chromatographic techniques.

P300132

The protective effect of eupatilin on indomethacin - induced cell damage in cultured feline ileal smooth muscle cells : Involvement of HO- 1 and ERK

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Chronic users of nonsteroidal antiinflammatory drugs frequently develop ulcerative lesions in the intestines. This study investigated whether eupatilin, a pharmacologically active flavone derived from Artemisia plants, prevents such side effect in vitro. MTT assay shows that the treatment of cultured feline ileal smooth muscle cells (ISMC) with 2.5 mM indomethacin for 2 hr decreased the cell viability to 43%. Pretreatment with eupatilin exhibited concentration - dependent inhibitory effects on cell death induced by indomethacin. Pretreatment with cycloheximide, an inhibitor of protein synthesis, attenuated the effect of eupatilin, suggesting that some proteins induced by eupatilin are responsible for the cytoprotection. Heme oxygenase - 1(HO- 1), known as an antioxidant enzyme, is a candidate since western blot analysis revealed that eupatilin - mediated HO- 1 induction occurred in concentration - dependent manners. PD98059, a MEK inhibitor, attenuated the eupatilin - induced HO - 1 expression and the effect of eupatilin on indomethacin - induced cell death. The data imply that cytoprotective action of eupatilin is partly due to eupatilin - mediated HO- 1 induction via ERK signaling in ISMC.

Grant(KRF - 2005 - 050 - E00008)

P300133

Anti - inflammatory, anti - angiogenic and analgesic activities of Ulmus davidiana var. japonica

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Using the methanol extract (UDE) of Ulmus davidiana var. japonica, some of its pharmacological activities were in vivo and in vitro elucidated. UDE exhibited strong antioxidant activity when assayed by a stable free radical 1,1 - diphenyl - 2 - picrylhydrazyl (DPPH). In a dose - dependent manner, UDE displayed potent anti - inflammatory activity against carrageenan - induced hind paw edema in rats, an acute inflammatory model. UDE dosedependently displayed a strong inhibition in the chick chorioallantoic membrane (CAM) angiogenesis. UDE also suppressed production of exudates and nitric oxide, a proinflammatory mediator, in the rat air - pouch model of acute inflammation. Analgesic activity of UDE was dose - dependently confirmed using the acetic acidinduced writhing test in mice. UDE significantly reduced the production of NO and the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase - 2 (COX - 2) in the lipopolysaccharide (LPS) - stimulated RAW264.7 macrophages. The results suggest that UDE has anti - inflammatory and analgesic activities possibly via its down - regulating activity on iNOS expression and antioxidant activity.

P300134

Anti - inflammatory and anti - angiogenic activities of Gastrodia elata Blume

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Gastrodia elata Blume has been traditionally used as a folk medicine for centuries in Oriental countries. Its ethanol extract (GEE) and subsequent fractions were used to evaluate their anti - angiogenic, anti - inflammatory and related activities. GEE potently inhibited angiogenesis in the chick chorioallantoic membrane assays, and its BuOH fraction was most inhibitory among the fractions. In a dose - dependent manner, GEE inhibited vascular permeability induced by acetic acid. GEE and its BuOH fraction contained inhibitory activities on production of exudates, leukocyte migration and nitric oxide (NO) level in rat air pouch model. GEE caused a dose - dependent inhibition of acetic acid - induced abdominal writhing in mice. In addition, GEE inhibited NO production and iNOS expression upon stimulation by lipopolysaccharide (LPS) in RSW264.7 macrophages. In summary, we demonstrate some novel pharmacological activities of Gastrodia elata, such as anti - angiogenic, antiinflammatory and analgesic activities, and in vivo and in vitro inhibitory activity on NO production.

P300135

Effects of paeoniflorin on monoamine levels in mice and rat Brain using HPLC - microdialysis

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Paeoniflorin (PF), a principal component from paeony roots, has been used as an antispasmodic and analgesic agent. From our previous study, we found that paeoniflorin showed antinociceptive effect on both the writhing response test and formalin test performed in mice. In the present study, the effect of paeoniflorin on monoamine neurotransmitters and their metabolites was investigated by using HPLC- microdialysis in mice and rats. PF increased norepinephrine (NE) and 3, 4 - dihydroxyphenylacetic acid (DOPAC) content in cortex, and increased the content of NE and decreased serotonin (5 - HT) content in medulla of the homogenized brain tissue. By microdialysis, paeoniflorin increased DOPAC and 5 - hydroxyindoleacetic acid (5 - HIAA) content and increased homovanillic acid (HVA), DOPAC and 5 - HIAA content in anesthetic rat cortex and striatum, respectively. It turns out that PF could activate the release of monoamines and increase their metabolites in mice and rat brain, which might account for its antinociceptive effects.

Key Words : Paeoniflorin, Monoamine Levels, HPLC - Microdialysis.

Acknowlegment : This study was supported by a grant from the National Science Council, (NSC 88 - 2314 - B - 039 - 007).

P300136

The Effects of Crinum asiaticum on the apoptosis induction and the reversal of multidrug resistance in HL - 60/ MX2

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The present study investigated the antiproliferative and chemosensitizing effects of Crinum asiaticum var. japonicum against multidrug resistance (MDR) cancer cells. The crude extract, chloroform (CHCl3) fraction, and butanol (BuOH) fraction of the C. asiaticum inhibited the growth of HL - 60/ MX2, mitoxantrone (MX) resistant HL - 60 cells. When the HL - 60/ MX2 cells were treated with the CHCl3 fraction and the BuOH fration, DNA ladder and sub - G1 hypodiploid cells were observed. Furthermore, the fractions reduced Bcl - 2 mRNA levels, whereas Bax mRNA levels were increased. These results suggest that the inhibitory effects of C. asiaticum on the growth of the HL - 60/ MX2 may arise from the induction of apoptosis. Treatment of the HL - 60/ MX2 with the fractions markedly decreased the mRNA levels of multidrug resistance protein (MRP) and breast resistance protein (BCRP), and increased the MX accumulation. From the results, the fractions of C. asiaticum seem to play pivotal roles as chemosensitizers. Taken together, components of C. asiaticum might have a therapeutic potential for the treatment of MDR leukemia.

Key word: HL - 60/ MX2, Crinum asiaticum, apotosis, chemosensitizer

P300137

The Influence of Commercial Preparations of Stevia rebaudiana (Bertoni) on glucose metabolism in mice

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Hypoglycaemic effect of two commercial products of Stevia rebaudiana Bertoni in mice was ivestigated. One group of mice was pretreated four days with stevia 200 mg/ kg and the other with 20 mg/ kg of stevioside. The changes in glucose level were provoked by glucose - tolerance test (500 mg/ kg, p.o.) and subcutaneous injection of adrenaline (0.2 mg/ kg). The same procedure of measuring blood glucose was applied on the mice with alloxan - induced diabetes mellitus. Blood glucose levels in mice pretreated with stevia and stevioside were lower compared

with control. Also, a smaller increase in this parameter compared to control was registered with pretreated mice in the glucose-tolerance test, pretreatment with stevioside being again more effective.

Pretreatment with stevioside caused no significant increase in blood glucose concentration after administering adrenaline, which was not the case with the animals pretreated with stevia and control. Pretreatment with stevia, and to a greater extent with stevioside, protected test animals from the toxic action of alloxan compared with controls.

P300138

Antileukemic activity of the resins of the Commiphora **sp.**

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Extracts from the Commiphora sp. are known to possess antipyretic, anti-inflammatory and hepatoprotective properties. The effect of the resins from 8 different Commiphora species was tested for antileukemic and anticoagulant properties. The antileukemic effects of the resins was tested on the chronic myelogenous leukemic cell line, K-562, using the MTT (1-(4'5-dimethylthiazol-2-yl)-3,5-diphenylformazan assay. IC_{50} values ranging from 37 µg/ml to 823.63 µg/ml and IC_{90} values ranging from 55 to 1800 µg/ml were obtained. The nitro-blue tetrazolium stain was used to determine whether differentiation was induced. Resins from all species showed differentiation induction. The automated Coag-A-Mate machine was used to determine the direct effects of the resins on the blood coagulation pathways in human plasma. No significant effects on either the extrinsic or intrinsic pathway was evident. Furthermore, no effect on fibrinogen levels or anti-Factor Xa activity was displayed.

KEY WORDS: Commiphora, anticoagulant, leukemia

ACKNOWLEDGEMENTS: The author wishes to acknowledge the HE Griffin Cancer Trust and the Wits Faculty Research Committee for grants that supported this research.

P300139

Protective effects of berberine on hydrogen peroxide-induced injury in rat PC12 cells

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In this study, we investigated the protective effects of berberine on cell death, generation of ROS and elevation of $[Ca^{2+}]_i$ induced by H_2O_2 in cultured rat PC12 cells. The cells treated with 150 µM H_2O_2 for 6 h underwent cell death as determined by MTT evaluation. The level of lipid peroxidation and antioxidant enzyme activities were measured by assay kits and apoptotic death was tested by DAPI nuclei staining. Pretreatment with berberine (0.01 µM-10 µM) for 24 h prior to H_2O_2 exposure significantly elevated the cell survival and antioxidant enzyme activities and decreased the level of MDA. It also significantly prevented the cells from H_2O_2-induced apoptosis, ROS generation and elevation of $[Ca^{2+}]_i$. These results suggest that berberine has protective effects against free radical-induced cell toxicity, which has therapeutic potential in treatment of oxidative damage-derived neurodegenerative disorders.

Key words: Berberine; H_2O_2; PC12

Acknowledgement: This work was supported by grants from National Distinguished Young Scientists of China (30425024), National Natural Science Foundation of China (30570556) to Dr. Chen J, and partly from Young Science Dawn Projection of Wuhan (20045006071-11).

P300140

Pharmacological characterization of 7-hydroxymitragynine, an alkaloid from Thai medicinal plant Mitragyna speciosa **: Discovery of an orally active opioid analgesic**

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7-Hydroxymitragynine (7-OHMG) was isolated as an opioid analgesic from Thai herbal medicine Mitragyna speciosa. In this study, we investigated antinociceptive, tolerance and gastrointestinal effect of 7-OHMG. 7-OHMG (0.5–2 mg/kg, s.c.) produced antinociceptive effect in mice tail-flick and hot-plate tests. When orally administered, 7-OHMG (2-8 mg/kg) also showed potent effects. These effects were about 5 and 15 fold more potent than that of morphine after s.c. and p.o. administration, respectively. Antinociceptive effect of 7-OHMG was completely blocked by pretreatment with µ-opioid selective antagonist. Analgesic tolerance to 7-OHMG was developed as was seen with morphine. Cross-tolerance to morphine was induced in mice rendered tolerant to 7-OHMG and vice versa. On the gastrointestinal transit study, 7-OHMG (1–4 mg/kg, s.c.) dose-dependently inhibited gastrointestinal transit. 7-OHMG is less constipating than morphine at the equi-antinociceptive doses. In conclusion, 7-OHMG induced potent antinociceptive effects, especially oral administration, and less constipating than morphine. 7-OHMG has promising characteristic as a novel analgesic.

Key words: 7-hydroxymitragynine, opioids, analgesia, morphine

P300141

ANTIOXIDANT ACTIVITIES OF SOME PHENOLIC ACIDS AND THEIR COMBINATIONS

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Antioxidant activities of tr-cinnamic (tr-CIA), p-coumaric (p-COA), o-coumaric (o-COA), ferulic (FA) and caffeic (CAA) acids and their combinations were examined. Antioxidant activities were studied by Rancimat Method, and beta-carotene/linoleic acid system. Free radical-scavenging properties were evaluated against 2,2-diphenyl-1-picryhydrazyl radical (DPPH*). Results were compared to those of synthetic antioxidants, BHA and BHT.

In Rancimat test, the addition of tested phenolic acids (CAA > BHA > FA > BHT) in olive oil significantly extended the induction time of lipid oxidation. Order for the scavenging activities of phenolic acids was CAA > BHA > FA > BHT and for their combinations was FA + CAA > BHA > pCUA + CAA > BHT. Phenolic acids with two-OH groups or a-OH and a-OCH3 groups bonded to aromatic ring, such as CAA, FA and their combinations showed higher activities. The results show that the antioxidant and anti-radical activity of phenolic acids correlated positively with the number of-OH groups bonded to the aromatic ring.

Keywords: Antioxidant activity, phendic acids

This study was supported by Research Fund of Anaddu University (040308).

P300142

Composition of conjugated linoleic acid and fatty acid n-3/n-6 ratio in Japanese Aigamo duck

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Conjugated linoleic acid (CLA) and alpha-linolenic acid (n-3) have various bioactivities, such as decreasing effects on body fat contents and anti-thrombotic or anti-platelet aggregation. In addition, there is competition mechanism between n-3 and n-6 fatty acid, and so the n-3/n-6 ratio seems to be more important in nutrition.

There is no report on the fatty acid composition in Aigamo ducks (Japanese cross-breed of mallards and domestic ducks). In the present study, we established measuring method for CLA by capillary gas chromatography and found, for the first time, 9c, 11t CLA in fatty acids obtained from the duck (0.41 ±0.04 mg/g of lipid, n = 5), but not in those from chicken. Analysis of total fatty acid composition of the duck indicated that the n-3/n-6 ratio of the ducks is higher than that of chicken, suggesting Aigamo duck could be a healthy food material, since the ratio would be taken seriously rather than fatty acid contents in recent nutritional science.

Key words: fatty acid, CLA, n-3/n-6, Aigamo duck

A Grant-in-Aid for COE from the Ministry of Education, Culture, Sports, Science and Technology of Japan (E-1)

P300143

INHIBITION OF MAST CELL-DERIVED HISTAMINE RELEASE BY FLOS MAGNOLIAE

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A number of Flos Magnoliae (FM) species have been reported as substitutes or adulterants for commonly used FM, although the differences in their pharmacological actions have not been reported. We have studied the effects of six commonly used FM species, M. biondii, M. denudata, M. sprengeri, M. kobus, M. liliflora and M. sargentiana, on compound 48/80-induced histamine release in rat peritoneal mast cells (RPMC). FM samples were collected from China and Australia. All FM species showed significant inhibitory effects on histamine release from RPMC measured by HPLC. The potency of individual FM species depends on the test concentrations. At 0.01 - 0.1 μg/ml, M. kobus and M. biondii showed a similar but more potent inhibition than other FM species. At a higher concentration (0.5 μg/ml), however, the effects of M. biondi, M. denudata, M. sprengeri and M. kobus were partly reduced. M. sargentiana was the least potent among six FM species tested. The results indicate that M. kobus and M. biondii may act better than other FM species against mast cell-derived histamine release in PRMC.

Document: Inhibition of Mast cell.doc

P300144

Effects St. John's wort on HPA axis control in rats after short- term and long- term treatment

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We have shown recently that a methanolic extract of St. John's wort (SJW) and hypericin have delayed effects on the expression of genes that are involved in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. A consistent body of data in the literature suggests that, among the components of SJW extract, hyperforin is one of the major active principle. In the present study it was therefore of interest to examine if hyperforin and a hyperforin-enriched lipophilic extract have delayed effects on HPA axis control centers similar to those of the methandic extract and hypericin. We used in situ hybridization histochemistry to examine in rats the effects of short-term (2 wks) and long-term (8 wks) oral administration of fluoxetine, a lipophilic CO_2- extract, and hyperforin-trimethoxybenzoate (TMB) on the expression of genes that may be involved in the desensitization of the HPA axis. Fluoxetine (10 mg/kg), given daily for 8 weeks but not for 2 weeks significantly decreased levels of corticotropin-releasing hormone mRNA by 22% in the paraventricular nucleus (PVN) of the hypothalamus and tyrosine hydroxylase mRNA by 23% in the locus coeruleus. Nor the CO2-extract (27 mg/kg) neither hyperforin-TMB (8 mg/kg) altered levels of gene transcription in brain structures relevant for HPA-axis control. The present data suggest that hyperforin is not involved in the regulation of genes that control HPA axis function.

P300145

Role of the nitric oxide signaling cascade in the bidogical actions of traditional Chinese Medicine

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Objective: Nitric oxide (NO) is an endogenous vasodilator and its deficient production from the endothelium is associated with a reduced vasodilator tone in pathological conditions. Many medcinal herbs exert their effects through the NO signaling pathway. In this study, the involvement of the NO signaling cascade in the actions of several traditional Chinese medicines, including Radix et Rhizoma Rhei (RR) and Radix Bupleuri (RB), was analyzed. Methods: Porcine coronary artery endothelial cell line (PCAEC) and primary porcine aortic endothelial cells (PAEC) were used. Cellular release of NO and cGMP were assessed using Griess reaction and enzyme immunoassays, respectively. Cell viability was assessed by (4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium (MTT) assay. Results: RB extract (50 mg/ml) stimulated NO release from PCAEC and PAEC cells after 30 minutes incubation when compared to control, without affecting cell integrity. Conclusions: Our results suggested that RB extracts possess beneficial vascular effect with therapeutic potential against cardiovascular disorders.

Key words: Endothelium, nitric oxide (NO), cyclic 3',5'-guanosine monophosphate (cGMP), traditional Chinese medicine

P300146

PHENOLS, FLAVONES and FLAVONOLS IN SOME HERBAL TEAS IN TURKEY and THEIR ANTIOXIDANT ACTIVITIES

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Four herbal infusions and their extracts with different polarities have been studied for their polyphenolic contents and antioxidant activities: Melissa officinalis L. subsp. officinalis, Helichrysum orientale (L.), Rosa canina L. and Matricaria chamomillae L. Total phenolic content was determined spectrometrically according to the Folin-Ciocalteu method and calculated as gallic acid equivalents (GAE). In addition, the contents of total flavonoids and flavonols were measured spectrometrically in extracts. Antioxidant activitiy was studied in an aqueous emulsion system of beta-carotene and linoleic acid by measuring the absorbance of the samples. The free radicalscavenging properties were also evaluated against 2,2-diphenyl-1-picryhydrazyl radical (DPPH). Results were compared those of an synthetic antioxidant, BHT. Antioxidant effects were correlated with the total amount of phenolic compounds contained in the extracts. In all these cases higher antioxidant activity was seen in the samples with higher phenolic content

Key words: Herbal tea, antioxidant activity, phenolic compounds

This study was supported by Research Fund of Anadolu University (Project No. 30353).

P300147

The effection of extraction from Tibet Medcine SJMD on the single nucleus-macrophage of the mouse

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Object: The study is on the effection of the effective ingredient in Tibet Medcine SJMD on the single nuleus-macrophage of the mouse with differdrt dosage.

Methods: Using the method of the mouse tail intracenous injection Bennhold Test, to prove the effection of SJMDs chloroform extract on the swallow function of the single nuleus-macrophage of the mouse and the weight of immune apparatus before and after medcine supply.

Results: The different dosage of the SJMDs chloroform extract conspicuous increase the swallow function of the mouses single nucleus-macrophage and enhance the weight of the thymus and milt remarkably. It proves the effective ingredient of SJMD could enhance the function of the mouses cellular immunity.

Key words: Bolengguazi; Single nucleus-macrophage; Sallow function

P300148

What is anti-convulsive effect of Scutellaria baicalensis from

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In present study, we investigate activities of baicalein, oroxylin A and wogonin on convulsion related behaviors such as myorelaxation, motor coordination, chemical induced seizure, and electro-shock seizure in mice.

Baicalein, oroxylin A and wogonin were intraperitoneally injected to mice Animals administered wogonin and baicalein exhibited significantly lower locomotor activities than control, but oroxylin A did higher activities.

Wogonin significantly reduced enduring time on the Rotarod and the horizontal wire, but oroxylin A increased them. Oroxylin A delayed the on-set time of sleeping induced by thiopental and also shortened sleeping time.

This results mean that baicalein and wogonin possess sedative and myo-relaxative activities but oroxylin A have awakening effect. Wogonin and baicalein significantly blocked convulsion induced by pentylenetetrazole (GABA antagonist) and electro-shock, whereas, didn't it induced by strechnine (glycine antagonist). Wogonin and baicalein induced hyperpolarization, but oroxylin A did depolarization. This results indicate that sedative or anti-convulsive effect of baicalein and wogonin was mediated by the action on GABAergic neuron.

P300149

Genistein induces nitric oxide release from vascular endothelial cells

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Estrogen is known to exert vascular protective effect through stimulating endothelial nitric oxide (NO) production. Genistein is one of the major phytoestrogen

present in soybeans. The aim of the investigation is to elucidate the effect of genistein on endothelial NO production. ECV 304, a human umbilical vein endothelial cell line, was employed as a model. The cells were treated for various time intervals with genistein at 10 - 6 M, the concentration achieved in blood plasma after consuming soy containing diet. NO release into culture medium was quantified by a chemiluminescence based method, and endothelial nitric oxide synthase (eNOS) expression in ECV cells was quantified by Western blot. Genistein induced NO release by 91 ±20% (n = 6, p < 0.05) and 26 ±4% (n = 5, p < 0.05) after 30 min and 1 hr, respectively. eNOS expression was not significantly changed in incubation from 4 to 48 hr (n = 2). Our results demonstrated that genistein exerted a short - term stimulatory effect on NO release by vascular endothelium, most likely via activation of eNOS. This effect of genistein is similar to the non - genomic vascular action of estrogen.

P300150

Therapeutic Beneficial Effects of Unique Natural Antioxidants (NAO)

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In our lab, we extracted, isolated and characterized unique natural antioxidants (NAO) from spinach and other medicinal plant sources. Several of these compounds, were identified as flavonoids and p - coumaric acid derivatives. These natural products, exhibit beneficial therapeutic effects, in both in vivo and in vitro systems.

Using TRAMP and SCID mice models, we elucidated the efficacy of the NAO in both preventing and delaying of prostate cancer in these animals. The effect of NAO on the molecular mechanism and cell cycle was demonstrated using human PCA cell lines. It was found that the NAO cause cell - cycle prolongation.

The anti - inflammatory effect of the compounds was tested in LPS induced sepsis model. Moreover, the specificity of the NAO and purified compounds, on the activity of LOX5, COX1 and COX2 was examined.

Strong evidence was found, that several natural components, are selective inhibitors of COX2.

The potential outcome of this work is discovery of unique natural antioxidants that will be efficient in preventing inflammation processes, and may be used in treating cancer diseases.

P300151

The Molecular Mechanism of EA - 1 inhibited growth in Human cervical cancer HeLa cell

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Euphorbia antiquorum (EA - 1) significant S phase arrest in HeLa and Ca Ski cells. The comet assay confirmed that EA - 1 could lead DNA fragment outflow form HeLa cell. Reactive oxygen species (ROS) were increased after cells treated with EA - 1, cyclosporine A and Allopurinol could decreased the levels of EA - 1 - induced ROS. Form western blotting analysis, EA - 1 could decrease cyclin dependent kinases Cdk2 and cyclin B1, cydin E and cydin A. EA - 1 increased the cyclin - dependent kinase inhibitors p21waf1/cip1, P27Kip. EA - 1 could increase the ATM, CHK2 and decrease Cdc25A, Cdc25C, Bcl - 2 and ERK - P levels. EA - 1 increased the JNK - P and P38 - P levels. EA - 1 also increased Bid and Bax pathway and increase cleaved - caspase 8, cleaved - caspase 9, cleavedcaspase 3 and cytochrome C protein levels. Furthermore, EA - 1 - mediated caspase activation was blocked by SP600125, but lethality was not diminished by SB203580. In conclusion, we suggest ATM induced DNA injury might as the major possible mechanisms of EA - 1 - induced S phase cell cycle arrest and caspases activation might as the major possible mechanisms of EA - 1 - induced apoptosis in human cervical cancer Hela cell lines.

P300152

Antiatherogenic and antihypertensive efficacy of Intractum Visci and its fractions in rats

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The aim of the study was to assess the influence of an ethanolic extract of fresh mistletoe plant (Intractum Visci - PhytoPharm Kleka S. A) on blood pressure and lipid profile in hypertensive rats. We found out that Intractum Visci after repeated intragastric administration reduced blood pressure in renal hypertensive rats and in spontaneously hypertensive rats (SHR). Moreover, the 5 subfractions of the water fraction obtained from Intractum Visci lowered blood pressure in the SHR rats. Additionally, Intractum Visci affected serum lipid profile by lowering LDL - cholesterol and increasing HDL - cholesterol. In rat aorta it was accompanied by enhancement of glycerol ester hydrolase and cholesterol esterase action which are involved in the metabolism of triacylglycerols and acylcholesterols, respectively. Among compounds isolated from phenolic subfractions, the major ones were identified as malic acid, 3 - O - caffeoylquinic acid and 4 - O - caffeoylquinic acid. It is remarkable that malic acid and caffeoylquinic acids have been shown previously to exert at least hypotensive activity.

P300153

Delayed protective mechanism of tetramethlpyrazine on rat's cardiomyocytes subjected to anoxia reoxygenation injury

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Aim: To investigate the delayed protective mechanism of tetramethlpyrazine (TMPZ) preconditioning on rat's cardiomyocytes subjected to anoxia - reoxygenation (A/R) injury. Methods: The primary cultured neonatal rat cardiomyocytes were precondtioned using TMPZ 100 μmol L - 1 for 3 hours and subjected to A/R injury after 24 hours. Viability, NF - kB activity, TNF - αcontent, ultrastructure, HSP70 expression in myocytes, and the activity of LDH in medium were measured. Results: A/R injury caused the decrease in the viability and the increases in the contents of LDH and TNF - α as well as the activity of NF - kB; HSP70 was of low expressed and the cell ultrastructure was hurt seriously. TMPZ preconditioning, however, significantly attenuated these changes. Moreover, it up - regulated the HSP70 expression. There was no significant difference between heat shock and TMPZ preconditioning in these indices. Conclusion: The possible mechanism of delayed cardioprotection is that TMPZ preconditioning up - regulates the express of HSP70, inhibits NF - kB activity and decreases TNF - αcontent.

Keyword: Tetramethlpyrazine; anoxia - reoxygenation; delayed protection; cardiomyocyte

P300154

Cardioprotective Effects of Sodium Ferulate Pretreatment on Isolated Rat Heart Injury

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To study the effect of sodium ferulate (SF) pretreatment on isolated rat heart injury and its mechanism. Isolated rat hearts were perfused for 15 min in Langendorff mode, with Kreb - Ringer's solution containing SF 1.69 mmol L - 1 or SF 1.69 mmol L - 1 concomitantly HOE140 1 μmol L - 1, L - NAME 100 μmol L - 1 and glibenclamide 30 μmol L - 1, respectively; then subjected to A/R injury. Heart rate, coronary flow (CF), left ventricular pressure and its first derivative were recorded. The activities of LDH, GSH - Px, SOD, the contents of MDA, NO and cGMP in CF or myocardium, and the area of myocardial infarction were measured. The cardiac function of SF pretreatment improved significantly, presenting increases on cardiac muscle contractility, the activities of SOD and GSH - Px, and the contents of NO and cGMP, in contrast, decrement of the area of myocardial infarction and the contents of MDA. The protective effect of SF was attenuated distinctly by glibenclamide, L - NAME or HOE140. The opening of ATP - sensitive potassium channels induced by the cGMP NO pathway may be an important mechanism in the cardioprotective effects of SF.

Key words: Sodium Ferulate, Ischemic preconditioning, Isolated rat heart

P300155

Protective Effects of Tetramethlpyrazine Preconditioning Mediated by Bradykinin on Isolated Rat Hearts

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Aim: To study the preconditioning effects and mechanisms of tetramethlpyrazine (TMPZ) on isolated rat heart subjected to anoxia - reoxygenation(A/R) injury. Method: Isolated rat hearts were perfused in Langendorff mode, and with TMPZ 100, 200, 400 μmol L-1 or with TMPZ 200 μmol L-1 concomitantly HOE140 1 μmol L-1 for 15 min, then subjected to A/R injury. Heart rate, coronary flow (CF), left ventricular pressure and its first derivative were recorded. The activities of LDH, GSH-Px, SOD and the contents of MDA in CF solutions or myocardium, the area of myocardial infarction were measured. Results: TMPZ 100, 200, 400 μmol L-1 preconditioning could make heart functions improved, moreover, the activities of LDH, contents of MDA and the area of myocardial infarction decreased, whereas, the activities of GSH-Px, SOD increased on the heart subjected to A/R injury, but after treating with HOE140, the protective effects of TMPZ were mainly cancelled. Conclusion: TMPZ can induce the cardioprotective effects of pharmacological ischemic preconditioning and the mechanisms may be relative with the enhancement of the activity of bradykinin system.

Key word: Tetramethlpyrazine, Bradykinin, Isolated rat heart

P300156

Pharmacological Mechanisms Involved in the Vasodilator Effects of Aqueous Extracts from Leaves of Echinodorus grandiflorus

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We investigated the effects of aqueous crude extracts of E. grandiflorus in the model of thoracic aortic rings from New Zealand rabbits prepared for measurement of isometric tension. Increasing concentrations of the extract (0.03 - 1.0 x 10-3 mg/mcL) induced a significant and dose - dependent vasodilator effect in endothelium-intact but not in endothelium-denuded rings, reaching the maximum relaxation of 80 ±5 % of noradrenaline - induced contraction. The vasodilator effect was partially inhibited by L - NAME 100 mcM (46 ±3 %) and methylene blue 20 mcM (45 ±3 %). On the other hand, the pre - treatment with indometacin enhanced the vasodilator effect. Pretreatments with atropine 10 mcM, glibenclamide 3 mcM, charybdotoxin 100 nM and verapamil 10 mcM did not alter the vasodilator effect. Finally, a PAF receptor antagonist, WEB 2086 (10 mcM), also inhibited relaxation induced by the extract. In conclusion, the aqueous crude extracts of E. grandiflorus present a marked vasodilator activity, partially dependent on NO synthesis/release and activation of PAF receptors. Moreover, the vasodilator effect did not appear to be related to the activation of cholinergic muscarinic receptors or an action on Ca^{2+} or K^{+} channels.

P300157

QUALITY ASSESSMENT OF RADIX SALVIAE MILTIORRHIZAE

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Radix Salviae Miltiorrhizae (RSM) is the dried root and rhizome of Salviae miltiorrhiza Bge (Laminaceae), and one of the most commonly used Chinese medicinal herbs. RSM has different varieties and some of them are grown in Australia. The aim of this study was to assess the quality of Australian grown RSM by HPLC fingerprinting and marker component assays. A quantitative analysis method was developed and validated, which then applied to the quality assessment of RSM from different sources. Similar chromatographic fingerprinting pattern was observed for different samples. Nineteen peaks (eight lipid - soluble components and eleven water - soluble components) were separately and selected as characteristic peaks for authentication. The relative retention time of these characteristic peaks was established as an important parameter for the authentication of RSM. The marker compounds studied include cryptotanshinone, tanshinone I, tanshinonne IIA, and salvianolic acid B. The contents of marker components varied between different samples. The results indicate that HPLC fingerprinting profile can be used for quality assessment of local grown RSM species.

P300158

Taste and its Relevance to Pharmacological Properties

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Ability of mammals to distinguish bitter taste is believed to be evolved from the need to detect poisonous substances. Logically, there seem to be an association between bitter taste and biologically active compounds. Thus, we examined the tastes of different classes of clinically used drugs. It was found that while most classes of drugs exhibit inconsistent taste properties, some groups of drugs display bitter or dominantly bitter taste. Those include: some classes of antibiotics such as the macrolides, tetracyclines, quinolones; some antiviral drugs such as the protease inhibitors and the nucleoside reverse transcriptase inhibitors; most anti - malarial drugs. It is therefore possible that bitter taste is evolved to protect organism from eating plants which interfere vital enzyme systems. We have built the three - dimensional models for bitter taste receptors by homology modeling. Our molecular docking studies show that the some antibiotics bind to a similar site in the taste receptors. In traditional medicine, bitter herbs are said to be effective in relieving "heat" and "dampness". Thus, the scientific logic of screening bitter substance for antibiotic, and antiviral agents is considered.

P300159

Structural Similarity between Human Bitter Taste Receptors and Histamine H1 - Receptor

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Bitter taste is the self - protection mechanism against poisonous substances evolved in mammals. Use of bitter substances to relieve inflammation - like symptoms has been used in tradtional Chinese medicine. In order to investigate the relevance between bitter taste and anti - inflammatory properties, we have built the three dimensional models of the bitter taste receptors and human histamine H1 - receptor, which regulates the allergic and hypersensitivity reactions in the human body. The bitter taste receptors exhibit very high structural similarities to the histamine H1 - receptor. The root - mean - square deviations among the bitter taste receptors and between the bitter taste receptors and H1 - receptor are less than 1.5 ?. A hydrophobic binding pocket similar to the H1 - receptor substrate binding pocket is present in the bitter taste receptors. However, two basic residues Lys76 and Lys78, which can interact with polar functions groups of substrates, are adjacent to the hydrophobic binding pocket, implicating the bitter taste receptors may have a broader substrate spectrum than the histamine H1 - receptor. This suggests that H1 antagonists are likely to bind to the bitter taste receptors.

P300160

Pharmacological screening of Cochlospermum vitifolium Sprengel: A potential agent for the treatment of metabolic syndrome

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Cochlospermum vitifolium is a tree that is used for the treatment of hypertension, diabetes and liver diseases. We assess the pharmacological properties of different extracts from C. vitifolium bark for the treatment of these illnesses. The hexane extract (HECv) (intact endothelium: IC50 = 14.42 ±5.90 microgram/ml, Emax = 92.71 ±8.9 %; denuded endothelium: IC50 = 27.94 ±4.0 microgram/ml, Emax = 78.68 ±4.6 %) as well as methanol extract (MECv) (intact endothelium: IC50 = 21.94 ±6.87 microgram/ml, Emax = 79.12 ±7.80 %) showed a significant vasorelaxation on rat aorta rings. On the other hand, MECv (120 mg/Kg) also showed a significant decrease of blood glucose levels ($p < 0.05$) on normoglycemic rats. Furthermore, a MECv (100 mg/Kg) showed a statistical decrease of serum glutamic pyruvic transaminase (GPT, 45 %) and alkaline phosphatase (APh, 15 %) ($p < 0.05$) in bile duct - obstructed rats. Finally, we isolated (§) - naringenin from MECv. Results suggest that C. vitifolium could be used as a potential agent against metabolic syndrome since it shows these pharmacological properties.

P300161

Studies on the Efficacy and Safety of Phlai gel

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Phlai (Zingiber cassumunar Roxb.) has long been used as an anti - inflammatory component in Thai traditional medicine. The safety and efficacy of Phlai gel, containing 10 % of Phlai extract for the treatment of inflammation were evaluated. Phlai gel could reduce croton oil - induced mouse ear edema and in carrageenan - induced rat hind paw edema as effectively as piroxicam gel, a standard drug. It caused minimal skin irritation when tested using OECD method. Repeated application of the gel using mouse ear irritation model did not cause skin irritation. It was non - allergenic when tested using Buehler's method. Subjective irritation

reaction was not observed when tested in the guinea pig model. The anti-inflammatory activity of Phlai gel applied topically in traumatic patients was comparable to that of piroxicam gel with regard to reduction in swelling size, redness score and pain relief. It was concluded that Phlai gel was a safe and effective anti-inflammatory preparation for clinical use.

P300162

The effects of oxymatrine on mice alimentary motor activity in vivo

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Objective: Our study was going to investigate the effects of oxymatrine on alimentary motor activity. Methods: The alimentary charcoal powder propelling model was used to test the motor activity of the mice. The drug was administered to the animals per os once a day and continuous for 3 days. After 30 minutes of the last administration the 5% charcoal powder was given per os. Then animal's intestine was separated after 20 minutes, and the length of the propelled charcoal powder was measured. Results: With the doses of 50 mg/kg, 25 mg/kg, oxymatrine could promote the charcoal powder propelling distance. The atropine and Morphine could not antagonize the increased alimentary motor activity. The alimentary charcoal powder propelling rate went to zero after used Ephedrine in this model. Conclusions: The oxymatrine could promote the alimentary motor activity in mice, and this alimentary motor activity of oxymatrine might be related to the receptor of the adrenergic receptor.

Key words: alimentary motility, oxymatrine.

Acknowledgement: Thanks for the Shandong Engineering Research Center of Natural Drug to provide the oxymatrine.

P300163

Butea superba: effects on penile erection and sperm

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Butea superba has been traditionally used to treat age-related problem including erection disorders. This study aimed to investigate effect of B.superba alcoholic extract on penile erection in aged male Sprague Dawley rats. The animals were pre-treated with the extract at various doses and the cavernous nerves were electrically stimulated. The intracavernous pressure was simultaneously recorded from the beginning. Sperm count was performed using a hemocytometer. Sperm motility was investigated in modified TCM199 medium. The results show that B.superba extract enhanced the penile erection with the most effective dose of 1 mg/kg B.W. Higher doses did not increase the erection. In addition, B.superba significantly increased the number of sperm and prolonged the motility of the sperm. These results suggest that B.superba is effective in penile erection and may be useful in the treatment of erectile dysfunction as well as in fertility.

Key words: Butea superba, penile erection, erectile dysfunction

Acknowledgement: We thank the Faculty of Pharmaceutical Sciences, Naresuan University and Mae Fah Luang University for financial support.

P300164

Pharmacological actions of GBE50, a new standardized preparation for Ginkgo biloba extract

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GBE50, a new preparation for Gingko biloba extract, has been granted of the category II Certificate of New Medicine by the State Drug Administration of China and several international patents, i.e. ZL95111763.7, U.S. Patent No. 6030621, etc. A series studies on different levels were performed to investigate its pharmacological actions. The effects of GBE50 were checked on two animal models with cerebral ischemic injury induced by middle-cerebral artery occlusion and cardiovascular injury by hyperlipemia, observing the pathological changes, biochemical parameters, expressions of TNF-α, IL-1β, HSP70 and caspase-3, etc. The cultured mice cerebral endothelial cell and rat cerebral neuron were used with determination of NOS, ET, SOD, etc. The rat brain mitochondrial function, cytochrome C release, anti-oxidation ability were examined. The influence of GBE50 on gene expression was determined by the gene chips with 13000 rat genes. The results proved that the GBE50 can produce good protection on different levels, showing its good prospect in the clinical application.

Key Words: GBE50, animal models, cell culture, mitochondria.

Acknowledgement: This study was funded by China "863" Project (2003AA2Z2032).

P300165

KFW, a traditional Chinese medicine remedy, protects SHSY-5Y neuronal cells against ischemia insult

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Cumulative evidence suggests that the Chinese herbal medicine might play a role in the prevention or treatment of cerebral ischemia. The aim of the present study was to investigate the effects of KFW, a Chinese medicine remedy, on preconditioned ischemia on neuronal cells. SHSY-5Y cells were cultured in glucose- and serum-free DMEM, and placed into an anaerobic chamber containing a gas mixture of 5% CO_2, 10% H_2, and 85% N_2 at different time courses to mimic cerebral ischemia. From our preliminary results, treatment with KFW dose dependently increase the viability of ischemic neuronal cells by MTT assay. Moreover, KFW reversed the increase of reactive oxygen species (ROS) and the decrease of the mitochondrial membrane potential (MMP) during ischemia insult. However, it did not significantly change intracellular calcium accumulation during ischemia insult. In addition, KFW did modify the expression of some caspases in the apoptotic cell death pathways. These data indicate that KFW could be used to protect neurons against ischemia.

Key words: Chinese herbal medicine remedy- KFW, SHSY-5Y neurons, ischemia.

P300166

ARACHIDONOYL-SERINE (ARA-S), A NOVEL BIOACTIVE LIPID MEDIATOR WITH VASCULAR PROTECTIVE PROPERTIES

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N-arachidonoyl-L-serine (ARA-S) is a recently identified endocannabinoid-like lipid with vasodlatory properties and which causes in vitro inhibition of ROI and NO formation in macrophages. In view of the preliminary results we tested this compound for cardioprotective activity.

Dose-response curves for left ventricular developed pressure (LVDP) were constructed to ARA-S, in isolated Langendorff-perfused rat hearts. After ischemic shock of 30 minutes and a 40 minutes reperfusion, ARA-S treated hearts had significantly increased LVDP as compared to controls. The infarct size was significantly smaller in hearts which underwent treatment with ARA-S as opposed to those untreated.

ARA-S is a novel member of a family of natural products which comprise an important emerging scientific field, that of biologically active lipids which are related to endocannabinoids. These molecules are important in a variety of physiological conditions; they may as well be involved in the homeostasis and protection of the cardiovascular system.

Key words: N-Acylethanolamines, Langendorff, endocannabinoids, bioactive lipids.

P300167

Hypocholesterolemic effects of Curcuma comosa in cholesterol-fed rabbits

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Hypercholesterolemia plays pivotal role in the pathogenesis of atherosclerosis. Recent study reported that antiinflammatory agents reduced plaque formation and improved vascular function in hypercholerdemic animal.

Curcuma comosa (CC) is used in folk medicine as anti-inflammatory agent. We investigated the effect of CC on cholesterol (C) levels, vascular function, TBARS and plaque formations in cholesterol-fed rabbits. Rabbits were fed with

C, C + simvastatin C + extract of CC or normal rabbit chow for 12 weeks. Plasma total C, LDL- C, HDL- C, triglyceride concentrations and TBARS formation were analyzed every 4 weeks. 12 weeks after the treatment the rabbits were sacrificed, vascular function and aortic plaque formation were determined. We found that extract of CC significantly lowered the cholesterol levels, TBARS formation, reduced aortic plaque formation and improved vascular function. The results suggest that CC possesses hypocholesterolemic effect, preserves the vascular function and retards atherosclerotic plaque formation. However, the mechanisms of their actions need further study.

Key words: Curcuma comosa, cholesterol, TBARS, aortic plaque

This work was supported by Thai NRC 2548

P300168

ESTROGENIC EFFECTS OF LYCOPENE AND BETA- CAROTENE ON ER(+) HeLa AND LNCaP CANCER CELLS

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Lycopene (lyc) is an anticarcinogenic and chemopreventive carotenoid bioactive compound which is present primarily in tomatoes. Estrogen receptor (+) prostate cancer cell (LNCaP) and cervix adenocarcinoma cell (HeLa) were prefer for testing estrogenic effects of lyco and beta- carotene. NIH3T3 fibroblast cells were used as control of normal tissues. MTT assay were performed as cytotoxic activity tests and mitochondrial activities of cells. Lyc and beta- carotene were applied at five different doses onto cells. Lyc gave rise to increase of mitochondrial activity of HeLa cells as opposite to that of in LNCaP and NIH3T3 cells. Beta- carotene was cause to increase of mitochondrial activity of LNCaP as opposite to that of in HeLa. Beta- carotene has no significant effect on NIH3T3 cells. It can be said that lyc can have estrogenic effect by inducing mitochondrial activity of HeLa cells and causes to decrease of mitochondrial activity of LNCaP cells similar to estrogen. It can be explain that because of estrogenic activity of lyc it can show anticarcinogenic effect on prostate cancer. Beta- carotene can't show any important anticarcinogenic effects on LNCaP and HeLa cells.

Key words: Lycopene, cancer, cell culture

P300169

Curcuminoids exhibit prophylactic effect on atherosclerosis in cholesterol- fed rabbits

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Curcuminoids, a group of phenolic compounds isolated from the roots of Curcuma longa Linn, exhibit a variety of beneficial effects on health and in preventing certain diseases. This study was aimed to examine the potential prophylactic effect of curcuminoids on experimental atherosclerosis in rabbits. Rabbits were fed diet containing no additive, 1% cholesterol or 1% cholesterol with 100 mg/kg/day of curcuminoid extract for 12 weeks. Plasma lipid levels were determined every 4 weeks. Endothelium- dependent vascular relaxations in isolated aortic rings, the severity of atherosclerosis in the thoracic aorta and the resistance to copper- mediated LDL oxidation in vitro were assesses after 12 weeks. Curcuminoid treatment produced significant reduction of atherosclerotic lesions, preserved impaired acetylcholine- mediated endothelium- dependent relaxations, increases the resistance of isolated LDL to copper- mediated oxidation in vitro with no significant change observed in levels of plasma lipids.

The results indicate that curcuminoid improves endothelial- dependent vasodilator function and prevents the development of aortic atherosclerosis in cholesterol- fed rabbits via reduction of vascular oxidative stress.

P300170

EFFICACY OF CHELATOR ALONGWITH ANTIOXIDANTS AGAINST BERYLLIUM INDUCED TOXICITY

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Therapeutic potential of Tiferron (Sodium- 4,5- dihydroxy- 1,3- benzene disolphonate) was evaluated in combination with α- Tocopherol, Piperine and Propolis against beryllium toxicity. Female albino rats were exposed to beryllium nitrate 1 mg/kg (ip) once a day daily for 28 days followed by therapy with Tiferron (300 mg/kg,ip), individually and in combination with α- Tocopherol (25 mg/kg,po), Piperine (10 mg/kg,po) and Propolis (200 mg/kg,po) respectively for 5 consecutive days after toxicant administration. Results revealed significant depletion in activity of SALP, while significant elevation was noticed in AST, ALT, LDH and γ- GT after toxicant administration. Significant rise was noticed in LPO and decrease in reduced GSH in liver and kidney. Tiferron in combination with Propolis exerted statistically more beneficial effects rather than other combinations to reverse alterations in the markers of oxidative stress and liver function tests concluding its therapeutic potential in treatment of beryllium induced toxicity.

Key words: Beryllium toxicity, Tiferron, Combination therapy.

P300171

Anti- diabetic activity of 3- O- methylursolic acid

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The anti- diabetic activity of 3- O- methylursolic acid (3- OMU) isolated from Euonymus alatus (EA) ether fraction was examined. 3- OMU augmented a glucose- stimulated insulin secretion from rat pancreatic islets in a dose dependent fashion. The mechanism to increase insulin secretion of 3- OMU was found out to be associated with ATP- sensitive K+ channel blockade, similar to the sulfonylurea. In a dose dependent manne, 3- OMU also inhibited a phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression in a H4IIE hepatoma cell, which was stimulated by cyclic AMP and dexamethasone. In addition, 3- OMU potentiated PPAR- gamma mRNA expression by 1.5 times compared to control 3T3- L1 adipocytes. Taken together, 3- OMU is expected to show the anti- diabetic effect through stimulating insulin secretion as well as ameliorating insulin resistance, and deserves to in vivo and human trial in future. Key words: 3- O- methylursolic acid; insulin secretion; insulin resistance; PEPCK; PPAR- gamma. This work was funded by Plant Diversity Research Center of 21st Century Frontier Research Program

P300172

THE INFLUENCE OF ANISE, CARAWAY, CORIANDER AND FENNEL ESSENTIAL OILS ON PENTOBARBITONE EFFECT

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The appearance of common usage of various herbal preparations in everyday practice and life imposes the question of possible interactions with drugs. The aim of this survey was to examine the influence of essential oils derived from anise, caraway, coriander and fennel on pentobarbitone induced sleeping time in mice. The animals were divided according to pretreatment regime (peroral application of 0.1 mL/kg of particular essential oil emulsion for p.o. use, during 5 consecutive days) into 5 groups: control (water) and anise, caraway, coriander and fennel group. Pentobarbitone (40 mg/kg) was intraperitoneally injected 2h after the oil application on 5th day. Pretreatment with all essential oils produce changes in pentobarbitone induced sleeping time and anise essential oil results in significant decrease of it. Regarding the fact that essential oils alone do not induce sleep and that their usage produce changes in pentobarbitone effect, we can conclude that the interactions between drug and fitopreparations containing these essential oils should be additionally examined.

Key words: pentobarbitone induced sleeping time, essential oils, anise, caraway, coriander, fennel.

P300173

Effects of quindic and anthraquindic compounds from Ventilago harmandiana Pierre on the production of inflammatory mediators in activated macrophages

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Aquinone and anthraquinone compounds from the heartwood of Ventilago harmandiana Pierre exhibited strong anti- inflammatory activities in the mouse ear edema model. The aim of study was to investigate whether anti- inflammatory activities of these compounds are mediated through the inhibition on TNF- alpha

and PGE2 production in activated human macrophages. Their effects on COX-2 and TNF-alpha mRNA expression were also investigated. TNF-alpha and PGE2 secretion from activated macrophages were measured using ELISA and EIA, respectively. The mRNA level was determined using RT-PCR. These compounds inhibited TNF-alpha and PGE2 production in activated human macrophages in a concentration and time-dependent manner without cytotoxic effects. Their mRNA expression was significantly inhibited by these compounds. These findings suggest that the inhibitory effects of the quinone and anthraquinone compounds from V. harmandiana on the production, in activated human macrophages, of TNF-alpha and PGE2 might be attributed, in part, to their anti-inflammatory activities.

Key words: Ventilago harmandiana, macrophage, TNF-alpha, PGE2
This research was supported by Government Funds

P300174

ALT-711 ameliorates diabetic renal injury in db/db mice through inhibition of NADPH oxidase-derived reactive oxygen species

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ALT-711, an advanced glycation end-products breaker, has been shown to attenuate renal injury in experimental diabetes. Since oxidative stress plays an important role in the development of diabetic nephropathy, we examined the effect of ALT-711 on oxidative stress in diabetic kidney. ALT-711 (2 mg/kg/day) was administered intraperitoneally for 12 weeks to 8-week-old db/m and db/db mice or for 4 weeks in 16-week-old db/db mice.

Mouse mesangial cells were stimulated with high glucose (HG) with or without ALT-711. Both early and delayed treatment with ALT-711 significantly attenuated renal expression of pentosidine, NADPH oxidase subunit, and nitrotyrosine proteins and features of diabetic nephropathy. In mesangial cells, ALT-711 effectively prevented HG-induced membrane translocation of NADPH oxidase subunits and generation of reactive oxygen species (ROS). ALT-711 was also found to directly scavenge H_2O_2 in test tube. Thus, the present study demonstrates that ALT-711 can prevent and reverse renal injury in a model of type 2 diabetes, in part, through inhibition of NADPH oxidase subunit activation and NADPH oxidase-derived intracellular ROS.

P300175

Apoptosis induction by acridone alkaloids and effects on reversal of multidrug resistance

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Aim: Acridone alkaloids constitute a small group of natural products found exclusively in the family Rutaceae. In the present work the anticancer activity of 9 acridone alkaloids (7 furanoacridones and 2 additional compounds) are characterized including apoptosis inducing and multidrug resistance (MDR) reversal capacity.

Methods: The antiproliferative effect of the tested drugs was determined by MTT assay using human cell lines (MCF7, HeLa and A431). MDR reversal activity was measured by rhodamine accumulation test on a Pglycoprotein expressing mouse lymphoma cell line. Apoptosis induction was proved by specific staining.

Results: Some of alkaloids have comparable cytostatic effect with the positive controls. Acridone alkaloids were found to induce apoptosis. Gravacridondiol and gravacridontriol inhibit P-glycoprotein substantially and have the capacity to increase the effect of other anticancer agents.

Conclusion: Our results indicate that acridone alkaloids can be a starting point of development of anticancer agents having both direct cytostatic and MDR reversing actions.

Key words: apoptosis, acridone alkaloids, multidrug resistance

P300176

Bacopa monniera Linn, a candidates for cognitive enhancer and neuroprotective agent against Alzheimer's disease

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Bacopa monniera Linn. has been widely used for various neurological disorders in traditional medicine for a long time. Recently, it has gained much attention due to its reputation as cognitive enhancer. In the present study, the effect of B. monniera on cognitive function both in healthy condition and in Alzheimer's disease were examined. Male Wistar rats were orally administered the alcoholic extract of B. monniera at various doses ranging from 20, 40 and 80 mg/kg B.W. The results showed that the extract significantly improved the cognitive function in healthy rats. The extract pretreatment for 2 weeks before the induction of Alzheimer's disease by bilateral injection of AF64A, acholinctoxin, via intracerebroventricular route could attenuate the cognitive impairment in Alzheimer's disease. These findings suggest that B. monniera may be a useful neuroprotective and therapeutic agent for Alzheimer's disease.

P300177

Neuroprotective effects of Ganoderma lucidum in human neuroblastoma SH-SY5Y cells.

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Ganoderma lucidum, an oriental fungus, is widely used for the promotion of health and longevity. Ganoderma lucidum has been shown to possess potent antioxidant activity with little or no side effects. The aim of this study was to investigate the effects of Ganoderma lucidum mycelium extracts on (1) neuronal cell viability, (2) neuronal cell differentiation and (3) neuronal cell protections. We used human neuroblastoma SH-SY5Y cells for studying these effects. Hydrogen peroxide was used to induce neuronal damage. Results showed that Ganoderma lucidum mycelium extracts had no cytotoxic effect. though it inhibited the growth of SH-SY5Y cells in a concentration dependent manner. In addition, they induced the neuronal cell differentiation and protected neuronal cells from hydrogen peroxide-induced damage. Our data demonstrate that the presence of neuroactive compounds in Ganoderma lucidum mycelium extracts that can induce the SH-SY5Y cell differentiation and protect SH-SY5Y cells from neuronal damage. Our results are compatible with the results from Cheung WM, etal. (FEBS Lett 2000; 486: 291-6), using rat pheochromocytoma PC12 cells.

P300178

BIOLOGICAL ACTIVITY DE BOERHAAVIA ERECTA L.

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A watery extract (EA) of Boerhaavia erecta L. was evaluated as antiviral by means of ultramicroanalític detection of the antigen of surface of the virus of the hepatitis B (HBsAg); as hepatoprotector in model of hepatic damage induced by tetrachloride of carbon in rats, antialérgico in isolated organs stimulated with histamina and acetilcolina and in bio models of bronchial spasm and cutaneous passive anafilaxia (APC) and antioxidant by means of the inhibition of lipid peroxidation, ferric reducing activity of plasma and scavering of 1-diphenyl-2-picrylhydrazyl. The EA inactived the HBsAg in standard serum and it suppressed the formation of the same one in the line PLC/PRF/5. The histopatology and values of activity of alarino amino transferasa show significant differences for the dose of 500 mg/kg. It was observed antagonistic properties of the receivers of the histamine as much in isolated íleon as in challenged guinea pigs and the APC was inhibited in rats. Also a good correlation between the percent of inhibition of malonildialdehído liberation and the logarithm of the concentration of the EA. The demonstrated biological activity, potentially linked, to antioxidant mechanisms, it grants to the EA of B. erecta, therapeutic interest in hepatic dysfunctions and al-

lergic processes.

Key words: Boerhaavia erecta L., antiviral activity, hepatoprotective activity, antihistaminic activity, antioxidant activity.

P300179

In Vivo Effects of Bifidobacteria on Aflatoxin B1 Absorption and Mutagencity

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This study was to investigate the in vivo protective effect of the dietary Bifidobacterium adolescentis 15 (Bifi) on aflatoxin B1 (AFB1), based on the assumption that the carcinogen binding effect of bifidobacteria evident in vitro may reduce the absorption and/or mutagenicity of AFB1. Thirty rats in 5 groups were either coadministered with 3 doses of Bifi and AFB1 or treated with AFB1 or vehicle only. Blood and feces were sampled before and after the treatment of the AFB1 for 25 days. The absorbed and excreted AFB1 was measured in blood and feces using liquid scintillation counting; the mutagenicity of the AFB1 was determined with the peripheral blood micronuclated reticulocyte frequencies using flow cytometry. The area under the curve between treatment groups over the sampling period was compared statistically. Except for the Bifi $5x10^{10}$ cfu/kg bw, which reduced blood AFB1 significantly (K-W Test, $p < 0.05$), no other difference was found between coadministration and AFB1 alone, and no dose-response was observed between the 3 Bifi doses. The absence of elimination and inhibition on AFB1 indicates the lack of the in vivo protection by the tested bifidobacteria.

Key words: bifidobacteria, aflatoxin B1, in vivo

P300180

RESVERATROL INHIBITS CONTRACTIONS TO ANGIOTENSIN II IN RAT AORTA

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Angiotensin II (AII) increases NAD(P)H oxidase activity which has been proposed as source of superoxide in vasculature. The aim of this study was to investigate the effect of long-term resveratrol treatment on the contractions to AII and NAD(P)H oxidase activity of aortic rings with endothelium from male rats.

Isometric tension was recorded in isolated aortic rings. Superoxide production was measured by lucigenin-enhanced chemiluminescence. Rats were administered resveratrol 50 mg/L in the tap water for 3 weeks orally. Dose-response curve for AII (10^{-10}-10^{-5} M) was obtained in aortic rings. Long-term resveratrol administration significantly decreased maximum contraction (E_{max}: 112.5 % vs 77.9 %) and sensitivity (EC_{50}: $1.1x10^{-8}$ M vs $1.6x10^{-8}$ M) to AII. Resveratrol (1, 10 uM) also significantly decreased AII, NAD(P)H and NADH-stimulated superoxide productions, comparable with that of DPI, in rat aorta. In conclusion, the results showed that resveratrol inhibits AII-induced contraction and NAD(P)H-derived superoxide formation demonstrating that decreased superoxide formation by resveratrol is positively correlated with decreased contraction to AII in rat aorta.

Key words: Angiotensin, resveratrol, contraction, superoxide

P300181

HYPOCHOLESTEROLEMIC EFFECT OF GREEN TEA AND ANTIOXIDANT EFFECT OF EPIGALLOCATECHIN GALLATE (EGCG) FROM GREEN TEA

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Green tea is known to have a positive effect in human health. The mechanism of action involves inhibition of lipid peroxidation by catechins of the tea, mainly EGCG. We aimed to evaluate the effect of green tea consumption on the lipid profile of healthy volunteers and the effect of specifically EGCG on the citotoxicity induced by hydrogen peroxide (H_2O_2) in isolated human fibroblasts. After oral administration of 1500 ml/day of green tea for 30 days, the lipid profile of 15 human volunteers was determined. Also, human fibroblasts in culture were subjected to H_2O_2 (3 mM) and EGCG in concentrations 0.03-0.3 mM, to evaluate cell viability (MTT assay). The consumption of green tea for 15 days resulted in a significant decrease in the levels of total cholesterol and LDL, but this effect was abolished after 30 days of treatment. EGCG showed a concentration-dependent-protection against cellular injury induced by H_2O_2 in human fibroblasts. In conclusion, green tea consumption seems to have an acute beneficial effect in human lipid profile and this seems to be mediated, at least in part, by the antioxidant effects of EGCG.

Key Words: tea, epigallocatechin gallate, antioxidant, hypocholesterolemic

P300182

Alkaloids from medicinal Geissospermum species inhibit serotonin (5HT) uptake by rat hippocampal synaptosomes.

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Several plants sold as Pão-Pereira, bitter South American medicinal species, are described as tonic, anticholinergic, sedative and anti-malaria. The Apocinaceae Geissospermum laeve Vell. Baill. is the most reputed yielding the alkaloids geissospermine (GSP), flavopereirine (FLP) and geissoschizoline (GSCh). This study focus on putative CNS activity of these alkaloids describing effects on [^{3}H]-5HT uptake by synaptosomes from rat hippocampal homogenates in 0.32 M sucrose. After 10 min incubation of the alkaloids (10^{-9}-10^{-5} M) with synaptosomes (0.5 mg protein mL^{-1}), at 37 °C, 4 nM [^{3}H]-5HT were added for 6 min and the specific radioactivity uptake measured in a β-counter comparatively to the effect of imipramine (IM, 10^{-10} -- 10^{-5} M). The results indicated that the compounds inhibited the amine uptake, FLP (IC_{50} = 1 µM) being less active than GSP (IC_{50} = 12 µM), GSCh (IC_{50} = 10 µM) and IM (IC_{50} = 60 nM). It is concluded that the alkaloids may potentiate the action of serotonin released from presynaptic hippocampal neurons mimicking the effect of antidepressive-like agents.

Key words: serotonin uptake - flavopereirine - geissospermine - alkaloid

Grants: FADA-UNIFESP, CNPq and FAPESP-BRAZIL

P300183

Molecular interaction of Geissospermum's alkaloids with α7 or muscle-type nicotinic receptors (nAChR) subtypes and with acetylcholinesterase (AChE).

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The action of bitter tropical Geissospermum species used in folk medicine for liver illnesses, malaria and occasional fever has been attributed to their alkaloids (Alk). G. laeve yielded geissospermine (GSP), geissoschizoline (GSCh) and flavopereirine (FLP) which in a general screening blocked nAChR noncompetitively and inhibited ChE. Interactions of the Alk with α7 nAChR from rat whole brain or muscle-type nAChR from diaphragm muscle (DIA), and with AChE from rat striatum homogenates were studied comparatively to galantamine (GAL) at α7 and AChE. Competition binding assays used [125I]-bungarotoxin (2 nM, 60 min, 25 °C); AChE activity was measured with the thiocholine method at 25oC. The Alk relative IC50 (uM) for the specific toxin binding at α7/DIA were: GSP (400/35); GSCh (602/100); FLN (145/54) and GAL (>104 M). For the AChE the IC50 (uM) were 100, 100, 5 and 0.8, respectively. The data indcated 1) the Alk affinities for DIA are low but higher than for α7 nAChR; FLP was the most active. 2) the Alk are weak AChE inhibitors, the most effective being FLP which was comparable to galantamine.

Key words: Nicotinic receptors – cholinesterase - alkaloids

Grants: FADA-UNIFESP, CNPq and FAPESP-Brazil

P300184

Effects of Liuwei Dihuang decoction on the balance of hypothalamus-pituitary-ovary axis

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Effects of Liuwei Dihuang decoction (LW) on the function of the hypothalamus-pituitary-ovary (HPO) axis were investigated. Radioimmunoassay was employed to quantify the level of estradiol. The level of luteinizing hormone (LH) was determined by Western blot. The results showed that the estrus cycle, as well as the diestrus of stress-loaded mice was significantly prolonged. Meanwhile, the level of pituitary LH in stress-loaded mice was significantly decreased, but the level of serum estradiol significantly elevated. Oral administration of LW showed significant reversal effects on the levels of pituitary LH and serum estradiol. Injection of corticosterone (CORT) decreased the levels of pituitary LH and

serum estradiol. Administration of LW showed significant improving effect on them. Our results indicated that hanging stress and CORT treatment both induced the imbalance of HPO axis, and LW was effective in restoring the balance of HPO axis.

Key words: Liuwei Dihuang decoction; hypothalamus - pituitary - ovary axis
Acknowledgement: This study was supported by the 973 Project of China (G1999054401, 2004CB518907) and the National Natural Science foundation of China (30200367).

P300185

Effect of Danggui Shaoyao Suan on cognition of Senescence - Accelerated Mice and long - term potentiation in rat hippocampal slices

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Danggui Shaoyao Suan (DSS), a traditional Chinese medicine prescription, have been proved to be effective to alleviate cognitive dysfunction in treatment of Alzheimer's disease (AD). However, the underlying mechanism is far from clear. In the present study, we observed the effect of DSS on learn and memory function in senescence - accelerated mice prone 8 (SAMP8), which is thought to be a useful model of human aging and AD.

After 3 month orally administration of DSS, the learning and memory ability of SAMP8 were ameliorated in the Morris water maze test, step down and step through test. Then the effect of DSS containing serum (DSSCS) on long - term potentiation (LTP) of CA1 subfield in rat hippocampal slices was studied. The results showed that DSSCS not only significantly enhanced LTP induction in normal slices but also ameliorated the inhibition of LTP by b amyloid. These results suggested that enhancing synaptic plasticity is one of mechanisms by which DSS alleviates cognitive dysfunction in AD.

Key Words: Danggui Shaoyao Suan; learning and memory; SAMP8; long - term potentiation
Acknowledgement: This work was supposed the Chinese National Key Project of Basic Research (2004CB518907).

P300186

The effects of oxymatrine on SD rat intestine motility in vitro

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Objective: To investigate the effects of oxymatrine on small intestine motor activity. Methods: 30 mm length of small intestine near the duodenum was cut immediately after the animals were knocked. Using tension sensor and transducer we measured the small intestine tone and contraction waves. Results: after administered the oxymatrine, the contraction amplitude and frequency increased markedly, and the increased contraction waves were related to the drug concentration. When the drug concentration up to 2 mg/ml in the infusion fluid, the intestine tone present lower for about 2 min, and then the High amplitude contraction waves persistent present. The atropine could not block this excited motor activity. Conclusions: the oxymatrine could increase the small intestine motor activity, and this effect could not be interrupted by atropine.

Key words: intestine motility, oxymatrine.
Acknowledgement: Thanks for the Shandong Engineering Research Center of Natural Drug to provided the oxymatrine

P300187

Intramuscularly administration of oxymatrine promotes the alimentary motility after enterorrhaphy of SD rats

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Objective: to investigate the effect of Oxymatrine on alimentary motility after enterorrhaphy of SD rats. Methods: after fasted for about 16 h, the animals were administered baralyme per os. Then anesthetized with chloral hydrate, the rat's abdomen was exposed via a midline incision. The enterotomy was carried out at the intestine 10 mm above the cecum, and the incision was closed with sutures. After administered the oxymatrine intramuscularly the animals were returned to their cages for recovery. The drug was continuously given once a day and until to 3th days after the operation. Results: with the doses of 25 mg/kg and 50 mg/kg, the first white defecation time was much earlier in drug treated groups than in control group. And the 3 days whole stool weight was much increased compared to the control. These results demonstrated that the oxymatrine could promote the alimentary motility after the enterorrhaphy of SD rats.

Key words: alimentary motility, oxymatrine, enterorrhaphy.
Ackonwledgement: Thanks for the Shandong Engineering Research Center of Natural Drug to provide the oxymatrine.

P300188

Green tea extract and its major polyphenol improve muscle function and resistance to stress in a mouse model for Duchenne muscular dystrophy

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Duchenne muscular dystrophy is a lethal muscular disorder caused by mutations in the gene for dystrophin, a cytoskeletal protein that contributes in the stabilization of muscle membrane. Here, the dystrophic mdx5Cv mouse model was used to investigate the effects of green tea extract, its major component (-) epigallocatechin gallate, and pentoxifylline on dystrophic muscle. Three - week old mdx5Cv mice were fed for either 1 or 5 weeks a control chow or a chow containing the test substances.

Histological examination showed a delay in necrosis of the extensor digitorum longus muscle in treated mice. Phasic and tetanic tensions of treated mice were increased, reaching values close to those of normal mice. Phasic to tetanic tension ratioes were also corrected. Finally, muscles from treated mice exhibited 30 to 50% more residual force in a fatigue assay. These results demonstrate that diet supplementation of dystrophic mice with green tea extract or epigallocatechin gallate protect muscle against necrosis, and stimulate muscle adaptation towards a stronger and more resistant phenotype.

P300189

Pharmacological research of (-) - epigallocatechin - 3 - gallate chelating with zinc for chronic renal failure

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There have been few effective chemicals applied to medicine involved in chronic renal failure (CRF) by so far.

Pharmacological research of (-) - epigallocatechin - 3 - gallate chelating with zinc (E05) in experimental CRF rat models (i.g. adenine, 5/6 nephrectomy) was studied in this paper. The renal function was measured by serum urea and creatinine (Cr), SOD and MDA in serum, liver and kidney, and pathologic alteration of kidneys. Cr level of the two models of CRF rats were significantly decreased after administration of E05 (6, 18, 54 mg/kg). E05 resulted in significantly lower Cr and urea in renal of CRF rats. SOD in blood, liver and kidney were raised and MDA were declined significantly. E05 had slight effect on albumin and cholesterol in serum, liver and kidney.

Pathologic lesion in E05 administration groups were significantly lessened in CRF rats induced by adenine intragastric administration. E05 exerts protective activity in rats with chronic renal failure, resulting in the improvement of renal function, against stress lesion and reducing the pathologic impairment.

Key words: (-) - epigallocatechin - 3 - gallate chelating; zinc; chronic renal failure

P300190

Cryptotanshinone inhibits cyclooxygenase - 2 enzyme activity but not expression

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Cryptotanshinone (CT), one of major constituents of tanshinones extracted from medicinal herb salvia miltiorrhiza Bunge, has been well - documented as antioxidative and anti - inflammatory effects. This study confirmed remarkable anti - inflammatory effect of CT in carrageeman - induced rat paw edema model. Since the action of CT on cyclooxygenase - 2 (COX - 2) has not been previously described, in the present study, we further examined the effect of CT on cyclooxygenases activities in the exogenous arachidonic acid (AA) - stimulated insect sf - 9 cells, which highly expressed human COX - 2 or human COX - 1, and on cyclooxygenases expression in the lipopolysaccharide (LPS) plus phorbol myristate acetate (PMA) - activated human U937 promonocytes. CT prevented the prostaglandin E_2 synthesis and reactive oxygen species generation catalyzed by COX - 2, without influencing COX - 1 activity in cloned sf - 9 cells. In PMA plus LPS - activated U937 cells, CT revealed negligible effects on expression of COX - 1 and COX - 2, on either mRNA or protein level. These results demonstrated that anti - inflammatory effect of CT is selectively direct towards enzymatic

activity of COX-2, but not towards the transcription or translation of the COX-2 genes.

Keywords: cryptotanshinone; Cyclooxygenase-2; prostaglandin E_2; reactive oxygen species

P300191

Polydatin protects brain tissues from ischemia-reperfusion injury via inhibition of cell adhesion molecules

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Objective: To evaluate the effects and mechanisms of polydatin in a model of focal ischemia reperfusion injury relevant to stroke. Methods: Rats were subjected to transient middle cerebral artery occlusion (MCAO) and reperfusion according to the intraluminal thread model. We assessed the neurological deficits of rats 24h postischemia in a blind fashion. After sacrifice, infarction volumes of the brain slices were calculated, furthermore, we determined the expression of cell adhesion molecules (CAMs) through immunohistochemistry and gene chips. Results: Neurological deficits and infarction volume 24h after reperfusion were significantly improved by polydatin. Moreover, we found that polydatin treatment was associated with a reduction in expression of CAMs, in particular ICAM-1, VCAM-1, L-selectin and Integrin α5. Conclusion: These results suggest that polydatin may be a potential agent for treatment of brain injury associated with stroke by inhibition of the expression of various CAMs.

Keywords: Middle cerebral artery occlusion; ischemia/reperfusion; polydatin; cell adhesion molecules

Acknowledgment: This study was supported by the National Natural Science Foundation, No: 30472178.

P300192

Effects of Leonotis leonorus aqueous extract on the isolated perfused rat heart.

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The aim was to determine the effect of Leonotis leonurus (LL) aqueous extract on the isolated perfused rat heart. ADR (1μM) significantly ($p < 0.05$) increased the LVSP by 40.6 ±2.67 mmHg, the LVDP by 43.90 ±3.49 mmHg and the HR by 22.49 ±5.58beats. DIG (2.5 ng/ml), significantly ($p < 0.05$) increased the LVSD by 9.46 ±5.04 mmHg, the LVDP by 9.65 ±5.11 mmHg and the HR by22.49 ±5.58bpm. LL (1.0mg/ml and 2.0mg/ml respectively) significantly ($p < 0.05$) increased the LVSP by 25.36 ± 8.10 mmHg, and 14.91 ±7.18 mmHg, the LVDP by 29.40 ±2.11 mmHg and 14.88 ±2.11 mmHg. L.L. also decreased the HR by 34.73 ±3.70bpm and 42.71 ±8.02 mmHg respectively. ADR effects reflect its positive inotropic and chronotropic effects. Digoxin shows a weaker positive inotropic effect and has little effect on the HR. At low concentrations LL produced a positive inotropic effect and a negative chronotropic effect. At higher concentrations (2.0mg/ml) LL dropped the values of all parameters to zero. It appears that at this concentration it contains constituents with toxic effects on the heart.

Key words: Leonotis leonurus, isolated perfused heart

Acknowledgement: The project is funded by the National Research Foundation (Grant # 2069540)

P300193

The reasearch on the bioactivities of betaine

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ABSTRACT: Object: to study the effect of betaine on EGF receptor and the lipotropic effect of betaine in hepatic steatosis induced by ethand in rats. Methods: using radioligand binding assay of receptor, comparing the binding of 125I-EGF to its receptor between the test group and the control group; Using the HPLC to determine the levels of S-adenosylmethionine in the rat liver cells to compare the differences between groups. Results: 26nmolL-1-5.2mmolL-1 betaine inhibit the binding of EGF receptor in a noncompetitive way, 0.5% betaine in the diet prevented hepatic steatosis induced by chronic dietary feeding. And promote the generation of Sadenosyl methionine compared with control group dramatically($P < 0.05$). Conclusion: betaine can inhibit the binding of EGF receptor and it has the ability to prevent the hepatic steatosis induced by ethanol.

KEY WORDS: betaine, epidermal growth factor(EGF receptor), S-adenosylmethionine

ACKNOLEDGEMENT: Thanks for the Committee of National Natural Science Foundation of China, by which the project is supported. (NO.30400352, NO. 30300284)

P300194

Involvement of GSK-3beta and DYRK1B in differentiation-inducing factor-3-induced phosphorylation of cydin D1 in HeLa cells

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Differentiation-inducing factors (DIFs) are putative morphogens that induce cell differentiation in Dictyostelium discoideum. We reported that DIF-3 activates glycogen synthase kinase-3beta(GSK-3beta), resulting in the rapid degradation of cydin D1 protein and slow reduction of cyclin D1 mRNA in HeLa cells. In this study, we investigated the effect of DIF-3 on cydin D1 mutants (Arg29Gln, Leu32Ala, Thr286Ala, Thr288Ala and Thr286/288Ala) to clarify the precise mechanisms by which DIF-3 degrades cyclin D1 in HeLa cells. We revealed that the phosphorylation of Thr286 and Thr288 were critical for cydin D1 degradation induced by DIF-3. Indeed, DIF-3 markedly elevated the phosphorylation level of cyclin D1, and mutations introduced to Thr286 and/or Thr288 prevented the phosphorylation induced by DIF-3. Depletion of endogenous GSK-3beta and dual-specificity tyrosine-phosphorylation regulated kinase 1B (DYRK1B) by RNA interference attenuated the DIF-3-induced cydin D1 phosphorylation and degradation. These results suggest that DIF-3 induces degradation of cydin D1 through the GSK-3beta- and DYRK1B-mediated threonine phosphorylation.

Key words: cydin D1, DIF, GSK-3beta, DYRK1B

P300195

Areca nut extract modulate amyloid precursor protein (APP) expression in vitro and in vivo

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Arecoline, an acetylcholine agonist and a major component of areca nut, has been shown with therapeutic potential for Alzheimer's disease (AD). AD is a neurodegenerative disease and characterized by the amyloid β (Aβ) deposits in the brain. The Aβ is formed after the cleavage of amyloid precursor protein (APP) by β and γ-secretase. In this report, we tested the hypothesis that areca nut extract (ANE) modulates the expression of APP. By using SK-N-SH neuroblastoma cells, arecoline treatment increased soluble APP (sAPP) levels as shown by Western blots with monoclonal antibody 22C11. On the other hand, ANE and fANE (ANE without arecoline) decreased sAPP levels with the same treatment protocol. However, oral administration of ANE to guinea pig at 2 and 10 mg/kg/day for 5 days significantly down regulated sAPP in CSF and total APP in hippocampus. Nevertheless, arecoline, ANE and fANE inhibited the aggregation capability of Aβ1-40 in vitro. This indicates that ANE down regulate the expression of APP in vivo, and this effect may not relate to arecoline. This study suggests that both ANE and arecoline modulates sAPP through APP processing but may through different pathways.

P300196

Protective Effect of Chinese Traditional Drugs on Acute Lung Injury Induced by LPS in Mice

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To build the experimental acute lung injury model and investigate the effects of some Chinese traditional drugs on acute lung injury in mice. Acute lung injury model was induced by lipopolysaccharide(LPS) intratracheal instillation in KM mice. Some Chinese traditional drugs were administrated 4 hours after LPS instillation. The protease activity and protein concentration in bronchoalveolar lavage (BAL) and the cells in the blood and BAL were measured after 24h LPS instillation. After given LPS, the number of the cells in the blood was not obviously changed; But in BAL, the number of leukocyte(WBC), neutrophil(GRAN) and lymphocyte(LYM), the protease activity and the protein concentration were significantly increased. Injections of Shengmai(SM), Shuanghuanglian, Chuanxiongqin(CXQ), Yinzhihuang, Fufangkushen, Xueshuantong and Yuxingcao could decrease the increased number of WBC, GRAN and LYM. The activity of protease was markedly depressed by SM, CXQ and Xingnaojing(XNJ). The pro-

tein concentration was markedly decreased by XNJ. The eight drugs all showed a protective effect on the acute injured lung induced by LPS in mice.

Key words: Chinese traditional drug; acute lung injury; LPS; protective effect

P300197

ANTIOXIDATIVE EFFICACY OF PROPOLIS EXTRACT AGAINST OXIDATIVE DAMADE INDUCED BY CARBON TETRACHLORIDE

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The purpose of this investigation was to explore whether or not propolis extract could prevent the hepatic damage caused by model toxicant i.e. carbon tetrachloride (CCl_4). Antioxidative efficacy of propolis extract was evaluated against acute (1.5 ml/kg, ip, once only) and subchronic (0.15 ml/kg, ip, 21 days) exposure to CCl_4. Toxicant exposure provoked marked elevation in the activities of serum transaminases, alkaline phosphatase and lactate dehydrogenase. Significant increased lipid peroxidation was observed after toxicant exposure. Drastic alterations were observed in enzymatic and non-enzymatic antioxidant defense system, which were estimated by reduced and oxidised glutathione, glutathione peroxidase and glutathione reductase in liver. Initial screening of Propolis extract at different doses (50, 100, 200 and 400 mg/kg, once only, po) revealed recoupment in acute study and found to be very effective in restoring all the parameters. Treatment with effective dose of Propolis extract (200 mg/kg, po) for 5 days after subchronic exposure of toxicant caused significant recovery. It was observed that Propdis exerts its beneficial hepatoprotective effect as a natural antioxidant due to the presence of polyphenols and flavonoids.

Keywords: Propolis, GSH cycle, CCl_4.

P300198

Protective effect of Daidzein against impairment of learning and memory induced by cerebral ischemia reperfusion in mice

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Numerous investigations indicated that the extract of Radix puerariae, Puerariae Isoflavone (PIF), significantly ameliorated the microcirculation and protected the neurons from the damage of cerebral ischemia. The protective effects of Daidzein (DZ), one of components of PIF, on the learning and memory impairment induced by cerebral ischemia –reperfusion (CIR) in mice were studied in this paper. The results showed that the administration of DZ (50 ~100 mg·kg^{-1}) reduced numbers of errors and prolonged the latency in step-down test and step-through test in mice performed CIR. In water maze test, the latency to find the terminal platform was decreased and the numbers of right reflect was increased in CIR mice with DZ. The increase of nitric oxide (NO) and enhance of nitric oxide synthase (NOS) activity in mice performed CIR were significantly prevented by administration of DZ. These results indicate that DZ has the effect of improving learning and memory impairment in mice performed CIR as one effective component of PIF and the regulation of NO and NOS activity may contribute to the protective effect.

Key words: Daidzein; Cerebral ischemia-reperfusion; Learning and memory

P300199

Effect of ethanol extract of Xanthoceras sorbifolia Bge's shell on learning and memory in impaired rats by bilateral carotid common artery occlusion

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We investigated the improvement effect of ethanol extract of Xanthoceras sorbifolia Bge's shell (XSBS) on learning and memory in rats performed bilateral carotid common artery occlusion (BCCAO). Oral administration of XSBS (31.4 ~125.7 mg.kg-1) started from 2nd day of the experiment. Y maze and Morris Water maze task were used to evaluate the learning and memory function of rats and the alterations in hippocampus morphology were assessed. The results showed that after administration of XSBS for 10 days the escape latency in directional swimming and working memory trial was shortened and in probe test the swimming time and distance in the target quadrant were prolonged and the numbers across the area of the platform were increased in rats with BCCAO. XSBS improved neurodegenerative changes and reduced the death of nerve cells in hippocampus. These results suggest that XSBS has improvement effect on learning and memory impairment in rats performed BCCAO and may be useful for the treatment of patients with learning and memory impairment.

Key words: Xanthoceras sorbifolia Bge; bilateral carotid common artery occlusion; learning and memory

P300200

Search for Anti-inflammatory & Anti-diabetic Agents from Australian & Chinese Medicinal Plants & Ethno-pharmacology Information

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Emerging evidences show that diabetes are inflammatory diseases. New drugs inhibiting cyclooxygenases (COXs) and activating peroxisome proliferator-activated receptors (PPARs) would be desirable. We report a systematic approach to search for the dual-action agents from medicinal plants of Australia and China. Forty Australian and 57 Chinese plants were recorded in two MS Access datasets after cross-cultural comparison of ethno-pharmacological information selected for their anti-inflammatory and anti-diabetic use in TCM and Australian Bush Medicine. From the datasets 29 species were selected for lab studies and 23 were shown to inhibit COXs; six also inhibited 5-lipoxygenase and phospholipase A_2. Further studies led to the discovery of a novel active racemosic acid from Ficus racemosa. Extracts and fractions from 3 Clematis species inhibited COXs and activated PPARs. It is anticipated that this systematic approach will increase the chance of finding dual anti-inflammatory and anti-diabetic agents from medicinal plants.

P300201

Effect of Puerariae Isoflavone on improving learning and memory impairment in rats performed bilateral carotid common artery occlusion

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We investigated the improvement effects of Puerariae Isoflavone (PIF), one of the extracts from Radix puerariae, on learning and memory impairment in rats with bilateral carotid common artery occlusion (BCCAO rats). Step-through test, eight-arm radial maze task and Morris water maze task were used to evaluate the learning and memory function of BCCAO rats. The results showed that oral administration of PIF (280-840 mg/kg) significantly improved the spacial learning and memory deficits, increased the activity of lactic dehydrogenase (LDH) and calcium pump (Ca^{2+}-ATPase) and decreased the content of lactic acid (LA) in the cerebrum of the BCCAO rats. The administration of PIF for 37 days also significantly reduced the histological lesions in the cortex and hippocampus CA3 region of BCCAO rats. These results suggest that PIF has the effect of improving learning and memory impairment in BCCAO rats and the improvement of the brain metabolism may be involved in the mechanism.

Key words: Puerariae Isoflavone; learning and memory; bilateral carotid common artery occlusion

P300202

Study of the pathway of apoptosis induced by arsenic trioxide in cancer cells

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ABSTRACT AIM To investigate the possible role of the mitochondrial transmembrane potential ($\Delta\psi_m$) and caspase3 in arsenic trioxide (As2O3) induced apoptosis of cancer cells. METHOD Namalwa, SGC7901 and Bcap37 cell lines were used as in vitro models. Apoptosis was confirmed by sub-G1 cells content as well as phosphatidylserine (PS) externalization. The $\Delta\psi_m$ was detected on flow cytometry through double staining of Rhodamine 123 (Rh123) and popidium iodide (PI). In addition, the effect of DEVD-CHO, a selective inhibitor of Caspase 3, on As2O3-induced apoptosis was studied. RESULT The As2O3 induced apoptosis closely associated with the externalization of the $\Delta\psi_m$ and the activation of Caspase 3. As2O3 induced cells necrosis when Caspase3 was inhibited. CONCLUSION As2O3 may selectively activates Caspase 3 after it induces externalization of $\Delta\psi_m$, which causes cancer cells apoptosis.

KEY WORDS: arsenic trioxide; apoptosis; Caspase3

P300203

Theaflavin ameliorates cerebral ischemia-reperfusion injury in rats through its anti-inflammatory effect and modulation of STAT-1

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Theaflavin a major constituent of black tea, possesses biological functions such as the anti-oxidative, anti-viral and anti-inflammatory. The purpose of this study was to verify whether theaflavin reduces focal cerebral ischemia injury in a rat model of middle cerebral artery occlusion (MCAO). Male Sprague-Dawley rats were anesthetized and subjected to a middle cerebral artery 2h occlusion and then a 24h reperfusion. Theaflavin administration (25 mg and 50 mg/kg, i.v.) ameliorated infarction by 40% ±9% and 62% ±8%, respectively. Theaflavin inhibited leukocyte infiltration, and expressions of ICAM-1, COX-2 and iNOS in injured brain. Phosphorylation of STAT-1, a protein which mediates intracellular signaling to the nucleus, was enhanced 2-fold over that of sham group and was inhibited by theaflavin. Our study demonstrated that theaflavin significantly protected neurons from ischemia reperfusion brain injury by limiting leukocyte infiltration and expression of ICAM-1, and suppressing upregulation of inflammatory-related prooxidative enzymes (iNOS and COX-2) in ischemic brain via, at least in part, reducing the activation of STAT-1.

[Key words] theaflavin; MCAO; COX-2; STAT-1

P300204

Effects of Ginkgdic acids on killing snail Oncomelania hupensis as new molluscicides

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This study was to check the effects of ginkgolic acids (GAs) extracted from the ginkgo exocarps on killing Oncomelania hupensis, the main intermediate for schistosomiasis. GAs is a group of 6-alkylsalcylic acids. The GA15:1, GA13:0 and rough extract of ginkgo exocarps were dissolved in alcohol first, and then diluted with natural water to different concentrations. The molluscicide niclosamidum (Nic) was used as the positive control.

The snails collected from a schistosome epidemic area in China were put into beakers containing 30 ml different concentrations of GA15:1, GA13:0, rough extract, Nic, 1% alcohol, or natural water. The snail mortality was recorded in 24 h and 24 h. The LD50 for GA15:1 (60%) was 20.46 mg/L (R=0.9568) and the LD50 for GA13:0 (95%) was 14.51 mg/L (R=0.9549). And the rough extract of ginkgo exocarps containing 5.46% GAs can kill all the snails in the concentration of 62.5 mg/L. The study on mechanism showed that GAs produced uncoupling effects on the snail mitochondrial oxidative phosphorylation.

#: China Patent Application Number 200610024040.5

Key Words: ginkgolic acids, Oncomelania hupensis, Schistosomiasis, molluscicide

P300205

Principium definition effective fraction of a herb and study of its procoagulant activity

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Objective To investigate the isolation, extraction, analysis of the effective fraction of a herb and blood coagulant activity in vitro and in local wound surface. Methods Part of the impurities were removed from the initial water extract by infusion extraction method in acetone-water (1:1) solution; Advanced purification was achieved by chloroform extraction and silicagel column chromatography. TLC was employed to determinate constituents of tannins, chromocors, alkaloids, organic acids, amino acids, volatile oils in the extract. The study on hemostatic property of the extract in wound surface (skin, liver, femoral artery) and in vitro was established in rabbits and Wistar Rats. Results The Extract was mostly composed of tannins (70.34%), a small quantity of organic acids and trace alkaloids. The clotting time was obviously shortened compared with positive control and saline group ($p < 0.001$). Conclusion The extract, determined by TLC, contained mostly tannins, a little organic acids and trace alkaloids. Chloroform extract played effective procoagulart in vitro and wound hemostatic role in our experiments, and it is effective fractions of this herb.

Key words: herb, extraction, TLC, coagulation test

P300206

Estrogenic extracts from Cajanus Cajan L. ameliorate ovariectomy-induced bone loss

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Purpose: we identify the effects of the extracts from Canjanus cajan on HOS TE85 osteoblast-like cells, marrow derived-osteoclast-like cells, and ovariectomy-induced bone loss rats. Method: By using MTT assay to test cell proliferation, ^{3}H-proline incorporation to investigate the formation of collagen, and by measuring the alkaline phosphatase (ALP) to evaluate the bone formation on HOS TE85 cell. Bone marrow cells were cultured to examine ECC's effects on the derivation of osteoclast cells. In vivo: 2 weeks after the ovariectomy, drugs were given to rats through stomach for 8 weeks, rats were killed, bodies and uterus were weighted. Serum estradiol, FSH, LH concentrations were measured. And Femoral morphology were observed. Results: In HOS TE85 cell, both ^{3}H-proline incorporation and cell count increased significantly after the treatment of ECC. derivedosteoclast cells were appeared much less. In vivo: we found improved femoral morphology in OVX rats, without affecting estradiol level. Conclusion: The ECC inhibited bone resorption, stimulated osteoblast acivity, and ameliorated bone loss on OVX rats.

P300207

Effect of Qingdaikeli on ulcerative colitis in rats and its mechanism

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We observed the effect of Qingdaikeli (QDK, traditional chinese medicines) on ulcerative colitis induced by trinitiobenzene sulfonic acid (TNBS) in rats and investigated its mechanism. The administration of QDK started from the 3rd of the experiment and serum TNF-α was detected by enzyme-linked immunosorbent assay (ELISA) on the 10th day of the experiment. On the 24th day, IL-1β and IL-4 levels were determined by ELISA and the content of adhesion molecule CD54 was determined with flow cytometer. The results showed that after administration of QDK (300~1200 mg.kg^{-1}) the serum level of TNF-α and IL-1β was significantly decreased, the level of serum IL-4 was increased and the level of colonic CD54 was decreased compared with model group.

The macroscopical observation showed that the ulcer area was reduced and histological examination revealed decrease of the infiltration of inflammatory cells into both the mucosa and sub mucosa in QDK-treated rats. In conclusion, QDK has protective effects on ulcerative colitis in rats and this effect may be caused by lowing the level of serum TNF-α, IL-1β and colonic CD54 and raising the level of IL-4.

Keywords: Qingdaikeli; ulcerative colitis; TNF-α; CD54

P300208

Discovery and pharmacology of conopressin-T, a novel Vasopressin-like peptide from Conus tulipa.

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The original discovery of two analogues of vasopressin from Conus venoms (named conopressins) was based on the "scratching" effect induced by these peptides upon intracerebral injection into mice. Conopressin-S was isolated from C. striatus, while conopressin-G was isolated from C. geographus venom. Here we describe the discovery and pharmacology of a novel new conopressin, conopressin-T (Con-T), isolated from the venom of C. tulipa. Con-T has a novel sequence that differs at two highly conserved residues found across the vasopressin-like peptide family. Synthetic Con-T and [L7P]-Con-T were tested for activity at the vasopressin/oxytocin receptor family using a radioligand binding assay to determine affinity and selectivity. Functional binding studies were also performed using a ERK phosphorylation based assay. The novel sequence of Con-T produced an interesting selectivity profile at the vasopressin/oxytocin receptor family.

P300209

D(+)β-3,4-Dihydroxyphenyl Sodium Lactate Increases Ischemia Induced Cell Proliferation and Survival in the Dentate Gyrus of Adult Gerbils

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Recent studies demonstrated that dentate neurogenesis increased after transient global ischemia and it is suggested that the increased neurogenesis contributes to the recovery of hippocampal function. D(+)β-3,4-Dihydroxyphenyl Sodium

lactate (DHPL) is a chemical compound isolated from the traditional Chinese herb Salvia miltiorrhiza Bge. Previous experiments in our laboratory demonstrated that DHPL has neuroprotective effect on cerebral ischemia brain injury in rats. In the present study, adult Mongolian gerbils were chronically treated with DHPL after ischemia, and the proliferation of cells in the dentate gyrus was examined. It was proved that bromodeoxyuridine (BrdU)-labeled cells in the dentate gyrus were significantly enhanced in number fdllowing DHPL treatment after 6 min global ischemia. In addition, the number of surviving BrdU-positive cells 40 days after ischemia also increased markedly in the DHPL group. This suggests that DHPL delivered to the brain well after stroke may have therapeutic benefits.

P300210

Protective effect of Veratrum nigrum var. ussuriense alkaloids on hepatic ischemia-reperfusion injury in rats.

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AIM; To investigate the protective effects of Veratrum nigrum L. var ussuriense Nakai alkaloids (VnA) on hepatic ischemia-reperfusion (I/R) injury in rats.

METHODS: Male Wistar rats were assigned into (1) sham operation group; (2) I/R group; (3) and (4) VnA treatment group(8,16μg/kg). Hepatic I/R injury was induced by 90 min ischemia and 4 h reperfusion. VnA was administered intraperitoneally 30 min before operation. The hepatocellular injury, oxidative stress, neutrophil recruitment were measured. The expression of liver ICAM-1 and E-selectin were performed..

RESULTS: Hepatic I/R injury was characterized by the histological evidence of liver edema, hemorrhage, PMN infiltration and elevated serum levels of AST and ALT. MPO activity significantly increased and the liver oxidant product were observed in high level. These changes were parallel to the positive expressions of I-CAM-1 and Eselection.

After administration of VnA, the histological evidence of liver injury was improved. The overexpressions of liver ICAM-1 and E-selection were suppressed

CONCLUSIONS: VnA ameliorates liver injury induced by IR.

KEY WORDS: hepatic ischemia-reperfusion; VnA; rats:

P300211

Effects of Taohong Siwu Decoction II in CAM assay and on B16 melanoma in mice and endothelial cells ECV304 proliferation

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Objective: To investigate the anti-angiogenesis action of The THSWDII. Methods: The CAM assay was adopted to study the anti-angiogenesis action of THSWDII; The MTT test was used to investigate its effect on proliferation of the human umbilical vein vascular endothelial cells ECV304; and the immunohistochemical method was used to observe the effect of THSWDII on the expression of KDR/Flk-1 and the microvessel density (MVD) of B16 melanoma in mice. Results: After treatment with THSWDII, the blood vessel index of CAM and the absorbency of ECV304 in the THSWDII 1mg/ml group and 2mg/ml group decreased significantly ($P<0.01$), the weight, the expression of KDR/Flk-1 and the MVD of B16 melanoma in mice reduced significantly in the THSWDII 5g/kg group, the 10g/kg group and the TSHSWD 10g/kg plus cyclophosphamide group ($P<0.01$). Conclusion: THSWDII has the actions of anti-angiogenesis, and inhibiting the proliferation of ECV304 cells and the growth of B16 melanoma. The clinical anti-tumour mechanism is considered to be related possibly to its anti-angiogenesis action by inhibiting the expression of KDR/FIK-1

P300212

Effect of Sodium Ferulate on Tumor Growth and Angiogenesis in Mouse H22 Model

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Objective To investigate the effect of sodium ferulate on tumor growth and angiogenesis in mouse H22 model. Methods H22 cells were inculate subcutaneous into KM mice. Animals were randomized for therapy on the second day and treated with sodium ferulate 200, 100, 50 mg/kg/d as well as sodium as control group for 14 days. The volume of tumor was measured at three days intervals. Tumor microvessel, VEGF and PCNA exoression were examed by immunohistochemical staining. VEGF mRNA translation was analyzed by RT-PCR. Proliferation of H22 cells and ECV304 cells in virto were examined MTT assay. Results In vivo, treatment with sodium ferulate inhibited expression of VEGF($P<0.05$), leading to a decrease in microvessel density, which also decreased the staining of proliferating cell nuclear antigen within tumor. In addition VEGF mRNA translution decreased dependently on the concentration of sodium ferulate. In vitro, sodium ferulate fail to inhibit the proliferation of both H22 cells and ECV304 cells. Conclusions Sodium ferulate inhibits tumor growth, angiogenesis as well as VEGF expression significantly in mouse H22 model. Also sodium ferulate inhibit proliferation of neither H22 cells nor ECV304 cells in vitro.

P300213

The essential oil and active constituents from Rhizomes Curcumae **inhibit cell growth via apoptosis in human HepG2 cells.**

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Curcumae belongs to the Family Zingiberaceae, the rhizomes of three species including Curcuma phaeocaulis, C. kwangsiensis and C. wenyujin are used as Ezhu, which is used for removing blood stasis and alleviating pain. In addition, the essential oil of Ezhu is reported to possess anti-tumor activity.

We prepared essential oil (O) and isolated three compounds namely Germacrone (G), Curcumenol (C) and Furanodiene (F) from Ezhu. The inhibitory effects as well as the underlying mechanisms of the compounds on human hepatocellular carcinoma cells (HepG2) were investigated. Results from MTT proliferation assay indicated that the proliferative capacities were strongly inhibited in the presence of the compounds (O, G, C, F) with IC50 at 7μg/ml, 40μg/ml, 60μM, and 30μM respectively. The suppression of cell growths medicated by above mentioned treatments were verified to be apoptotic, based on the appearance of DNA laddering and TUNEL assay. In addition, change in mitochondrial membrane potentials and expression of apoptotic markers, Bcl-2 and Bax were analyzed to investigate the apoptotic pathways in HepG2. These results indicate that Ezhu would be a potential candidate for development of efficacious anti-tumor drugs.

Keyword: Rhizoma Curcumae, Ezhu, Apoptosis.

P300214

Anti-excitotoxicity Effects of Zi-Bu-Pi-Yin Recipe in vitro and Mechanisms

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To study at evaluating protective effects of Zi-Bu-Pi-Yin (ZBPY) recipe on hippocampal neurons in excitotoxicity system model, furthermore exploring the deferring decrepitude mechanisms of ZBPY recipe. We established Glu excitotoxicity system model of primary hippocampal neurons with 100μM Glu and 10μM glycine on the 12th day. Experiments presented here included ZBPY recipe group, cholesterol group, ZBPY+chlolesterol group and MK-801 positive control group which generally accepted as anti-excitotoxicity drug. The degree of neuron damage was evaluated by LDH efflux in supernatant. The protective effect of ZBPY recipe drug serum (5%) on neurons damaged by Glu was studied by serum pharmacology. Intracellular cholesterol was detected by High Performance Liquid Chromatography (HPLC) and the cholesterol in supernatant was detected with enzymatic method, the results were used to analysis the positive effects on homeostasis of cholesterol in excitototic injury neurons. The mechanisms of the protective effect on excitototic injury of ZBPY recipe are related to the regulation of cholesterol homeostasis in neurons.

Key words: Zi-Bu-Pi-Yin recipe; serum pharmacology; excitotoxicity; hippocampal neuron

Acknowledgements: This work was supported by National Natural Science Foundation of China Grant 30472255.

P300215

Inhibition mechanism of growth of human pancreatic cancer by d-limonene

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To study the Inhibition mechanism of d-limonene on the growth of human pan-

creatic cancer. Metastasis model simulating human pancreatic cancer was established by orthotopic implantation of histologically intact human tumor tissue into pancreatic wall of nude mice. From fifth day after implantation, control, 5-FU group (30 mg/kg/d), D-limonene group (15 ml/kg/d), combined treatment group (both D-limonene and 5-FU) were respectively administered every other day for seven weeks. Eight weeks after implantation, tumor size, inhibition rate and apoptotic index (AI) were calculated through orthotopic tumor weight, MVD and VEGF were measured, and the expression of p53, bcl-2, bax, nm23, CD44V6, PCNA, NF-κBp65, cytochrome-C, Caspase-3 were detected respectively by means of immunohistochemistry and the Western-blot. These data suggested that D-limonene can induce the apoptosis of pancreatic cancer cell by adjusting the protein expression of correlative gene. The inactivation of NF-κB and the release increase of cytochrome-C and the activation of caspase-3 signal pathway is one of mechanisms on pancreatic cancer cell apoptosis by d-limonene in nude mice.

Key words: Pancreatic cancer; D-limonene; Apoptosis.

Acknowledgements: This work was supported by National Natural Science Foundation of Liaoning Province of China Grant 20042128.

P300216

Anti-oxidant and anti-inflammatory activity of roots of Asparagus racemosus.

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Objective: To study anti-oxidant and anti-inflammatory activity of different extracts of roots of Asparagus racemosus Willd.

Materials and methods: Different extracts of A. racemosus viz. Crude Extract (CE), Methanolic Fraction (MF) and Precipitated Aqueous Fraction (PAF), were prepared. Anti-oxidant activity was evaluated by (1) DPPH scavenging (2) Nitric Oxide Scavenging and (3) Lipid peroxidation induced by iron-ADP system method. Whereas the anti-inflammatory activity was measured by % reduction in carrageenan induced hind paw oedema. The extent of potency of extract was compared with Ascorbic acid and Diclofenac sodium (50 mg/kg) for antioxidant and anti-inflammatory activity respectively.

Result: There was significant antioxidant activity observed in MF compared to other extracts, which was comparable with that of standard Ascorbic acid. Also there was significant decrease in inflammation corresponding to mean displacement volume compared to standard Diclofenac sodium.

Conclusion: MF of roots of Asparagus racemosus showed both anti-oxidant and anti-inflammatory activity in comparison with standard reference drug.

P300217

Contractile activity of the scorpion Androctonus crassicauda venom on isolated rat vas deferens.

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Scorpion venoms are one of the sources of dangerous envenomations but on the other hand they are rich sources of biologically active compounds such as proteins and peptides, especially active on the endocrine, immune, cardiovascular and on the central and autonomic nervous system. Androctonus crassicauda (Oliver 1807) is one of the most venomous scorpion species and a cause of envenomation leading deaths at the Middle East region of the world. Freeze dried venoms of Androctonus crassicauda was reported to be active on the isolated rat vas deferens but not on isolated rat gastric fundus and ileum in our previous studies. The venom was shown to exhibit a significant contractions of the vas deferens. In this study, 10^{-3} and 10^{-4} mg/mL of freeze dried whole venom was investigated on the prostatic and epididymal parts of isolated rat vas deferens. As a result Androctonus crassicauda venom was observed to elicit more contraction on the prostatic part of vas deferens. To the best of our knowledge, this is the first report on this differential activity of the venom on segments of the rat vas deferens which the mechanism awaits to be investigated.

Key words: Scorpion, Androctonus crassicauda, isolated vas deferens, contraction.

P300219

Differential Gene Expression and Regulation in Clinically Drug Resistant Isolates of Candida albicans from Bone Marrow Transplanted Patients Using cDNA Microarrays

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Fungi have emerged as the fourth most common pathogens isolated in nosocomial bloodstream infections and Candida albicans is the most common human fungal pathogen. Only a few antibiotics are effective in the treatment of fungal infections. In addition, the repetition and lengthy duration of Fluconazole therapy has led to an increased incidence of azole resistance and treatment failure associated with C. albicans. To investigate the mechanism of drug resistance and explore new targets to treat clinically resistant fungal pathogens, we examined the large-scale gene expression profile of two sets of matched fluconazole-susceptible and -resistant bloodstream C. albicans isolates from bone marrow transplanted (BMT) patients for the first time by microarray analysis. More than 198 differential expressed genes were identified and they were confirmed and validated by RT-PCR independently. Not surprisingly, the resistant phenotype is associated with increased expression of CDR mRNA, as well as some common genes involved in drug-resistance such as CaIFU5, *CaRTA2* and CaIFD6. Meanwhile, some special functional groups of genes, including ATP binding cassette (ABC) transporter genes (*IPF7530*, *CaYOR1*, *CaPXA1*), oxidative stress response genes (*CaALD5*, *CaGRP1*, *CaSOD2*, *IPF10565*), copper transport and iron mobilization related genes (*CaCRD1/2*, *CaCTR1/2*, *CaCCC2*, *CaFET3*) were found to be differentially expressed in the resistant isolates. Furthermore, among these differential expressed genes, some co-regulated with CaCDR1, *CaCDR2*, *CaIFU5*, such as CaPDR16 and CaIFD6, which have a DRE-like element and may interact with TAC1 in the promoter region, were first indicated to be candidates to the targets of transcription factor TAC1. These findings may shed light on mechanisms of azole resistance in C. albicans and clinical antifungal therapy.

KEY WORDS: Candida albicans, Microarray, Drug resistance, Bone Marrow Transplant, Differential gene expression

P300220

Antidiarrheal effect of the methanolic extract of Kratom (Mitragyna speciosa Korth.)

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Kratom (Mitragyna speciosa Korth.) has constipation and analgesic effects. The study was aimed to investigate in vivo antidiarrheal effect of the methanolic extract of Kratom leaves. Dried leaves of Kratom were extracted with methanol, then lyophilized and dissolved in the vehicle. Six groups of wistar rats (n=10) were orally administered the extract (50, 100, 200 and 400 mg/kg), loperamide 3 mg/kg or vehicle one hour later, castor oil (2 ml) was feeded and animals were placed in each individual cage. The severity of diarrhea was recorded as frequency, ranking scores (++; copious, +; mild and 0; no diarrhea) and fecal weight 8 hr after castor oil administration. The methanolic extract of Kratom (100-400 mg/kg) caused a significant and dose-dependent reduction in diarrheal frequency (64.8-85.2%), diarrheal score (65.8-85.1%) and fecal weight (72.2-88.2%). At the dose of 200 and 400 mg/kg of the extract produced the same effect as loperamide. The methanolic extract of Kratom exhibited antidiarrheal effect in caster oil induced diarrheal model, which may be due to anti-electrolyte permeability.

Key words: Kratom, Mitragyna speciosa Korth., antidiarrheal effect
Acknowledgement: Thai Government Research Fund

P300221

Vasorelaxant effect of pinocembrin on the rat thoracic aorta

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The aim of present study was to evaluate the vasorelaxant effects of pinocembrin and its possible mechanisms in isolated rat aortic rings. Pinocembrin induced re-

laxation in aortic rings pre-contracted with norepinephrine (NE, 1 μM) or KCl (60 mM), with pEC_{50} value 4.37 ± 0.02 and 4.52 ± 0.04. Pretreatment with pinocembrin also inhibited contractile responses to NE and KCl. The vasorelaxant effect of pinocembrin was attenuated significantly by endothelium removal or incubation with L-NAME (100 μM), but was uninfluenced by the presence of propranolol (10 μM) or indomethacin (5 μM). In endothelium-denuded rings, the vasorelaxant effect of pinocembrin was partially inhibited by glibenclamide (10 μM), tetraethylammonium (5 mM) and 4-aminopyridine (100 μM). Pinocembrin also reduced NE-induced contraction in Ca^{2+}-free solution and inhibited contraction produced by increasing external calcium in Ca^{2+}-free medium plus 60 mM KCl. Our results suggest that pinocembrin induces relaxation in rat aortic rings through an endothelium-dependent pathway, involving NO, and also through an endothelium-independent mechanism by opening K^+ channels and blockade of Ca^{2+} channels.

Key words: pinocembrin, vasorelaxant, NO

P300222

Pharmacokinetic Study of Scymnol Sulfate in Mice.

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Traditional Asian medicine has long recognised the therapeutic potential of shark bile containing 5β-scymnol sulfate (SS), which is a potent topical therapy for the treatment of hyperseborrhoea, and a hepatoprotective agent when taken internally. In a preliminary study the pharmacokinetic properties of this bile sterol in a mammalian system was investigated by administering ^{14}C-labelled SS (70 mg/kg) to male Swiss mice via oral, intravenous or intraperitoneal routes and monitoring for up to 48 hours, before the collection of organs, blood, urine and faeces. Tissues were enzymatically digested prior to liquid scintillation counting. SS was well tolerated and caused no behavioural or gross morphological changes, although an increase in liver somatic index was observed. Irrespective of dosage route, SS underwent rapid hepatic extraction from the blood and was secreted into bile, demonstrating choleretic properties and enterohepatic recirculation. The predominant route of elimination was via faeces, with radioactivity still detectable at 48 hours. In conclusion, the pharmacokinetics of SS in the mouse appears to be similar to that of endogenous bile salts.

Key words: scymnol sulfate, bile, pharmacokinetics

P300223

The anxiolytic-like effects of the total flavones Scutellaria baicalensis Georgi in experimental animals

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Scutellariae Radix, the dried root of Scutellaria baicalensis Georgi (Labiatae), was a commonly used traditional Chinese medicine in clinical practice. The putative anxiolytic activity of total flavones of the dried root of Scutellaria baicalensis (TFSB) was examined in male mice by using a number of experimental paradigms of anxiety. In the elevated plus-maze test, TFSB (30.0 mg/kg, 100 mg/kg, p.o., 7days) had a modest anxiolytic-like effect. It increased the percentage of entries into open arms and of time spent on open arms. In the light/dark test, TFSB (30.0 mg/kg, 100 mg/kg, p.o., 7days) prolonged the time spent in the light area. In the open-field test, TFSB (30.0 mg/kg, 100 mg/kg, p.o., 7days) had a modest anxiolytic-like effect. It not only prolonged the time spend in centers but also increased the times the mice ran to centers without altering the locomotor activity of the animals (evaluated by squares). Thus, these findings indicated that TFSB exhibits significant anxiolytic effects.

Key words: baicalin; anxiety; Elevated plus-maze; light/dark box

Acknowledgement: This study is supported by the project of Key-Laboratory for New Drug Screen of Liaoning Province.

P300224

Extract from Arca granosa Linnaeus inhibits proliferation of human tumor cell lines of kidney and lung origin

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This study examined the mechanisms of an extract of Arca granosa Linnaeus inhibits tumor growth. The extract inhibited proliferation of six human tumor cell lines of various origins. Cell lines Ketr-3 and A549, which were of kidney origin, and the NCI-H460 cell line, which was of lung origin, were more sensitive to the extract than the HepG-2, MCF-7 or MGC-803 cell lines, which were of other origins. Flow cytometric analyses showed that the extract blocked various phases of the cell cycle in Ketr-3, A549 and NCI-H460 cells and inhibited DNA synthesis in these lines. We consider the extract from Arca granosa Linnaeus to be a novel antitumor agent that is especially effective in kidney and lung-tumor cell lines.

Key words: Antitumor; Proliferation; Arca granosa Linnaeus

Acknowledgement: Project supported by Qingdao technology Bureau (№05-1-HY-81 and 2005SKI-04)

P300225

Protective Effect of Total Flavones of Buckwheat Flower on Carbon Tetrachloride-induced Hepatic Impairment[1]

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Objective: The protective effect and possible mechanism of total flavones of buckwheat flower on experimental hepatic impairment in mice were studied. Methods: The hepatic impairment model of mice was induced by injecting carbon tetrachloride subcutaneously every 4d for 7 times. Meanwhile, mice in the two treatment groups were given TFBF at dosages of 0.04 $g \cdot kg^{-1} \cdot d^{-1}$ and 0.02 $g \cdot kg^{-1} \cdot d^{-1}$ respectively through intragastric injection, mice in the positive control group were treated with methionine by contrast. Next the day CCl_4 was lastly injected, half of the mice were killed. The contents of alanine aminotransferase (ALT) in serum and ALT, superoxidase dismutase (SOD), glutathione (GSH), malonaldehyde (MDA), triglyceride (TG), total cholesterol (TC) in liver tissue, the liver indexes (LI), and the hepato-pathologic changes were examined. The rest mice were given identical treatment for another 2 weeks. Results: TFBF could inhibit the rising of serum ALT, liver MDA, TG, TC, LI, and the lowering of liver SOD and GSH in CCl_4-induced hepatic impairment mice. It could obviously ease the hepato-pathologic damages as well. Conclusion: TFBF could effectively protect the hepatic impairment in CCl_4-induced mice.

Key Words: Buckwheat; flavone; hepatic impairment; carbon tetrachloride

[1]Project supported by the science committee of Hebei Province(№03276421)

P300226

POLYPHENOLIC COMPOUNDS ISOLATED FROM Rubus koreanum MIQUEL INHIBIT CATECHOLAMINE RELEASE FROM THE ISOLATED RAT ADRENAL GLAND

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The purpose of the present study was to investigate whether polyphenolic compounds isolated from liquors, which is brewed from Rubus koreanum MIQUEL, may affect catecholamine release from the isolated perfused rat adrenal medulla. Taken together, these experimental results obtained from the present study demonstrate that PCRK greatly inhibits the CA secretion evoked by stimulation of cholinergic receptors and the membrane depolarization from the isolated perfused rat adrenal gland. It seems likely that the inhibitory effect of PCRK is mediated by blocking the calcium influx into the rat adrenal medullary chromaffin cells as well as by the inhibition of Ca^{2+} release from the cytoplasmic calcium store, which is relevant to the blockade of cholinergic receptors.

P300227

Effects of Rosa roxburghii Extract on Proliferation and Differentiation in Human Hepatoma SMMC-7721 Cells and CD34+ Haematopoietic Cells

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Rosa roxburghii Tratt is an herbal medicine with anticancer potential. This study investigated the effects of ethanol extract and a triterpene of Rosa roxburghii on proliferation and differentiation in human hepatoma SMMC-7721 cells and in umbilical cord blood CD34+ haematopoietic stem progenitor cells. Both extracts inhibited the proliferation of hepatoma cells in a concentration- and time-dependent manner, and decreased the release of alpha-fetoprotein from hepatoma cells. Apoptosis was increased only at the highest dose of the ethanol extract in hepatoma cells. Both extracts of Rosa roxburghii did not affect the differentiation of cord blood CD34+ cells to granulocyte and monocyte, as evidenced by flow

cytometry analysis of CD11b and CD15. The ethanol extract slightly inhibited proliferation of cord blood CD34+ cells, but no the triterpene. Thus, the triterpene and ethanol extract of Rosa roxburghii are effective in the inhibition of human hepatoma SMMC-7721 cell growth, without affecting the differentiation of CD34+ cells. The triterpene has less toxicity to human bone marrow depression than the ethanol extract of Rosa roxburghii, and it appear to be a better anticancer drug.

Key words: Rosa roxburghii extract, proliferation, differentiation, human, hepatoma SMMC-7721 cells, umbilical cord blood CD34+, haematopoietic cells

P300228

Ganoderma lucidum polysaccharides peptide (GLPP) protects ECV304 cells from oxidantive injury

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AIM: To study the protective effects of Ganoderma lucidum polysaccharides peptide(GLPP) on the ECV304 cells injured by reactive oxygen specices(ROS), derived from ter-butylhydroperoxide(tBOOH) in vitro. METHODS: Cultured ECV304 cells were injured by ROS, derived from tBOOH. The survival rate of cells was measured by MTT assay, and the morphological change of cells were observed under light and electron microscopes. The percentage of apotosis of ECV304 cells, labeled with AnnexinV/PI was measured by flow cytometry. RESULTS: GLPP(12.5, 50, 100 mg·L^{-1}) could reduce foam formation in cells and inhibit the apotosis and necrosis of ECV304 cells. The survival rate of cells was increased. Under the electron microscope it was found that GLPP (100 mg·L^{-1}, for 24hr) could protect the organelle such as mitochondria from injury and cells from apotosis by tBOOH. The result of flow cytometry showed the percentage of apotosis of cells was decreased in the group treated with GLPP. CONCLUSION: GLPP had significant protection effects on ECV304 cells from oxidantive injury.

KEY WORDS: Ganoderma lucidum polysaccharides peptide, ECV304 cells, oxidantive injury, tBOOH

P300229

The Effects of vitexia-rhamnoside on Human Umbilical Venous Endothelial Cells In Vitro

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Objective: To observe the effect of vitexia-rhamnoside (V-R) on Human umbilical venous endothelial cells (HUVECs) damaged by hypoxia and reoxygenation.

Methods: HUVECs were prepared by enzyme digestion for culture. And HUVECs were damaged by ischemia and reperfusion following hypoxia and reoxygenation. Enzyme kinetics, fluorescent staining methods, and patch-clamp technique were used to detect the lactic dehydrogenase(LDH) activity in supernatants, cytoplasmatic Ca^{2+} concentration and rectifier current (I_K) of HUVECs respectively.

Results: V-R with all concentrations in this experiment exhibited morphological protective effects on HUVECs damaged by hypoxia and reoxygenation, and decreased LDH activity in supernatants from 19.00 ±1.94u/L to 8.81 ±12.75u/L. Cytoplasmatic Ca^{2+} concentration was increased from 43.51nmol/L to 151.24nmol/L following hypoxia and reoxygenation and V-R degraded this increase to normal levels (45.83 ~60.69nmol/L). Electrophysiological data indicated that V-R possessed dual effects on the delayed rectifier current (I_K) of HUVECs. I_K increased from 1187.02 ±246.08 pA to 2229.48 ±496.45 pA at the dosage of 10^{-3} mol/L, from 732.73 ±105.06 pA to 1056.80 ±652.05 pA at 10^{-4} mol/L, and at 10^{-5} mol/L, I_K decreased from 1080.02 ±303.73 pA to 768.21 ±193.41 pA.

Conclusion: V-R can protect HUVECs against ischemia/reperfusion injury, and showed a dual effect (stimulating/inhibiting) on membrane K^+ channels of HUVECs.

Key words: vitexia-rhamnoside; endothelial cell; hypoxia and reoxygenation; K^+ channels

P300230

Triptdide, a diterpenoid triepoxide, from Tripterygium wilfordii Hook.f. suppresses inflammation and cartilage destruction in collagen-induced arthritis mice

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Chinese herbal remedy Tripterygium wilfordii Hook.f.(TWHF) has been reported to be therapeutically efficacious in the treatment of rheumatoid arthritis (RA) but its in vivo actions have not been clarified. The purpose of this study was to investigate the effects of triptolide, a diterpenoid triepoxide extracted from TWHF, on inflammation and cartilage destruction in collagen-induced arthritis (CIA) model mice. Histological examination demonstrated that triptolide significantly reduced the inflammatory responses and cartilage damage in the joint tissues. Interestingly, triptolide down-regulated the expression of matrix metalloproteinases-13 and -3, which are considered to be key enzymes in the pathological destruction of cartilage, and simultaneously up-regulated tissue inhibitors of metalloproteinases-1 and -2 expression in the joints. Moreover, triptolide inhibited prostaglandin E2 production via selective suppression of the production and gene expression of cyclooxygenase (COX)-2, but not COX-1. The levels of interleukin (IL)-1β, tumor necrosis factor αand IL-6 were also decreased by triptolide in the joint tissues and sera as well as down-regulation of their mRNAs in the joints. In addition, triptolide treatment in vivo was able to reduce an abundance of nuclear factor-κB, the transcriptional factor closely related to the inflammatory process, in articular cartilage and synovium in CIA mice. These results suggest that triptolide exerts novel chondroprotective and anti-inflammatory effects on RA, and the therapeutic action of TWHF on RA is, in part, due to the triptolide activities.

Key words: Triptolide; Inflammation; Cartilage destruction; Collagen-induced arthritis mice

P300231

Effect of Chaihu and its Extract On Monoaminergic Neurotransmitters in Brain of Liver-qi Stagnated Syndrome Rats with Chronic Restrained stress

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Chaihu is widely used in reliving symptoms in exterior and interior, soothing the live and elevating the collasped yang. In order to explore the mechanism of chaihu's soothing liver, the monoaminergic neurotransmitters in the brain of liver-qi stagnated syndrome were measured in Chaihu and its extract treated rats. Male SD rats were randomly divided into six groups after being tamed one week, and establishing the liver-qi stagnated syndrome rat. The treatment group is given medicine once everyday after having been modeled one week, and Xiaoyaosan is as positive drug. Four weeks later, the level of NE and DA in the rats' wet brain is determined with fluorescence spectrometry. The results indicated that the abnormal behavior of rats were improved in Chaihu, Saikosaponin and positive treatment groups compared to model rats. In the treatment groups, the rat's brain level of NE and DA had a significant increase. The level of NE did not show a difference between the chaihu, saikosaponin and the Xiaoyaosan, but the efficacy of Chaihu is better than the recipe of Xiaoyaosan in the level of DA. Chaihu's property of soothing the liver is supported by our assay. It is probable that saikosaponin is the active composition of soothing liver.

Key words: Norepinephrine; Dopamine; Radix Bupleuri; Saikosaponin

P300232

Selective effects of long-term administration of St. John's wort and isolated compounds on β-adrenergic binding in rat frontal cortex

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Since down-regulation of central β-adrenergic receptors has been widely considered a common mechanism of antidepressant we used quantitative radioligand receptor-binding-studies to examine in rats the effects of short-term (2 weeks) and long-term (8 weeks) administration of different St. John's wort (SJW) extracts and isolated compounds on β-adrenergic binding in rat frontal cortex. The effects were compared to the standard antidepressants imipramine and fluoxetine. [^{125}I] CYP binding to beta-adrenergic receptors was found to be decreased after short as well as after long-term treatment with imipramine. Long-term treatment with fluoxetine elicited a marked increase in β-adrenergic binding

in the frontal cortex. Similar to fluoxetine, [^{125}I] CYP binding to β_2- adrenergic receptors was found to be increased after 8 weeks with a lipophilic CO_2- extract. Short- term treatment with a methanolic SJW extract slightly decreased β_2- adrenoceptor binding, no effects were observed after 8 weeks. Treatment with hypericin led to a significant down- regulation of β_1- adrenergic receptors in the frontal cortex after 8- weeks while hyperforin was ineffective in both treatment paradigms. A flavonoid- fraction, free of hypericin and hyperforin but enriched in hyperoside, isoquercitrin, and quercitrin- caused a pronounced effect on β_2- adrenergic receptor down- regulation after 2 weeks. However, pure hyperoside, isoquercitrin or miquelianin alone had no effect on β_2- receptor binding. The inactivity of the single flavonoids may be explained by either effects of so- far untested compounds in the flavonoid- fraction or a synergistic action of substances in combination. To our knowledge this is the first study that systematically investigates the effects of SJW extracts and distinct compounds on β- receptor- regulation.

P31. Molecular Pharmacology and Toxicology

P310001

The Effect of the Combination of Salvianolic Acid B and Panax Notoginseng Saponins on Myocardial Apoptosis after Myocardial Infarction in Rats

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OBJECTIVE: Salvianolic acid B (SalB) and panax notoginseng saponins (PNS), the major ingredients of the Compound Danshen Formula, have previously shown their protective properties against ischemic heart disease. To investigate the mechanism of SalB and PNS against myocardial apoptosis after myocardial infarction (MI).

METHODS: Rats of occluding the left coronary artery treated with SalB, PNS and the combination of SalB and PNS for one week and were tested for heart function by hemodynamic and echocardiography studies. RNA in the ischemic region of left ventricular was isolated for Affymetrix arrays and RT- PCR.

RESULTS: The combination of SalB and PNS can decrease LVDP, LVDs, LVPWs, LVESV, and increase ±dp/dtmax, EF, FS. The combined administrations can significantly potentate myocardial apoptosis. Of the apoptosis related genes tested, Bcl2a1, IL- 6, IL- 18, JAK- 2, Stk17b, Spp1, Birc4, Bcl- 2, Bax, caspase- 8, STAT- 3 and Pawr increased significantly in MI compared with control, whereas the combination attenuated these expressions.

CONCLUSION: Suggesting a mechanism involved the downregulation of these apoptosis related genes expression.

Key Words: SalB, PNS, cardiomyocyte apoptosis, MI

P310002

The effect of lipopolysaccharide and tumour necrosis factor alpha on D- galactosamine- induced apoptosis in rat hepatocyte culture

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Rat hepatocytes were incubated in culture with D- galactosamine (GalN), lipopolysaccharide (LPS) + GalN or tumor necrosis factor alpha (TNF- α) + GalN. Caspase- 3 activity and cytochrome c (cyt.c) in hepatocyte cytosol, viability and nitric oxide (NO) produced were estimated. Hepatocytes were investigated morphologically using Annexin- V. GalN produced an increase in caspase- 3 activity with no change in cyt.c.

Combined treatment with LPS + GalN or TNF- α+ GalN did not increase caspase- 3 activity as compared to GalN. Hepatocyte viability was decreased with increasing caspase- 3 activity. GalN treatment increased medium NO levels while combined treatment produced slight increase in NO production. Apoptosis was confirmed morphologically and by Annexin- V binding. Caspase- 3 activity accompanied by morphological features of apoptosis represents sensitive markers for hepatocyte apoptosis. Considering the fraction of cells completing apoptosis while others that turned toward necrosis and the dual role of NO, caution should be exercised in data interpretation and combinations of different test methods should be applied.

Key words: Apoptosis, D- galactosamine
Supported by GA ČR 305/05/2425 and VZ MSM 0021620807.

P310003

Genotoxic assessment of TheraCIM- h- R3 by means of the bone marrow micronucleus test

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TheraCIM- h- R3 is a humanized growth factor receptor monoclonal antibody (mAb) in development for the treatment of head and neck tumours in which malignant cells over- express the Epidermal Growth Factor receptor. In order to assess the genotoxic potential of this mAb it was performed the bone marrow micronucleus test in Cenp:NMRI mice. It was established three dose levels (5.7, 28.5, and 57 mg/Kg body weight) and two control groups (negative: saline, positive: cyclophosphamide 40 mg/Kg body weight). All substances were administered via intraperitoneal injection scheduled in two treatments at 24- hour intervals, and samples were collected 24 hours following the final treatment. The proportion of immature among total (immature + mature) erythrocytes was determined for each animal by counting 500 erythrocytes, and 1000 immature erythrocytes per animal were scored for the incidence of micronucleated immature erythrocytes. Statistical analysis of the results allowed establishing that TheraCIM- h- R3 did not show genotoxic or citotoxic effect in the bone marrow cells of the used mice.

Key words: TheraCIM- h- R3, tumours, micronucleus test

P310004

Propionyl- L- carnitine Prevents the Progression of Cisplatin- Induced Cardiomyopathy in a Carnitine- Depleted Rat Model

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This study is to investigate whether carnitine deficiency is a risk factor during development of cisplatin (CDDP)- induced cardiomyopathy and whether propionyl- L- carnitine (PLC) could offer protection against this toxicity. A six groups of adult male Wister rats were used. In the carnitine- depleted rat model, CDDP induced dramatic increase in serum cardiomyopathy enzymatic indices, CK- MB and LDH, as well as progressive reduction in total carnitine and ATP content in cardiac tissue. PLC supplementation resulted in a complete reversal of the increase in cardiac enzymes, TBARS and NOx, and the decrease in total carnitine, GSH and ATP, induced by CDDP, to the control values. Moreover, histopathological examination of cardiac tissues confirmed the previous results. In conclusion, data from this study suggest for the first time that carnitine deficiency and oxidative stress are risk factors and should be viewed as mechanisms during development of CDDP- related cardiomyopathy and that supplementation with PLC, prevents the progression of CDDP- induced cardiotoxicity.

P310005

Pharmacophore Models Implicating 2- PAM as a Source for Chemically- induced Parkinson's Disease

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2- Pralidoxime chloride (2- PAM) is the US FDS- approved drug for acute organophosphate poisoning. Yet 2- PAM has a striking resemblance to the metabolites of 1- methyl- 4- phenyl- 1,2,3,6- tetrahydropyridine (MPTP). MPTP and its derivatives have been shown to be sources for chemically- induced Parkinson's disease. Pharmacophore models including shape constraints have been developed for MPTP and its more potent derivatives. 2- PAM and other oximes used to treat organophosphate poisoning were fit to these models. The results show that 2- PAM is the only oxime to fit these models, implicating it as another source for chemicall induced Parkinson's disease. Anecdotal data from both Gulf War veterans and the farm migrant worker community provide further cause for concern in the continued use of 2- PAM.

P310006

Amnesia induced by halothane is related with an increase of the 5 - HT1A and galanin receptors function in the limbic areas of the rat brain.

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The effect of halothane 'anaesthesia in the memory and in the rat brain 5 - HT_{1A} and galanin (GAL) receptors were determined. Rats (n = 12, adult male Sprague - Dawley) were anaesthetized with halothane (HAL) (3 - 5 mmHg in 50 % O_2 - 50 % O_2N, 30 min). The memory capacity (8 - arm labyrinth) and brain 5 - HT_{1A} and GAL receptors (autoradiography techniques with ^{3}H - 8OH - DPAT and ^{125}I - human - porcine galanin) were determined 24 h after. HAL increased the time spent in: the first choice (+ 50 %), in the first 1 and 8 accuracy choices (+ 53 %, + 67 %), and the error choices total number (+ 136 %). HAL diminished the time spent eating (- 26 %), and the accuracy choices total number (- 44 %) and the first 8 choices accuracy choices number (- 42 %) ($p < 0.05$). HAL increased the affinity of GAL receptors in hippocampus and amygdala (Ki increment 82 % and 151 %) ($p < 0.01$). HAL increased the 5 - HT_{1A} receptors affinity of fronto - parietal cortex, hippocampus CA1 and amygdala (K_d reduction - 66 %, - 88 % and - 63 %) while reduced receptors affinity of dorsal raphe (Kd increment + 224 %) ($p < 0.01$). Amnesia induced by halothane in rats are linked to an increased neurotransmission of the galanin and the 5 - HT_{1A} receptors in limbic areas.

Key words: halothane, brain, 5 - HT_{1A}, galanin

P310007

Low Doses Of Diclofenac Induces Hepatocellular Changes In Rat Treated In Vivo

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Diclofenac is reported to have cytotoxic effects and induces apoptosis in various cell lines. This study is conducted to investigate the mechanism of apoptosis in liver of rat treated with diclofenac. Rats were dosed with 3, 5 and 10 mg/kg diclofenac in saline for 15 days. Livers were then removed, weighed and processed for histopathological analysis. 10 mg/kg diclofenac after 15 days treatment found to trigger the accumulation of inflammatory cells such as lymphocytes and nuetrophils. This was observed mainly in the centrilobular region. Apoptotic cells were observed following TUNEL assay and were also found to be the hepatocytes at the centrilobular area. This evidence was supported by the changes seen in the mitochondria membrane observed using TEM.

P310008

Regulation of hypoxia - induced iNOS expression by PI - 3 kinase and Hypoxia inducible Factor - 1alpha in microglia

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Exposure of microglia to hypoxia induces cellular activation and animal studies have shown that neuronal cell death is correlated with microglial activation following ischemia - reperfusion. In the current work, we investigated the mechanism involved in the production of NO and the expression of inducible NO synthase (i-NOS) by hypoxia in microglia. Exposure of microglial cell line BV - 2 as well as primary mouse microglial cultures to hypoxia followed by reoxygenation induced the production of NO, indicating that hypoxia could lead to the inflammatory activation of microglia. Moreover, the molecular analysis of these events indicated that iNOS expression was regulated by PI3 kinase/AKT/mammalian target of rapamycin (mTOR) signaling pathway and the activation of hypoxia inducible factor - 1alpha (HIF - 1alpha). In addition, up - regulation of HIF - 1alpha was also found after cerebral ischemia induced by permanent occlusion middle cerebral artery in mice. Thus, hypoxia may also promote neuronal injury indirectly via microglial activation during cerebral ischemia.

Key words: HIF - 1alpha, iNOS, PI3 kinase

Acknowledgement: This work was supported by grants from NSC

P310009

The use of RNA interference to reduce nucleoside transport in rat C6 glioma cells.

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Adenosine is a neuromodulator in brain. It is a metabolite of ATP and it initiates receptor - mediated signaling events. In cell culture experiments, we have found that neurons and astrocytes have different roles in that stimulated rat forebrain neurons release adenosine per se whereas astrocytes salvage this adenosine. Thus, nucleoside transporters can mediate adenosine efflux from neurons but adenosine influx into astrocytes. The present study was performed to test the hypothesis that RNA interference can inhibit nucleoside transporter expression in astrocytes, reduce adenosine salvage and promote adenosine receptor signaling. We used the Block - it RNAi Designer program (Invitrogen) to design 10 short hairpin RNA sequences for the equilibrative nucleoside transporter type 2 (ENT2, Slc 29A2). These sequences were cloned into the pENTR U6 vector. After verifying insert sequences, C6 glioma cells were transfected with empty vector or shRNA vectors. Inhibition of adenosine uptake ranged from 10 - 50 % in cells transfected with shRNA sequences compared to wild type or vector - transfected cells.

Supported by the Canadian Institutes for Health Research

P310010

RasGRP confers phorbol ester sensitivity to EL4 lymphoma Cells

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EL4, a mouse lymphoma cell line, exists in variants that are either sensitive or resistant to phorbol 12 - myristate 13 - acetate (PMA). PMA induces robust Erk MAPK activation, IL - 2 production, and growth arrest in sensitive, but not resistant. EL4 cells. The objective of this study was to test the hypothesis that RasGRP, a Ras guanine nucleotide exchange factor that binds PMA, is responsible for PMA sensitivity in EL4 cells. Ras activation was assessed by a Ras - GTP pulldown assay, Erk activation and RasGRP expression were tested by immunoblotting, and IL - 2 production was quantified by ELISA. A siRNA was used to inhibit RasGRP protein expression. The results of this study showed that RasGRP protein is expressed at much higher levels in sensitive than in resistant cells. The full extent of PMA - induced Ras activation is observed only in cells expressing RasGRP. Introduction of siRNA for RasGRP into sensitive cells suppresses PMA - induced Ras and Erk activation, blocks PMA - induced IL - 2 production, and abolishes PMA - induced growth arrest. We conclude that PMA sensitivity, as previously defined for the EL4 cell line, is conferred by expression of RasGRP. (Supported by NIH CA094144 - 01)

P310011

Interaction of Dopamine D1 Receptors with PSD - 95

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Objective: To study whether postsynaptic density protein 95 (PSD - 95) can interact with dopamine D1 receptors (D1Rs) and modulate D1R function. Methods: Transient and stable expression in HEK293 cells, receptor binding, cAMP accumulation, immunocytochemistry, co - immunoprecipitation, RT - qPCR. Results: 1) A direct interaction between D1Rs and PSD - 95; 2) Coexpression of PSD - 95 can ①increase D1 - EYFP receptor surface level without change of the transcription level (18.7 %). ②Decrease DA - induced D1 - EYFP receptor internalization (from 52.5 % to 20.5 %). ③Increase D1R agonist SKF38393 induced accumulation of cAMP (12.1 %). Conclusion: This study provides the first evidence that PSD - 95 can interact with D1Rs and enhance D1R mediated signal transduction by increasing D1R suface level and by reducing agonist - induced endocytosis of D1Rs. Changes in PSD - 95 expression, such as those observed in schizophrenia, may also cause alterations in D1 receptor - mediated signaling.

Acknowledgments: This work was supported by Ministry of Science and Technology (973 - 2003CB5154000); National Natural Science Foundation (30223130); and Chinese Academy of Sciences (Bai - Ren - Ji - Hua)

Key words: D1 - EYFP, PSD - 95, interaction

P310012

Activation of Serotonin Transporters by Pro-Inflammatory Cytokines Interleukin-1beta and Tumor Necrosis Factor-Alpha: A role of p38 MAPK

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Our recent study demonstrated that activation of p38 MAPK induces a catalytic activation of the serotonin(5-HT) transporter (SERT). Since inflammatory cytokines can activate p38 MAPK, we hypothesized they might also activate SERT. Using 5-HT transport assays, we found that IL-1β and TNF-α stimulated 5-HT uptake in both a rat raphe cell line, RN46A, and in mouse midbrain and striatal synaptosomes. We found that IL-1β stimulated 5-HT uptake in a dose- and time-dependent manner, effects abolished by IL-1ra, an antagonist of the IL-1 receptor, and by SB203580, a p38 MAPK inhibitor. TNF-α also dose- and time-dependently stimulated 5-HT uptake that was only partially blocked by SB203580. Western blots showed that IL-1β and TNF-α activated p38 MAPK, in an SB203580-sensitive manner. IL-1β induced a decrease in 5-HT Km, while TNF-α stimulation involved in a change in both 5-HT Km and SERT Vmax. We conclude that pro-inflammatory cytokines can acutely regulate neuronal SERT activity via p38 MAPK-linked pathways.

Key words: serotonin, transporter, cytokines, p38 MAPK

ACKNOWLEDGMENTS: NARSAD award to CBZ; NIDA DA07390 to RDB., and the OCD/TS Program to WAH

P310013

Comparison of Binding Kinetics of Antimuscarinic Agents at the M_2 and M_3 Muscarinic Receptors

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The association and dissociation binding kinetics of [^{3}H] NMS, [^{3}H] QNB, [^{3}H] tiotropium, [^{3}H] LAS-34273, and [^{3}H] glycopyrrolate at 37 °C at the human M_2 and M_3 receptors expressed in CHO cell membranes. The association binding kinetic studies performed at concentrations close to the affinity constant (K_i) of each ligand demonstrated that these compounds behaved as typical muscarinic antagonists with rapid association for [^{3}H] NMS, [^{3}H] QNB, [^{3}H] LAS-34273, [^{3}H] tiotropium, and [^{3}H] glycopyrrolate (hM_2 receptor $t_{1/2}$ on = 0.6, 1.4, 1.1, 2.7, 1.9 min, respectively; hM_3 receptor $t_{1/2}$ on = 2.2, 5.3, 4.4, 14.4, 6.6 min, respectively). However, there was a clear subtype specific distinction among compounds with respect to the dissociation kinetics. When displaced by the antagonist atropine (10 μM), the rank order of dissociation ($t_{1/2}$) at the hM_2 receptor was [^{3}H] QNB (44 min) > [^{3}H] tiotropium (34.9 min) > [^{3}H] LAS-34273 (13.8 min) > [^{3}H] glycopyrrolate (4.3 min) > [^{3}H] NMS (1.0 min). At the hM_3 receptor, it ranged from minutes to hours of dissociation ($t_{1/2}$) with the rank order; [^{3}H] tiotropium (536 min) > [^{3}H] QNB (253 min) > [^{3}H] LAS-34273 (78.3 min) > [^{3}H] glycopyrrolate (31.9 min) > [^{3}H] NMS (5.5 min).

Key words: binding kinetics, muscarinic antagonist, M_2, M_3

P310015

Inhibition of N-acetyltransferase activity and gene expression in HepG2 cell lines by Solanine

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AIM: to examine whether or not Solanine could affect arylamine N-acetyltransferase (NAT) activity and gene expression (NAT mRNA) in human liver cancer cell lines(HepG2). METHODS: The NAT activity was examined by high performance liquid chromatography and the gene expression of NAT was determined by polymerase chain reaction(PCR). RESULTS: Solanine displayed a dose-dependent inhibition to cytosolic NAT activity and intact HepG2 cells. Time-couse experiments indicated that N-acetylatin of 2-AF measured from intact HepG2 cell were inhibited by Solanine for up to 48h. Using standard steady state kinetic analysis, it was demonstrated that Solanine could decrease the Vmax of NAT activity but same Km in HepG2 cells. Solanine decreased mRNA NAT expression in examined HepG2 cells. CONCLUSION: Solanine could inhibited NAT activity and NAT1 mRNA expression, It was a possible uncompetitive inhibitor to NAT in intact HepG2 cells.

KEY WORDS: Solanine; N-acetyltransferase; NAT1 mRNA

ACKNOWLEDGEMENT: This work is supported by the National National Science Foundation of China(No. 30400591, 30400352)

P310016

Expression of Human Fusion GPR81-Gi1α with Baculovirus System

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As a member of human orphan G protein-coupled receptors (oGPCR), GPR81 is known little in its ligand pairing, pathophysiological significance and other aspects including as a potential therapeutic target etc.. In this experiment, we profiled the tissue distribution of GPR81 mRNA in human fetus by RT-PCR, fused GPR81 with human Gi1α by overlap PCR and established a Bac-to-Bac baculovirus expression system in Sf9 cells for GPR81-Gi1α fusion protein. It was showed that GPR81 has no intron, encodes a 346 amino acids protein with seven transmembrane domains, maps to human chromosome 12p24 and has the highest homology with nicotinic acid receptor. GPR81 mRNA was the most abundant in human fetal liver and heart, but little in lung and intestine. Western-blot analysis indicated GPR81-Gi1α fusion protein could be properly expressed in Sf9 cells and 72h of infection time and 5 of multiplicity of infection (moi) is optimal for expression. The establishment of the expression system for GPR81-Gi1α protein set a solid basis for high through-put ligand screening and function exploration of GPR81.

Key words: GPR81; Gi1α; Bac-to-Bac expression system

Acknowledgment: Supported by a grant (No. 30171096) from NSFA

P310017

The inhibition potency of PPARalpha, gamma and alpha/gamma agonists on the uptake and efflux processes in liver

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To predict possible drug-drug interactions via hepatic membrane transporters and elucidate the mechanism of the cholestatic liver damage induced by PPARgamma agonists, we examined the inhibitory effects of PPAR agonists on hepatic uptake and efflux transporters using in vitro transporter expression systems. Furthermore, after i.v. administration of PPARgamma agonists, the concentrations of plasma bile acid and administered drug in plasma and liver were measured in rats. In vitro analyses revealed that the inhibition potency for each transporter depends on the individual drugs, in the order of PPARgamma > PPARalpha/gamma > PPARalpha. The inhibitory effects of pioglitazone and rosiglitazone on human BSEP-mediated taurocholate transport were much more potent than those on rat Bsep-mediated transport. We also observed an increase in plasma total bile acids and taurocholate in rats after administration of troglitazone, while pioglitazone and rosiglitazone produced no significant increase in the plasma bile acid concentration.

Key words: PPAR agonists, transporter inhibition, drug-induced cholestasis

Acknowledgement: We appreciated Sankyo Co. and Merck KGaA for providing us PPAR agonists.

P310018

Investigation of Hepatotoxicity of Three Pyrrolizidine Alkaloid-containing Traditional Chinese Medicinal(TCM) Herbs

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Pyrrolizidine alkaloid (PA) is wildly distributed in different plants worldwide. Most of the naturally occurring PAs cause liver toxicity and/or cancer. However, there is limited information on PA-containing TCM herb induced hepatotoxicity. The present study investigated hepatotoxicity of three PA-containing TCM herbs, namely Ligularia hodgsonii, Gynura segetum and Crotalaria sessiliflora. The results demonstrated that all three PA-containing herbs caused liver damage after single and multiple dosage of their water extracts to rats. Hepatotoxicity was dose-dependent, in particular PA content-dependent, and also correlated to the formation rate of toxic pyrrolic metabolites of PAs generated in the liver. Moreover, metabolic activation rate varied markedly in different PAs, suggesting that PA-containing TCM herbs may cause hepatotoxicity to different extents depend-

ing on structure and content of PAs in the herbs. Therefore, quality control of PA - containing herbs should be developed based on the type and quantity of PA present in individual herbs.

Key words : Pyrrolizidine alkaloid, TCM herbs, hepatotoxicity
Acknowledgement : Support from CUHK Direct Grants (2041071 &2041150) is acknowledged.

P310019

ALTERED INTESTINAL SMOOTH MUSCLE CONTRACTION AND INTRACELLULAR CALCIUM IN CROHN'S DISEASE

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Contractile behaviour and intracellular calcium (Ca) were investigated in intestinal smooth muscles from patients undergoing surgery for Crohn's disease (CD) as well as for intestinal cancer; the latter group was used as control. Circular muscles (CM) obtained from CD patients were more likely to exhibit spontaneous contraction than their control counterparts. Both diseased and control longitudinal muscles (LM) exhibited spontaneous contraction; the frequency of the contraction was significantly higher in diseased LM. In both control and diseased muscles, the cholinergic agonist carbachol (Carb) elicited contraction in a concentration - dependent manner; maximal Carb - induced contraction was decreased by 34% and 21% in diseased CM and LM, respectively. Diseased smooth muscle strips showed a patchy distribution of high - Ca areas, which contrasts to the more uniform distribution of Ca in control preparations. On average, resting Ca was higher in diseased strips. Carb - induced elevation of Ca was reduced in diseased preparations. These data suggest that alterations of Ca homeostasis underly altered intestinal motiliy in CD patients.

P310020

A role for Rho kinase in vascular contraction evoked by sodium fluoride

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Fluoride has been known to produce robust vascular contractions. We hypothesized that Rho kinase plays a role in vascular contraction evoked by sodium fluoride. In both physiological salt solution and calcium - free solution with 2 mM EGTA, cumulative addition of NaF induced vascular tension in concentration - dependent manners. Single administration of NaF (8 mM) slowly increased vascular tension over 20 minutes in parallel with the phosphorylation level of 20 kDa myosin light chain (MLC20) and the target domain of myosin phosphatase (MYPT1). The Rho kinase inhibitor Y27632 decreased vascular tension induced by 55 mM KCl, 1.0 μM phenylephrine, or 8 mM NaF, but not by 1.0 μM phorbol dibutyrate. Y27632 also decreased the level of phosphorylation of MLC20 and MYPT1 induced by 8 mM NaF. The protein kinase C inhibitor Ro31 - 8220 inhibited vascular tension induced by 1.0 μM phorbol dibutyrate, but not by 8 mM NaF. These data suggests that Rho kinase plays an important role in vascular contraction evoked by sodium fluoride.

Keyword : fluoride, contraction, Rho kinase, Y27632

P310021

Cytoprotective Role of Liver Fatty Acid Binding Protein in Acetaminophen Induced Cytotoxicity

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INTRODUCTION: Liver Fatty Acid Binding Protein (L - FABP) was recently identified to contain antioxidant activity in L - FABP transfected Chang liver cells. In this study we investigated the L - FABP antioxidant effect on the detoxification process of acetaminophen (AAP). AAP metabolism is known to be associated with the release of reactive oxygen species (ROS). METHODS: Chang liver cells, stably transfected with pcDNA3 - L - FABP and pcDNA3 vector, respectively were used in determining the viability of hepatocytes (using the cell proliferation reagent WST - 1) following oxidative stress studies. Chang liver cells were seeded onto 96 - well plates and treated with AAP at different concentrations for various times. RESULTS : AAP treatment induced a significant change in cell viability between pcDNA3 - L - FABP transfected and pcDNA3 transfected Chang liver cells. pcDNA3 - L FABP transfected Chang liver cells were more resistant to AAP induced cell toxicity. CONCLUSION: Our results show that pcDNA3 - L - FABP transfected cells were more resistant to oxidative stress induced by AAP metabolism. Therefore, L - FABP may have a cytoprotective role in AAP induced cytotoxicity. This study was supported by a grant from the CIHR.

P310022

Visualization of thromboxane A2 receptor alpha and beta isoform heterodimerization using fluorescence resonance energy transfer (FRET).

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Thromboxane A2 (TXA2) promotes platelet aggregation and bronchoconstriction following the interaction with two alternatively splice variants of the G protein - coupled TXA2 receptor, termed TPalpha and TPbeta. To visualize the intracellular trafficking of TPalpha and TPbeta in living cells we generated a series of chimeric receptors fused to the green, cyan, and yellow fluorescent proteins (GFP, CFP and YFP). Following individual transient transfection into HEK293 cells, TPalpha - GFP and TPbeta - GFP showed surface expression and signalling efficiency similar to their respective untagged counterparts. Upon U46619 agonist exposure cells expressing TPbeta - GFP displayed an intracellular punctate fluorescence whereas TPalpha - GFP - expressing cells showed homogeneous surface fluorescence, thus only TPbeta undergoes agonist - induced vesicle - mediated endocytosis. In contrast, TPalpha - GFP was efficiently internalised when co - expressed with untagged TPbeta, thus suggesting the formation of hetero - dimers. This was confirmed by detecting FRET between co - expressed TPbeta - CFP and TPalpha - YFP at the surface of unstimulated cells and intracellularly upon U46619 agonist exposure.

Keywords : GPCR, dimerisation, FRET.

P310023

Hydrogen sulfide inhibits cell growth of human lung fibroblast cells

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It is now becoming increasingly clear that hydrogen sulfide (H_2S) is naturally synthesized in many mammalian tissues and that substantially elevated biosynthesis of this gas occurs during chronic inflammation, stress and shock. However, the mechanism by which H_2S is up regulated remains largely unknown. The aim of this study was therefore to assess the effects of H_2S on cell growth and function. To this end, we treated human normal lung fibroblast cells with different concentrations (10 - 70 uM) of NaHS (H_2S donor) and cell cycle alterations, DNA damage and various proteins were studied. A significant dose dependent increase in cell death, apoptosis and reduction in number of cells in the G1 phase was observed after 12 h of NaHS treatment. The G0/G1 phase of the cell cycle was found to be more sensitive than the S and G2 phases. A time - dependent induction of p53 and release of cytochrome c into the cytosol from the mitochondrial membrane was also observed. Our findings suggest a molecular basis for cell cycle - dependent alterations of H_2S and may play a critical role in apoptosis and cell proliferation.

P310024

Toxicity to repeated dose of the humanized monodonal antibody R3, during six months by intravenous route in Cercopithecus aethiops sabaeus monkeys.

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The humanized monoclonal antibody R3 (mAb h - R3), is a product destined to the treatment in human patients by intravenous route, to diminish or eliminate malignant cell transformations associated to the Epidermal Growth Factor receptor overexpression. The objective was to evaluate the toxicity of the mAb h - R3 administration by intravenous route to 18 Cercopithecus aethiops sabaeus monkeys during six months. Three experimental groups were utilized: group Control, and two groups treated to low and high dose of 2.85 and 28.57 (mg/Kg) respectively. Deaths were not observed, the body weight had a significant increase for

weeks, there were not toxic effects in the Haemotological and Biochemical parameters. In the electrocardiography registrations, it was observed a light increment of the cardiac frequency in treated animals. There were neither neurotoxic effects on the studied variables nor macro and microscopic lesions in the skin.

Key words: Toxicity, Cercopithecus aethiops sabaeus, monkeys, monoclonal antibody

P310025

Effects of Propylthiouracil on the Steroidogenesis in Adrenocortical Cells

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The acute effects of PTU on plasma corticosterone and the early rate-limiting step of steroidogenesis in adrenal cells were studied. Rats were catheterized and the blood samples were collected after infusion with saline or PTU. Rat zona fasciculata-reticularis (ZFR) cells were treated with PTU in the presence or absence with ACTH. Media were collected for corticosterone RIA and the cells were collected for Western blot. Infusion of PTU diminished the plasma corticosterone concentrations without changing T4 levels. Both basal and ACTH-stimulated corticosterone release as well as steroidogenic acute regulatory (StAR) protein expression was attenuated by PTU. Also, PTU inhibited the P450scc enzyme activity (a 50% decrease in the Vmax). We also isolated ZFR cells to observe the SF-1 activity by electrophoresis motility shift assay (EMSA) and the ERK1/2 expression. Both basal- and ACTH-stimulated SF-1 activities were attenuated by PTU. Moreover, PTU increased the phospho-ERK1/2 expression. These results suggested that PTU acutely diminished the cortcosterone secretion by (1) activation of ERK protein, (2) inhibition of SF-1 activity, and (3) affection of the early rate-limiting step of steroidogenesis.

P310026

Cytotoxic activities of DPE on human cervical adenocarcinoma and ovarian cancer cells by induction of apoptosis

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Indian Mockstrawberrg Herb (IMH), the herb of Duchesnea indica (Andr.) Focke and Duchesnea chrysartha (Zollinger & Moritzi) Miquel, is commonly used to treat cancer in China for centuries. The objective of our study was to demonstrated its anti-cytotoxicity on cancer cells in vitro and elucidate the underlying mechanism. We evaluated the cytotoxic activities of Duchesnea phenolic extract (DPE) using MTT assay, morphological observation, DNA fragmentation by electrophoresis and flow cytometric analysis. The results showed that DPE at 20-160ug/ml for 72h dose-dependently suppressed the proliferation of Hela, skov-3, HEC-1B and BGC-823 ($p < 0.05$). The induction of chromatin condensation appearance, DNA fragmentation, accumulation of sub-G1 phase and S cell cycle arrest in DPE treated Hela and skov-3 cells evidenced that the cytotoxicity is through activation of apoptosis. Taken together, our study suggests that DPE could inhibit proliferation of cancer cell lines via blocking cell cycle in S phase, inducing apoptosis.

Key word: Duchesnea indica (Andr.) *Focke;* Apoptosis; Cell cycle; Cytotoxic

Acknowledgement: Special thanks to the financial support for this work from the National Key Basic Research and Progress Projects (973), Ministry of Sciences and Technology of China (2004CB72030).

P310027

In vitro discrimination between sedative and non-sedative histamine H1 receptor antagonists by an intact cell binding assay.

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We investigated the changes in the binding properties of sedative and non-sedative antihistamines by histamine (HA)-induced internalization of histamine H1 receptors (H1Rs) in intact human U373 MG astrocytoma cells. Internalization of H1Rs was induced without their degradation by treatment with 0.1 mM HA for 30 min at 37 °C. The binding properties of [3H] mepyramine, a cell-penetrating radioligand for H1Rs, were not changed by the HA pretreatment. The displacement curves for sedative antihistamines (6 drugs tested) against [3H] mepyramine binding were not changed by the HA pretreatment. In contrast, the displacement analyses for non-sedative antihistamines (5 drugs tested) showed that their affinities for H1Rs were reduced by the HA pretreatment, which was prevented under hypertonic conditions where the clathrin-mediated receptor internalization was inhibited. These results suggest that non-sedative antihistamines have the lower affinities for the intracellular H1Rs than for the cell surface H1Rs, possibly due to their less accessibility through the biomembrane. This intact cell binding assay might provide us a novel method for screening sedative and non-sedative antihistamines in vitro.

P310028

IN VITRO ANTIPLASMODIAL ACTIVITY AND CYTOTOXICITY OF NEW N-ALKYL AND N-BENZYL 1,10-PHENANTROLINES DERIVATIVES

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Previous study showed that 1,10-phenantroline skeleton was active in vitro on chloroquine-resistant and sensitive strain of Plasmodium falciparum. Based on the skeleton, 8 derivatives of N-alkyl and N-benzyl 1,10-phenantrolines have been synthesized. This study was conducted to evaluate in vitro antiplasmodial activity and cytotoxicity of these compounds. The in vitro antiplasmodial on chloroquine-resistant P. falciparum strain (FCR-3), chloroquine-sensitive P. falciparum strain (D10) and cytotoxicity test on Vero cells were determined by radioactive method after 24 and 72 h incubation periods, and were expressed by the 50% concentration inhibiting of the parasite or cell growth (IC_{50}). Cytotoxic/antiplasmodial ratio was calculated to evaluate its safety. The highest antiplasmodial activity was observed for (1)-N-benzyl-1,10-phenantrolinium iodide with IC_{50} 0.08-0.59 µM, IC_{50} on Vero cells was 2207.77-126631.51 µM, and cytotoxic/antiplasmodial ratio showed that this compound was safe (9199.04-214629.67).

Key words: 1,10-phenantroline, P. falciparum, antiplasmodial, cytotoxicity

P310029

PROSTAGLANDIN ETHANOLAMIDES (PROSTAMIDES), PHARMACOLOGICAL BASIS OF THE ANTI-GLAUCOMA DRUG BIMATOPROST

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Prostaglandin F2alpha-ethanolamide (prostamide F2alpha) is biosynthesized from anandamide in a two step process involving COX-2 and PGFs. Prostamide F2alpha and its structural analog bimatoprost appear pharmacologically distinct from prostaglandins (PGs). Fluorescence confocal microscopy studies demonstrate that FP receptors and putative prostamide sensitive receptors reside on different feline iris cell populations. Moreover, selective prostamide antagonists have been discovered. AGN204396 selectively antagonizes the effects of prostamide F2alpha and bimatoprost in the feline iris but does not alter the response to PGF2alpha and selective FP receptor agonists.

Bimatoprost is also differentiated from FP receptor agonist at the clinical level in that bimatoprost is effective in glaucoma patients that are unresponsive to latanoprost. Studies on gene regulation in human ciliary muscle cells have implicated the CCN gene Cyr 61. Studies on CCN gene regulation have also provided further pharmacological differentiation in that the prostamide analog bimatoprost upregulates Cyr 61 and PGF2alpha produces upregulation of both Cyr 61 and CTGF.

P310030

Identification of Relaxin-3/Insulin-Like Peptide 7 (INSL7) as a ligand for orphan G-protein Coupled Receptors GPCR135 and GPCR142

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Hundreds of orphan G-protein coupled receptors (GPCR) have been found by searching the human genome database. Among them is GPCR135 (SALPR), whose cognate ligand(s) has (have) not been identified. We have identified both rat and porcine brain extracts that stimulated 35S-GTPgS incorporation in cells over-expressing GPCR135. Peptide purification, followed by N-terminal sequence analysis of the ligand from porcine brain revealed that the ligand is relaxin-3 (aka INSL7), the most recently identified member of the insulin/relaxin family. We recombinantly expressed and purified the human relaxin-3 peptide, which potently stimulates GTPgS binding in GPCR135 over-expressing cell membranes with an EC_{50} value of 0.25 nM. In addition, relaxin-3 inhibits

cAMP accumulation in GPCR135 expressing cells with an EC_{50} of 0.35 nM. ^{125}I-Relaxin-3 binds GPCR135 at high affinity with a Kd value of 0.31 nM. We tested all known peptides in the insulin/relaxin superfamily and found that relaxin-3 is the only ligand that activates GPCR135. Further studies showed that GPCR142, another orphan GPCR that is homologous (43% sequence identity) to GPCR135, is an additional receptor for relaxin-3.

P310031

A Novel Protein Geranylgeranyltransferase-I Inhibitor with High Potency, Selectivity and Cellular Activity

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Inhibiting protein prenylation can modulate signaling proteins, including oncogenes like Ras and Rho. The largest class of prenylated proteins contain a CaaX motif at their c-termini and are subject to a maturation process initiated by attachment of an isoprenoid by either FTase or GGTase-I. Inhibitors of FTase (FTIs) are subject of intensive development and have efficacy in clinical trials. While GGTase-I inhibitors (GGTIs) received less attention, evidence suggests GGTIs may augment therapies using FTIs and could treat additional diseases. Here we characterize a selective, potent, and cell-active GGTase-I inhibitor, GGTI-DU40. Analysis revealed that inhibition by GGTI-DU40 is competitive with the protein substrate; the Ki for inhibition is 0.8 nM. Studies indicate GGTI-DU40 blocks prenylation of geranylgeranylated CaaX proteins. Treatment of breast cancer cells with GGTI-DU40 inhibited thrombin-induced cell rounding via inhibition of Rho proteins without effecting parallel mobilization of calcium via Gbetagamma. These studies establish GGTI-DU40 as a prime tool for interrogating biologies associated with GGTase-I and define a novel structure for this emerging class of experimental therapeutics.

P310032

Intracellular cys-430 is a target for mercury and reactive oxygen species in the P2X2 purinoceptor channel

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Trace metals allosterically modulate P2X receptors. Extracellular histidines are critical in the copper and zinc modulation, but are not involved in the mercury action. To identify the site of mercury action, we used P2X4/2 receptor chimeras that contained the extracellular sequence of the P2X4 but the transmembrane and intracellular domain of the P2X2 receptor. While the ATP-gated current in the P2X4/2a chimera was potentiated by mercury, the P2X4/2b receptor, a splice variant chimera lacking a 68 amino acid segment in the carboxyl end, was resistant to mercury but as sensitive to copper or zinc as the P2X4/2a variant. This observation suggested that the site of mercury action could be one of the 68 additional amino acids of the P2X4/2a variant, contained a single cysteine residue. Site directed mutagenesis of Cys-430 for alanine, both in the wild type P2X2 and the P2X4/2a receptor were resistant to mercury, but not to trace metals. Hydrogen peroxide increased 3-fold the ATP-evoked currents in the P2X2 or the P2X4/2a receptors; its action was abolished in the C430A mutants. Methanethiosulfonate alkylation abolished the mercury or the peroxide potentiation. Funded by CRCP-FONDAP and MIFAB Institute.

P310033

Effects of Calcium Antagonists on Human Equilibrative Nucleoside Transporters

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Objective: To study the effects of nimodipine and other calcium antagonists on human equilibrative nucleoside transporter (hENT)-1 and hENT2. Methods: We have cloned hENT1 and hENT2 and expressed them in nucleoside transporter-deficient PK15NTD cells. [^{3}H] Adenosine uptake by PK15NTD/hENT1 and PK15NTD/hENT2 cells was measured in the presence of different concentrations of calcium antagonists. Results: Nimodipine inhibited hENT1 and hENT2 with IC_{50} of 150 nM and 30 uM, respectively. Kinetic studies revealed that nimodipine decreased Vmax of adenosine uptake without change on Km. Other dihydropyridines were less potent in the inhibition of hENT1. Interestingly, nifedipine and nitrendipine were more effective than nimodipine in inhibiting hENT2 but nicardipine and felodipine had no effect on hENT2. Verapamil (a phenylalkylamine) and diltiazem (a benzothiazepine) showed negligible effects on hENT1 and hENT2. Conclusions: Nimodipine is a non-competitive inhibitor of hENT1 and hENT2. Other dihydropyridines also inhibit nucleoside transporters and their potencies may be related to the ester groups at C-3 and C-5 positions of pyridine ring and nitro group at benzene ring.

P310034

Analysis of coupling of M2 muscarinic acetylcholine receptors to Gi/o, Gs and Gq heterotrimeric GTP-binding proteins

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We have shown recently in our laboratory that activation of individual subtypes of muscarinic acetylcholine receptors leads to changes in several second messenger pathways via coupling to different G-protein subtypes (Michal et al. 2001 Br J Pharmacol 132: 1217-1228; Michal and Dolezal 2005 J Neurochem 94 (Suppl): P. 520). To study interaction between muscarinic M2 receptor and different G-proteins in detail we adopted scintillation proximity assay (DeLapp et al. 1999 J Pharmacol Exp Ther 289: 946-955). We show that under identical conditions different agonists activate different sets of G-protein subtypes. The extent of activation of Gs and Gq G-proteins does not correlate with the magnitude of stimulation of preferentially coupled Gi/o G-protein. On the other hand, it correlates with the magnitude of allosteric intraction between agonist and GDP on receptor-Gprotein complex. We conclude that conformations of M2 receptor induced by interaction with agonists are agonist specific and differ in interaction with G-proteins.

Supported by project AV0Z50110509, grants GACR305/05/0452 and LC554.

P310035

SREBP-1 involved in the effect of curcumin on cholesterol efflux in lipid-loaded cells derived from vascular smooth muscle cells

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Aim To observe the role of SREBP-1 in curcumin-induced cholesterol efflux at lipid-loaded cells derived from VSMCs. Methods Cultured VSMCs were exposed to ox-LDL to form lipid-loaded cells and then were challenged to various concentration of curcumin for different time. HPLC was used to measure the levels of total cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE). Western blot was employed to determine the expression of SREBP1 and caveolin-1. Results ox-LDL (50 mg/L) incubation for 48 h promoted cellular levels of TC, FC and CE, increased cellular lipid droplets, and decreased expression of SREBP-1 and cavedin-1 in VSMCs. Treatment of curcumin decreased the levels of TC, FC and CE in dose-dependent manner, and with the peak at 24 hr, which was accompanied by an decreased cellular lipid droplets. Furthermore, curcumin (25 μmol/L for 24 hr) promoted the expression of SREBP-1 and caveolin-1 in VSMCs. ALLN, an inhibitor of SREBP-1 activity, significantly attenuated the effects of curcumin. Conclusion Curcumin inhibited ox-LDL induced cellular accumulation through increasing the expression of SREBP-1 and caveolin-1 in VSMCs.

Key words: SREBP-1, caveolin-1, curcumin, cholesterol.

P310036

Daxx Downregulation Involved in the Inhibition of Apoptosis in THP-1 Macrophage by Probucol

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Aim To study the correlation between Daxx expression and the antiapoptotic effects of probucol. Methods THP-1 derived macrophages were exposed to ox-LDL to induce apoptosis, which was determined by flow cytometry analysis and acridin orange staining. RT-PCR and indirect immunofluorescence were used to detect Daxx and caspase-3 expression. Results THP-1 macrophages exposed to 100 mg/L ox-LDL for 48 hr results in typical morphologic changes of apaptosis, including condensed chromatin and shrunken nucleus. Ox-LDL treatment markedly increased Daxx expression in a time-dependent manner, and accelerated Daxx translocation from cytoplasm to nucleus. Probucol (50 μmol/L) pretreatment for 4 hr before ox-LDL stimulation significantly inhibited Daxx expression and THP-1 macrophages apoptosis. Furthermore, ox-LDL enhanced caspase-3 expression at both mRNA and protein levels without translocation. Probucol attenuated ox-LDL-stimulated caspase-3 expression. Conclusion

Probucol inhibited the apoptosis by down-regulating Daxx expression and nuclear translocation.

Key words Daxx; apoptosis; probucol; THP-1 macrophage.

This work was supported by the National Natural Science Foundation of China (30470719).

P310037

Toxicological assessment of a N-Glicolil GM3 based-vaccine

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The Center of Molecular Immunology of Cuba has developed a ganglioside based-vaccine using the N-Glicolil GM3. With the aim of it's toxicologic evaluation it was performed acute and repeated dose intramuscular administration in Sprague Dawley (SD) rats. All animals were inspected daily for clinical signs. Body weight and rectal temperature were measured during the administration of vaccine. Blood samples were collected for hematological and serum biochemical determinations. Gross necropsy was accomplished on all animals at the end of study, and histological examination was performed on tissues from the repeated dose study. No significant adverse clinical findings were noted in any study and no significant differences were found in mean body weight and rectal temperature between groups. All treated rats showed tissue hardening and an inflammatory reaction around the administration site. Vaccine treated animals showed an increased of total leucocytes, neutrophils, and spleen weights. No other tissues showed signs of toxicological lesions. In conclusion, N-Glicolil GM3 vaccine was found to have a low toxicity in SD rats.

Key words: N-Glicolil GM3, ganglioside, toxicity, rats.

P310038

Role of Cyclophilin A in Cellular Cholesterol Accumulation in Macrophages Induced by Oxidized Low Density Lipoprotein

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Aim To study the roles of cyclophilin A on cellular cholesterol accumulation in macrophages derived from RAW264.7 monocyte induced by ox-LDL. Methods RAW264.7-derived macrophages were pretreated with 75 mg/L probucol for 30 min and followed by 75 mg/L ox-LDL treatment for 48 hours. Western-blot and immunofluorescence were used to detect the expression of cyclophilin A. Oil Red O staining, HPLC and liquid scintillator accounting were performed to determine the cellular lipid droplets, total cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE). Results Ox-LDL treatment increased cellular levels of TC, FC, and CE, and also increased cellular accumulation of lipid droplets, but decreased cyclophilin A expression by 54.5 ±6.3%($p < 0.05$). Probucol pretreatment significantly decreased the cellular levels of TC and CE companied by an obvious abatement of lipid droplets. Furthermore, probucol promoted cyclophilin A expression by 81.3 ±3.6 %($p < 0.05$). Conclusion Probucol deceases cellular cholesterol accumulation induced by ox-LDL, which might be due to its up-regulation on cyclophilin A expression.

Key words Probucol; cyclophilin A; lipid-loaded cells; ox-LDL.

P310039

The heart-specific miRNA expression in the human bone mesenchymal stem cells (hMSC) induced by 5-aza

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OBJECTIVE: MicroRNAs (miRNAs) are involved in differentiation or cell proliferation in several organisms. The present study is to investigate the heart-specific miRNA expression in the hMSCs induced by 5-aza. METHODS: The hMSCs were isolated from human bone marrow and cultured for 2 weeks, then the cells were cultured continuously in addition with 5-aza for another 2 to 3 weeks. Then the total RNA from cultured cells was extracted and human cardiomyocytes were used as controls separately. Primers used for first-strand synthesis are: for miR-208, 5′-CTT GAG ACA CCG TAA GTC CA-3′, For miR-181a, 5′-AAC AGA AAG CAA GGA ACA GTG A-3′, For miR-143, 5′-ACA AGT GGC TGA TAG TAT GGA-3′, For miR-206, 5′-CTC TTG CTT CCT TGG TGA GG-3′, similar designs For miR-1-1 and miR-1-2. The PCR products were also analyzed by DNA sequencing identification. RESULTS: All the 6 miRNAs were amplified from the human cardiomyocytes, only miR-181a was expressed in the hMSCs, and miR-208, miR-181a, miR-143 and miR-206 were expressed in the hMSCs after incubation with 5-aza. CONCLUSION: The heart-specific miRNAs were involved in the process of cardiomyocyte differentiation from hMSCs, and miR-1-1 and miR-1-2 not-expressed may be also necessary for the cardiomyocyte generation.

KEY WORDS: hMSCs, Cardiomyocyte, MicroRNA.

ACKNOWLEDGMENTS: This work was supported by NSFC (30271287, 3030042, 30571850) and GDNSF (015015, 04102307, 033189).

P310040

Role of rPAF in inflammation and apoptosis in drug-induced model of liver injury. Effects of rPAF-AH in liver regeneration and oxidative stress.

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Aim of this study was to investigate the effects of PAF inactivator, recombinant PAF-acetylhydrolase (Rpaf-AH) on post acetaminophen-treatment functional outcome of the liver in the rat. The control group received a toxic dose of acetaminophen (3.5g/Kg BW) and the rPAF-AH-treated group received furthermore a dose of Rpafah (10 mg/Kg BW). The animals were sacrificed at time points of 56, 66, 72, 84 and 96 after acetaminophen treatment. The hepatic injury was evaluated by determination of degree of liver inflammation and apoptosis. Liver regeneration was estimated by hepatocyte mitotic index. Hepatic levels of malondialdehyde (MDA) and serum superoxide dismutase (SOD) activity were also measured as indicators of tissue damage and as parameters of oxidant-antioxidant balance. The positive effects of rPAF-AH were expressed by (1) high decrease of hepatic injury (2) diminution of regenerating activity and (3) reduction of oxidative stress. These results indicate that the use of PAF inactivator enhances liver's recovery from acetaminophen intoxication and attenuates the severity of experimental liver injury providing important means of improving liver function following acetaminophen intoxication.

P310041

Comparison Of The Solid Phase and Liquid-liquid Extraction Method For Toxicological Screening Using Gas Chromatography/Mass Spectrometry

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Toxicological screening due to chemical substances and drugs can only be achieved using gas chromatography/mass spectrometry. We investigated the effectiveness of liquid-liquid extraction method for drug screening in poisoning suspected cases.

55 materials either blood and urine or both from patients were obtained from various departments, of Gulhane Military Medical Hospital. Samples were extracted by liquid-liquid (LL) extraction method using cyclohexane/ethylacetate. We used acidic, basic and neutral extraction for each sample. Each extract injected in 1 microlitre to GC/MS and offline analysis were performed.

Mean extraction time of each sample was nearly an hour and analysis time of each extract (acidic, basic, neutral) was 25 minute. We found 25 poisoning subjects of 55 cases. The involved drugs were antidepressants, stimulants, local anesthetics, analgesics, antihistamines, hypnotic. Male patients (39 of 55) were considerably higher, this might be due to demographic features.

GC/MS drug screening using LL extraction method in biological samples of patients offers short, easy and inexpensive laboratory diagnosis of the poisoning case.

P310042

Consequences of cobalt chloride induced stabilization of HIF-1α in primary cultures of mouse astrocytes

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Cobalt chloride (CoCl2) is able to stabilize Hypoxia-Inducible Factor-1α (HIF-1α) and in that way mimic some aspects of hypoxia. Exposure of primary as-

trocytes to CoCl2 (0.2 - 0.8 mM) for 24 h resulted in cytotoxicity evidenced by dose - dependent ATP depletion. Both apoptotic and necrotic cells were detected in the culture. Stabilization of HIF - 1α was followed by an increased expression of genes such as pro - apoptotic factor Nip3, inducible nitric oxide synthase (iNOS) and heme - oxygenase 1 (HO - 1). Pre - incubation of astrocytes with bongkrekic acid, an inhibitor of the mitochondrial permeability transition (MPT) pore, reduced ATP depletion significantly. Our data suggest that the action of Nip3 on mitochondria, involving MPT pore opening, is crucial for the apoptotic process caused by Co^{2+}. By contrast, pre - treatment with iNOS inhibitors did not prevent ATP depletion. Caspase activation and oxidative stress contributed modestly to toxicity. Thus, exposure to Co^{2+} in vitro induces several features also associated with the deleterious effects of low oxygen in vivo, e.g. cell death by apoptosis and necrosis, stabilization of HIF - 1α and increased expression of Nip3, iNOS and HO - 1.

Key words: Apoptosis, HIF1α, Nip3

P310043

INFLUENCE OF CYTOCHROME P - 450 INDUCTION ON RAT MALE GONADOTOXIC EFFECTS

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Introduction: Important roles in xenobiotics multilevel influence on spermatogenesis have highly reactive metabolites and oxygen active forms produced during their biotransformation with cytochrome P - 450.

Aim: investigaton of pyrazinamide (cytochrome P - 4502E1 inductor) effects on functional, morphologic and biochemical parameters of rat testis.

Methods: Pyrazinamide (500, 1000, 2000 mg per kg) was administered to rats during all period of spermatogenesis. Lipids, nucleic acids, histones contents, morphologic changes, functional state of spermatozoid were investigated in testis.

Results: Pyrazinamide (1000 and 2000 mg/kg b.w.) lowered spermatozoids number to 22,5%, spermatogonia - to 38,8%, increased spermatocytes meiotic activity to 87,8%, caused changes of cholesterol, DNA, RNA and histones contents. Dose 2000 mg/kg b.w. also caused degenerative changes in testis seminiferous tubules.

Conclusions: xenobiotics effects on male reproductive system tightly connected with its cytochrome P450 2E - dependent metabolism.

Key words: pyrazinamide, cytochrome P450 2E, rat testis

P310044

SSRI TREATMENT INDUCES A NEUROGENIC RESPONSE IN RAT CEREBELLAR GRANULE CELLS

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Neuronal proliferation can be induced by pharmacological stimuli as shown in in vivo and in vitro. Here we investigated whether a neurogenic response could be elicited in cerebellar granule cells (CGC) cultures upon treatment with selective serotonin reuptake inhibitors (SSRI). Immunocytochemical analysis of CGC revealed the presence of granule neurons, glial cells, and a cell component termed round cells. Fluoxetine (1 μM) increased cell proliferation, as assayed by [^{3}H] - thymidine incorporation, but the BrdU cell - culture labelling revealed that only the round cell component accounted for this effect. In view that round cells owns a wide serotoninergic profile and that the fluoxetine - induced proliferation was oriented in a neurogenic fashion (both evidenced by immunocytochemistry and/or PCR), the analysis of the molecular mechanisms r + evealed that this effect seems to be triggered by 5 - HT1A receptor through ERK1/2 and CREB. Present findings show that round cells in CGC cultures proliferate and differentiate in response to fluoxetine, and that this effect may be mediated, at least in part, by CREB activation through activation of MAPK/ERK cascade.

Key words: Cerebellar granule cells; SSRI; ERK; CREB

P310045

INHIBITION BY N - ETHYLMALEIMIDE OF SUBSTRATE UPTAKE AND LIGAND BINDING BY THE HUMAN NOREPINEPHRINE TRANSPORTER (hNET) IS DUE TO DIFFERENT MECHANISMS

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The norepinephrine (NE) transporter (NET), which is responsible for re - uptake of NE, is a target for cocaine, desipramine or nisoxetine (NIS). Although NE transport and NIS binding are irreversibly inhibited by N ethylmaleimide (NEM), it is unknown whether both blocking effects are due to interactions of NEM with the same SH - group of the NET. Therefore, we studied in cells expressing the hNET or hNET - Cys/Ala mutants the effects of NEM on [^{3}H] NE uptake and [^{3}H] NIS binding.

We show that i) the IC_{50} of NEM for inhibition of [^{3}H] NE uptake is more than 30 - fold lower than that for [^{3}H] NIS binding, ii) the two inhibition curves were characterized by clearly different Hill coefficients, iii) half maximum inhibition was reached much faster for [^{3}H] NE uptake, and iv) cocaine or NET substrates were able to protect only [^{3}H] NIS binding, but not [^{3}H] NE uptake, from inactivation by NEM. All hNET - Cys/Ala mutants (not including the functionally essential Cys176 and 185) were active and sensitive to NEM, questioning the importance of NET SH - groups in NEM action. Inhibition of NE uptake by NEM is probably due to inhibition of Na+/K+ - ATPase, which creates the driving force for NE uptake.

Key words: NET, NEM

P310046

Stimulation of chromaffin cell scinderin gene promoter increases stimulation - induced actin disassembly and exocytosis.

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Chromaffin cell (CC) F - actin disassembly allows movement of vesicles (CV) towards exocytotic sites. Scinderin (Sc), a Ca^{2+} - dependent F - actin severing protein, controls F - actin. Sc gene has been clone and its product, Sc, has three actin, two PIP2 and two Ca^{2+} sites. Sc levels were modified by stimulation of Sc gene promoter. Sc promoter has four dioxin responsive elements (DRE) for transcription factor aryl hydrocarbon receptor (AhR). An oligonucleotide with DRE sequences was gel shifted (EMSA) by untreated or TCDD (2,3,7,8 - tetrachlorodibenzo - p - dioxin, a ligand for AhR) treated CC nuclear extracts into a complex blocked by unlabelled, probe. EMSA and Westerns indicated AhR in CC. CC treatment with 10 nM TCDD or 10 μM ATRA (all - transretinoic acid) increased Sc expression, F - actin disassembly and exocytosis. The results demonstrate: the first characterization of Sc - promoter; an increased SCIN transcription following TCDD or ATRA that resulted in potentiation of stimulation - evoked F - actin disassembly and neurosecretion, effect due to an increase in CV at release sites. Therefore, Sc controls the size of the CV pool at release sites.

P310047

Understanding the Relationship between Metabolism and Toxicity for Bioreductive Drugs: a Study on the Anti - Tumour Prodrug CB 1954

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Bioreductive drugs such as CB 1954, nilutamide (anti - tumour) and nimesulide (NSAID) cause hepatotoxicity possibly due to activation by endogenous reductase(s). The aim of this study was to investigate the relationship between bioactivation and hepatotoxicity using CB 1954 as a model compound. Drug administration in mice caused a dramatic increase in both alanine (ALT) and aspartate aminotransferase (AST) and in rats only a slight increase in AST. Histopathological examination of the livers revealed centrilobular hepatocyte injury in mouse but periportal (biliary) damage in rat. Aerobic incubation of CB 1954 with mouse or rat liver S9 resulted in formation of cytotoxic 2 - and 4 - nitroreduction metabolites, which were also seen in vivo. In conclusion, both mice and rats are susceptible to the hepatotoxicity of CB 1954, perhaps via different mechanisms, which may involve endogenous bioactivation. These models may be used to investigate potential host toxicity of other bioreductive drugs, some of which are under development in ACSRC.

Key words: bioreductive, metabolism, hepatotoxicity, CB 1954

This work is supported by the Auckland Medical Research Fund and New Zealand Top Achiever Doctoral Scholarship.

P310048

Interaction of the mu - opioid receptor with synaptophysin influences receptor internalization and signaling

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New insights into opioid receptors may refine the use of opiates and/or develop a new therapy to resist opiate addiction. In search of proteins regulating mu-opioid receptor (MOR1) endocytosis, synaptophysin (Syp) was found to bind to rat MOR1 in yeast two-hybrid assay. Coimmunoprecipitation experiment and bioluminescence resonance energy transfer (BRET) assay confirmed that MOR1 constitutively interacts with Syp in transfected HEK293 cells. Here we show that overexpression of Syp enhances the internalization of MOR1. Conversely, overexpression of a Syp truncation mutant prevents agonist-mediated internalization of MOR1. The observed effects of Syp on MOR1 internalization might result from the interaction between Syp and dynamin, which recruits dynamin to the plasma membrane for the fission of clathrin-coated vesicles. In addition, Syp-augmented trafficking of MOR1 leads to an attenuated agonist-induced receptor desensitization and a faster receptor resensitization. Taken together, our findings strongly suggest that synaptophysin plays a role in the regulation of MOR1 trafficking and signaling.

Key words: mu-opioid receptor, synaptophysin, internalization, desensitization

P310049

Phospholipase D2-Phosphatidic Acid-Diacylglycerol Pathway Is Involved in Agonist-induced delta-Opioid Receptor Endocytosis

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Receptor endocytosis after agonist exposure is one important regulation process of opioid signaling. In investigating delta-opioid receptor (DOR) endocytosis in DOR and PLD2 coexpressing HEK293 cells, we found that DOR physiologically interacted with phospholipase D2 (PLD2) and the DOR agonist DPDPE activated PLD2. Quantitative internalization assay and confocal microscopy results showed that overexpression of PLD2 or heterologous PLD2 activation strongly enhanced agonist-induced DOR endocytosis, whereas overexpression of a catalytically inactive mutant PLD2 or replacement of the PLD2 product phosphatidic acid (PA) with phosphatidylbutanol blocked DOR endocytosis. These suggest that PLD2 activity is required for agonist-induced DOR endocytosis and PA plays a crucial role. PA and diacylglycerol (DAG) can be converted to each other by PA phosphohydrolase and DAG kinase respectively. Inhibition of PA phosphohydrolase attenuated DPDPE-induced DOR endocytosis. Conversely, inhibition of DAG kinase increased DPDPE-induced DOR endocytosis. Therefore the function of PA for DOR endocytosis appears to be played PA-derived DAG. We can conclude PLD2-PA-DAG pathway is involved in agonist-induced DOR endocytosis.

P310050

In Vivo Analysis of hUGT1A1 Homodimerization Using Fluorescence Resonance Energy Transfer (FRET).

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UDP-glucuronosyltransferase 1A1 (UGT1A1) is essential for the biliary excretion of bilirubin, and genetic deficiencies in UGT1A1 cause Crigler-Najjar syndrome. Recent findings suggest homodimerzation of UGT1A1, and mutant UGT1A1 may act as a dominant negative protein in vivo. In order to investigate homodimerization of UGT1A1 in vivo, FRET technique was used to determine protein-protein interaction by generating hUGT1A1 C-terminal tagged fusion proteins with monomeric cyan fluorescent protein (1A1-CFP) and monomeric yellow fluorescent protein (1A1-YFP). The 1A1-CFP and 1A1-YFP cDNA constructs were cotransfected in Cos-7 cells and analyzed for increase in FRET. Cotransfected cells ranged from 40-100 % FRET signal in the cell, indicating 1A1-CFP/1A1-YFP homodimerization in vivo. In addition, 1A1-HA and 1A1-CFP constructs were cotransfected into Cos-7 cells and coimmunoprecipitation using HA-tagged resin beads confirmed the intermolecular interaction of 1A1-CFP with 1A1-HA. In conclusion, this FRET technique can be used to investigate other potential UGT1A isoform homo/heterdimerization complexes in vivo. Supported by USPHS Grant GM49135.

Key words: UDP-glucuronosyltransferase, dimerization, FRET, UGT1A1

P310051

Geranylgeranyl pyrophosphate accelerates the decay of endothelial nitric oxide synthase mRNAs

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Statins are the competitive inhibitor of HMG-CoA reductase and decrease the level of mevalonate to deprive intracellular sterols. In this study, we examined the effects of lovastatin on the expression of eNOS gene in HUVEC-derived cell line, EA.hy926. Lovastatin (25 μM) increased the levels of eNOS mRNAs to approximately 3 fold, which could be prevented by either mevalonate (300 μM) or geranylgeranyl pyrophosphate (GGPP, 20 μM). The mRNA levels were determined by real-time PCR and comparative △△Ct method. In the presence of a transcription inhibitor, either mevalonate or GGPP accelerated the decay of eNOS mRNA. In order to determine whether cis-acting elements are necessary for the decay of eNOS mRNA, four different chimeric gene constructs which contain a part of the human eNOS cDNA were prepared using pEGFP-C2 (Promega). The data from transfection experiments shows that cis-acting elements, which regulate the GGPP-mediated decay of eNOS mRNA, are located in the 3'-terminal including the 3'-untranslated region. Our data indicates that lovastatin increases NO production in endothelial cells by stabilizing the eNOS mRNA.

Key word: Statins, eNOS, mRNA stability

P310052

Huperzine A May Have No Neuroprotective Effect on Ischemic Brain Damage

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Acetylcholinesterase (AChE) inhibitors were used to treat Alzheimer's disease. Recent report showed donepezil had a neuroprotective effect in rats. In this experiment we investigated whether huperzine A could attenuate the ischemic brain damage. Sixty SD rats were divided into 4 groups. One was MCAO (middle cerebral artery occlusion) models given saline and the other 3 were administered huperzine A for 7 days at dose of 0.1 mg/kg, 0.2 mg/kg and 0.4 mg/kg respectively. 30 min after last oral administration the ischemia was made by MCAO. The infarct area of brain was observed. 5 0f 15 brains each group were homogenized and AChE was determined by ELISA with monoclonal antibody 2E6 directed specifically to brain AChE but neither reacted with AChE from erythrocyte nor did with butyrylcholinesterase from serum. The results showed that the amount of AChE in all huperzine A groups was higher than that of model rats. But the infarction area in animals administered huperzine A was not different from that of model rat. It suggested that AChE inhibitor may upregulate the expression of AChE and huperzine A might not have neuroprotective effect on brain ischemia injury.

P310053

Exocytotic glutamate is released by exocytosis from glia particles freshly prepared from the adult rat when subjected to mild depolarizing stimuli

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Previous work has shown that glial sub-cellular particles (gliosomes) represent a viable astrocytary preparation, which exhibits ~30 nm non-clustered cytoplasmic vesicle and contains most of the proteins of the exocytotic machinery. Increasing of internal gliosomal [Ca^{2+}] efficiently stimulated glutamate release and the vesicular fusion rate (J Neurochem 96: 656-668, 2006).

We show here that KCl (15, 35 mM), 4-aminopyridine (0.1, 1 mM) or veratrine (1, 10 microM) induced Ca^{2+}-dependent glutamate release from gliosomes. KCl increased gliosomal membrane potential and cytosolic [Ca^{2+}]. KCl also induced glutamate release and intracellular [Ca^{2+}] increase in cultured astrocytes prepared from adult but not from neonatal rats, particularly after their conditioning with neurons. The KCl-evoked glutamate release and [Ca^{2+}] increase in gliosomes and astrocytes were prevented by blocking the Na^+/Ca^{2+} exchanger. The present results suggest that the ability of gliosomes and cultured astrocytes to trigger glutamate exocytosis by mild depolarization is linked to in situ maturation of glial cells.

Key words: Gliosomes, Glutamate release, Exocytosis, Cultured adult astrocytes
Supported by Italian Ministry of University

P310054

SUPERIORITY OF LIQUID-LIQUID EXTRACTION FOR TOXICOLOGICAL SCREENING IN GAS CHROMATOGRAPHY/MASS SPECTROME-

TRY ANALYSIS

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Gas chromatography/ mass spectrometry (GC/ MS) is the main device for toxicological screening. Although solid phase extraction (SPE) methods have been developing each day, the comparison of the liquid - liquid (LL) and SPE has not been explored for rutin toxicological screening of biological samples.

We compared LL and SPE method for the most encountered toxicological drugs in the emergency department using recovery values in GC/ MS analysis.

We found that the recovery of an antidepressant amytriptilline, a non - steroidal anti - inflammatory drug diclophenac and an antihistamine chlorpheniramine maleat was 96.71 ±2.62, 90.99 ±3.84, 93.43 ±3.15 for LL and 87.05 ± 9.33, 81.43 ±1.76, 80.97 ±3.85 for SPE respectively. The difference between the LL and SPE extraction was statistically significant ($p < 0.01$).

In conclusion, our results show that the recovery values of LL extraction used in this study is higher than SPE, thus offers an inexpensive and acceptable extraction method for the screening of toxicological emergency.

P310055

CCG- 1423, A Small Molecule Inhibitor of the Galpha13/RhoA Signaling Pathway

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Lysophosphatidic acid stimulates G13/ Rho - dependent cellular processes. The rhoGEF, leukemia - associated (LARG), and the serum response factor (SRF) coactivator MKL1 are oncogenes involved in the G13/ Rhodependent transcriptional pathway. A high - throughput SRE - luciferase screening assay was done and a small molecule compound, CCG- 1423, was identified as a pathway inhibitor (IC_{50} - 1.6uM). CCG - 1423 inhibits downstream of Rho, but upstream of SRF, by inhibiting SRE - luciferase stimulated by G12QL, G13QL, RhoAGV, RhoC - GV, and MKL1, but not SRF - VP16. CCG - 1423 shows specificity by not inhibiting GAL4 - luciferase stimulated by GAL4 - VP16 or by a GAL4 - MKL1 transactivation domain fusion. In addition, CCG - 1423 potently (< 1uM) inhibited LPA - induced DNA synthesis in PC - 3 prostate cancer cells, but not SKOV - 3 ovarian cancer cells. It inhibited PC - 3, but not SKOV - 3 matrigel invasive activity. It also inhibited LPA - stimulated cell growth of rho - dependent cancer cell lines, but not rho - independent cancer cell lines. CCG - 1423 should be a useful tool to disrupt rho - mediated responses in cancer.

Key words: Drug Discovery, Rho, Transcription, Cancer

Supported by: NIH R01 GM89561 and the UM Comprehensive Cancer Center

P310056

Functional change of dendritic cells postinfection by recombinant retrovirus carrying fragment of human telomerase reverse transcriptase gene

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To explore the possibility of hTERT as a tumor associated antigen in demritic cell - based immunotherapy, a fragment of hTERT was amplified by RT - PCR and subcloned into retroviral expression vector pLXSN, which was transfected into PT67 packing cell line by lipofectamine. The recombinant retrovirus was transfected into DCs. The level of IL - 12 was determined by ELISA, the abilities of DCs to stimulate allogeneic lymphocyte proliferation were evaluated with MLR, and CD80, CD83, CD86 and HLA - DR were detected by flow cytometry. CTL assays were performed with CytoTox 96 Non - Radioactive Cytoxicity Assay. The results showed that hTERT - DCs had no effects on its secretion of IL - 12 and its stimulation ability in allogeneic lymphocytes reaction and expressed significantly lower levels of CD83. Specific CTLs showed higher cytotoxicity against telomerase positive target cells than negative target cells. Our results indicated that DCs infected with the recombinant retrovirus may inhibit DCs selves' maturation, and that hTERT - DCs do not change their functions of activating and stimulating lymphocytes proliferation, and priming autologus T lymphocytes to generate Specific CTL against hTERT.

Key words: human telomerase reverse transcriptase; recombinant retrovirus; dendritic cells; immunotherapy

(Supported by grants from Natural Science Foundation of Guangdong province, No: 32876)

P310057

Crocetin inhibits angiotensin II - induced vascular smooth muscle cell proliferation via extracellular signalregulated kinases 1/2 pathway

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In the present study, we investigated the effect of crocetin, a natural carotenoid compound isolated from Gardenia jasminoids Ellis, on angiotensin II (Ang II) - induced vascular smooth muscle cells (VSMCs) proliferation and extracellular signal - regulated kinases 1/2 (ERK1/2) activation. 3 - [4,5 - dimethylthiazol - 2 - yl] - 2,5 - dephenyl tetrazolium bromide (MTT) and [^{3}H]thymidine incorporation assay showed that crocetin inhibited Ang II - induced VSMCs proliferation significantly. In - gel kinase assay indicated that Ang II elicited rapid increase of ERK1/2 activity, which was suppressed by crocetin markedly. Western blot and cell - based enzyme - linked immunosorbent assay (ELISA) demonstrated that crocetin inhibited the phosphorylation of ERK1/2 by Ang II. Indirect immunofluorescent technique also showed that crocetin inhibited nuclear translocation of ERK1/2 induced by Ang II. These findings suggest that the inhibition by crocetin on Ang IIinduced VSMCs proliferation can be attributed, at least in part, to its inhibitory effect on ERK1/2 pathway.

Key words: ERK1/2; MAP kinases; Crocetin; Angiotensin II

Acknowledgements: We thank Dr. Yuqing Wu in Nanjing Medical University for the technical assistance.

P310058

Toxicological evaluation of *Morinda citrifolia* **(Noni) in rats**

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Morinda citrifolia (Noni) has being traditionally taken not only for a variety of medical problems but also as a general tonic and restorative. In order to perform its toxicologic assessment were undertaken acute and repeated dose oral studies in Cenp:SPRD rats. Administration was performed by gavage, establishing a Treated and a Control group. Used doses were 2000 mg/ kg of body weight in acute toxicity study and 1000 mg/ kg in the repeated dose study (28 days). It was accomplished daily clinical examinations, besides weekly determination of body weight, water and food consumption. Clinical pathology parameters were analyzed at the end of the test period. All animals were subjected to gross necropsy, and a histological examination was performed. There were no deaths, pathological findings, nor clinical sign alterations. Clinical pathology results reflected slight variations between groups in some parameters, not being of biological meaning. It could be concluded that tested substance is not toxic under our experimental conditions.

Key words: Morinda citrifolia, Noni, toxicity, rats.

P310059

Ketamine and Etomidate Enhance the Activity of Two - pore - domain K+ Channel TREK- 1

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TREK- 1 (TWIK - related K^+ channel) is a two - pore - domain potassium channel expressed highly in the human central nervous system and has been proposed to play an important role in neuroprotection and general anesthesia. Previous studies have shown that TREK - 1 can be activated by several anesthetic agents such as halothane, nitrious oxide, choloroform. However, whether ketamine and etomide affect TREK - 1 channel is not characterized. The purpose of this study is to investigate the action of ketamine and etomidate on TREK - 1 channel. The whole - cell patch - clamp recordings were used in this study. Both ketamine and etomidate could enhance the currents passed in Chinese hamster ovary (CHO) cells stably expressing TREK - 1. Clinically relevant concentrations of ketamine increased outward currents with an EC_{50} of 10 μM, whereas etomidate enhanced the channel activity with an EC_{50} of 1.8 μM. These results suggested

that TREK-1 might play, at least in part, a role in the general anesthetic process of ketamine and etomidate.

P310060

The effect of polysaccharide nucleic acid fraction of bacillus calmette guerin (BCG-PSN) on the chronic urticaria

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Objective: To evaluate the effect of BCG-PSN on the chronic urticaria and investigate the mechanism of it. Methods: Observe the mast cell degranulation by microscope; evaluate the inhibition ratios of locus ceruleu; using radioimmunoassay to detect the level of cAMP in mast cell after treated with BCG-PSN; using ELISA to measure the levels of IL-4 and INF-γ after allergized from 1d to 21d; Results: BCG-PSN can inhibit mast cell degranulation and when the concentrations from 10-6 mg/ml to 10-2 mg/ml, the inhibition ratios are from 36.92% to 68.18%; the inhibition ratios of locus ceruleus of different dose groups are significantly higher than that of the control group($p < 0.05$); the level of cAMP increased significantly ($P < 0.05$) after using BCG-PSN; the serum levels of INF-γ were increased significantly ($P < 0.05$), and IL-4 were decreased dramatically ($P < 0.05$) in different dose groups after allergized 14d. Conclusion: BCG-PSN can prevent and cure the chronic urticaria and the mechanism may be related to regulation and modulation of BCG-PSN to Th1/Th2 cytokines imbalance, which then enhances the cellular immunity.

Key words: BCG-PSN; chronic urticaria; Th1/Th2.

Thank members of Pharmacology for their help!

P310061

Expression of SPATA4 Gene Enhances Cells Resistance to Apoptosis Induced by Etoposide and Taxol

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SPATA4 gene was first cloned from testis cDNA library by creating mouse cryptorchidism model and making use of subtractive hybridization. Results of in situ hybridization assay confirmed that human SPATA4 was expressed in seminiferous tubules, more precisely in Sertoli cells. The apoptosis of Hela cells and Hela/SPATA4 cells induced by etoposide and taxol have distinctive differences which were detected by MTT assay and flow cytometry detection. The cells that express SPATA4 gain the ability of resistance to apoptosis compared with the wild type. All the results above demonstrated that SPATA4 gene may play an important role in the regulation of spermatogenesis as a Sertoli-specific gene. Although we have found that SPATA4 gene possesses anti-apoptosis effect, how it executes the effect has not been clarified. As a result, our further study about SPATA4 gene will focus on elucidating the mechanism of anti-apoptosis.

Key words: SPATA4, Sertoli-specific expression, apoptosis

P310062

Repeated doses toxicity assay of *Dermatophagoides siboney* allergen extract in mice.

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Allergen extracts are used for hyposensitiveness and immunotherapy treatments, reducing significantly the clinical symptoms of the disease. The objective of this work was to evaluate the toxicity of *Dermatophagoides siboney* allergen extract after it's repeated subcutaneous administration to Cenp:NMRI mice. There were established two experimental groups, Control and Treated (20 animals each). Animals were daily observed to detect toxicity signals. At the end of the assay there were carried out hematological and blood chemistry exams, besides an anatomo-pathological examination. There were not detected any significant variations in corporal weight or in water and food consumption. Hematological analysis did not show any variation, but blood chemistry study showed variations in uric acid, urea and glucose, not being of biological relevance. Anatomo-pathological results showed hemorrhagic and inflammatory lesions in both experimental groups. It could be concluded that the used dose of 166.6 UB did not cause lethality or toxic effects in the Cenp:NMRI mice.

Key words: allergens extract, Dermatophagoides siboney, toxicity.

P310063

Possible protective mechanism of *Phyllanthus amarus* Schum & Thonn. aqueous extract on paracetamol-induced hepatotoxicity in rats

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Hepatoprotective mechanism of *P. amarus* was studied by determining the amount of paracetamol and its metabolites (glucuronide, sulfate, cysteine and mercapturic acid conjugates) in urine, pentobarbital-induced sleeping time and hepatic reduced glutathione in rats pretreated with *P. amarus* aqueous extract. Antioxidant activity was also tested. It was shown that the extract neither changed the amount of any paracetamol metabolites nor the sleeping time, but increased the hepatic reduced glutathione. The extract posseses the DPPH radical scavenging activity with IC_{50} of 45 μg/ml and the iron chelating activity. The total phenolic content as tannic acid equivalent was 3.56%. The results suggested that the hepatoprotective mechanism of *P. amarus* aqeuous extract was neither related to the inhibition on cytochrome P450, nor the induction on sulfate and/or glucuronide conjugation of paracetamol, but partly due to the protective effect on the depletion of hepatic reduced glutathione and also its antioxidant activity, especially the radical scavenging and iron chelating activity which might be related to the high phenolic content.

P310064

Studies on arsenic trioxide induces autophagy in human leukemia cell lines HL60

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Autophagy is the bulk degradation of proteins and organelles essential for cell homeostasis and may play an important role in tumorogenesis. Here, we investigated the mechanisms of autophagy in As_2O_3-induced death of HL60 cells. The proliferation of HL60 cells was evidently inhibited after As_2O_3 treatment and autophagy was induced detected by both MDC staining and TEM. The autophagy inhibitor 3-MA has opposite effects in As_2O_3-induced death of HL60 cells: if 3-MA was added 30 min after As_2O_3, it attenuated the death of HL60 cells; whereas if it was added 1h before As_2O_3, it potentiated As_2O_3's cytotoxicitiy in HL60 cells, mitochondrial membrane potential of HL60 cells collapsed, the expression of cathepsin B or D increased, and cell cycle was also delayed. The results suggested that As_2O_3 induced the autophagy of cell line HL60 and the mechanisms of autophagy were differented in it: it acted as a protective mechanisms in forepart of As_2O_3-induced death and induced apoptosis lately possibly due to the decrease of cathepsins activity. There was a mutual regulation between apoptosis and autophagy in death signaling process mediated by mitochondria.

KEY WORDS As_2O_3; autophagy; HL60; 3-MA

P310065

Nogo-66 and myelin-associated glycoprotein (MAG) inhibit the adhesion and migration of Nogo-66 receptor expressing human glioma cells

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Malignant gliomas are common and aggressive brain tumours associated with significant morbidty and mortality. We showed in this report that substratum adherence and migration by human U87MG glioma cells in culture were significantly attenuated by the extracellular domains of Nogo-A (Nogo-66) and the myelin-associated glycoprotein (MAG). U87MG cells contained significant amounts of endogenous Nogo-66 receptor (NgR), and treatment of the cells with phosphatidylinositol-specific phospholipase C (PI-PLC) or NgR antibodies resulted in an increase in their ability to adhere to, or migrate through, Nogo-66- and MAG-coated substrates. Nogo-66 and MAG may therefore modulate glioma

growth and migration by acting through the NgR, a phenomenon that has potential therapeutic implications.

Keywords: glioma, myelin-associated glycoprotein, Nogo-66, Nogo-66receptor.

P310066

MOLECULAR INTERRELATIONSHIPS BETWEEN THE GASTRIC MUCOSAL PROTECTIVE EFFECTS PRODUCED BY CAPSAICIN AND OTHER DRUGS IN RATS.

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Background: NSAIDs produce gastric mucosal damage, which can be prevented by diffferent antisecretory drugs and capsaicin. Aim: To compart the interrelationship between the capsaicin vs. other drugs-induced gastric mucosal protective properties. Methods: Indomethacin (20 mg/kg sc. given) was applied to produce gastric mucosal damage in 4 h pylorus-ligated rats, with or without application of betanechol, histamine and pentagastrin. The gastric acid secretion and mucosal damage (number and severity) were detected. Capsaicin, atropine, cimetidine, prostaglandins, PPI, β-carotene were applied to inhibit gastric acid secretion and gastric mucosal damage. Results: 1. Capsaicin inhibited both the basal and stimulated gastric secretion and gastric mucosal damage; 2. the capsaicin prevents the IND-induced gastric mucosal preventive effect is higher extent than those produced by other atropine, cimetidine, prostaglandin, PPI and β-carotene. Conclusions: The capsaicin-induced gastric mucosal protective effect differ from that produced by other gastric inhibitory drugs and scavanger.

Key words: gastric mucosal damage; gastric mucosal protection; capsaicin; antisecretory drugs; scavanger (Grant: RET-II C8/2005).

P310067

Emodin induces apoptosis in HK-2 cells through caspase3-dependent pathway

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Aim: To know the mechanism of cytotoxic effects on HK-2cells by emodin(1,3,8-trihydroxy-6-methylanthraquinone). Methods: Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) staining. Induced-apoptosis cells were quantitated and the ratio of hypodiploid cells were examined by FACScan flow cytometry. the integrity of genomic DNA was analyzed by agarose electrophoresis. The enzymatic activity of caspase 3 was detected by a colorimetric substrate, Ac-DEVDPna. Results: In vitro emodin induces apoptosis in HK-2 cells, accompanied by the dose-and time-dependent appearance of characteristics of apoptosis including increases in DNA ladder intensity and the ratio of hypodiploid cells. Emodin at apoptosis-inducing concentrations causes an increase of caspase 3 activity. The caspase 3 inhibitor, Ac-DED-CHO, attenuated emodin-induced changes above-mentioned. Conclusion: Our experiments provide evidence that emodin is harmful to kidney through induction of apoptosis in HK-2 cells in caspase3-dependent manner.

Key words: emodin; HK-2; apoptosis; caspase3

P310068

Retinoids Activate the RXR/SXR-mediated Pathway and Induce the Endogenous CYP3A4 Activity in Huh7 Human Hepatoma Cells

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Steroid and xenobiotic receptor (SXR)/retinoid X receptor (RXR)-mediated pathway regulates the transcription of genes encoding xenobiotic-metabolizing enzymes such as CYP3A4. To evaluate the effects of retinoids on RXR/SXR-mediated pathway, transient transfection assays were performed using human hepatoma Huh7 cells with a reporter driven by a RXR/SXR consensus binding element (ER-6). The acid forms or the direct precursor of acid (aldehyde) (9-cis-RA, 9-cis-retinal, 13-cis-RA, and all-trans-RA) exhibited a greater or similar potency than rifampin. RXR may serve as a silent or an active partner of SXR. Furthermore, retinoids can increase CYP3A4 enzyme activity in Huh7 cells. An in vitro drug-drug interaction test showed that 9-cis-RA elevates the covalent binding of N-acetyl-p-quinoneimine, a toxic intermediate formed in acetaminophen phase I metabolism. Taken together, retinoids activate RXR/SXR-mediated pathway and regulate the expression CYP3A4. Thus, retinoids potentially could cause drug-drug interactions when they are administered with other CYP3A4 substrates.

Key Words: retinoid; retinoid X receptor; steroid and xenobiotic receptor; CYP3A4

P310069

Adducts of electrophilic metabolites with CYS^{34} of human and bovine albumin: a method to monitor S-thiolation by drug metabolites implicated in Adverse Drug Reactions (ADRs)

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Sulfamethoxazole (SMX) is an antibacterial sulfonamide that is converted to N-hydroxyl-SMX (SMX-HA) and electrophilic metabolites. We hypothesize that electrophiles react with cysteine thiols of proteins to form a hapten that can be involved in the initiation of immunologically based ADRs. SMX-HA and 2,4-dinitrochlorobenzene (DNCB) react with Cys^{34} in human or bovine albumin at pH 7.0-8.5 in 1:1 to 100:1 molar ratio under nitrogen. Western blots were performed by SMX/2,4-dinitrophenyl antibodies to determine the adducted protein. Densitometry analysis showed a linear relationship between antibody binding and SMX-HA/DNP-P with albumin conjugation up to 130 nM ($r = 0.995$, $P < 0.01$) or 20 nM ($r = 0.974$, $P < 0.01$), respectively. Currently, additional sites (to CYS^{34}) of reaction of DNCB and SMX-HA with albumin are being characterized in trypsin digests by micro-LC/tandem mass spectrometry. Digestion of the adducts yields the Cys-S-DNP or Cys-SMX-Pro-Phe tripeptide. Inclusion of a synthetic deuterated albumin adduct in the initial pronase incubation mixture will permit quantitation of the amount of metabolite S-thiol adduct present in a patient's blood treated with SMX (or another drug of interest).

P310070

The role of β$_1$-integrin receptor in apoptosis induced by ofloxacin in rabbit chondrocytes

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Quinolones (QNs)-induced arthropathy is a major toxic effect in immature animals that has restricted its clinical application. However, its exact mechanism is still unclear. We investigated the mechanism of ofloxacin-induced chondrocyte injuries, focusing on the question whether QNs may induce apoptosis and role of β$_1$-integrin receptors. Juvenile rabbit joint chondrocytes cultured in alginate microspheres were incubated with ofloxacin at 0, 2, 5, 10, 20, 40 μg/ml for 96 hours. Analysis of apoptosis were performed using fluorescent dye staining and DNA ladder. β$_1$-integrin receptors and other signal proteins expression were determined by RT-PCR and/or immunoblotting. Ofloxacin induced apoptosis in a time and concentration-dependent manner, accompanied by degradation of poly (ADP-ribose) polymerase, caspase-3 cleavage and DNA ladder formation. β$_1$-integrin, erk1/2 and Grb2 were significantly reduced but mRNA of β$_1$-integrin was no difference by ofloxacin. Therefore, ofloxacin affect the functions of β$_1$-integrin receptors and subsequently inactivates the MEK1/2 pathway, resulting in apoptosis. Supported by China Natural Science Foundation 30500641.

Key words: ofloxacin, Chondrocytes, Apoptosis, β$_1$-integrin.

P32. Proteomics

P320001

Proteomic analysis effects of tetramethylpyrazine on irradiated QMSC1 cells.

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Tetramethylpyrazine is the active ingredient of a Chinese herbal medicine. In this study, tetramethylpyrazine was tested for its radioprotective activities in QMSC1 cells. The proliferation of QMSC1 cells was measured by MTS assay kit and flow cytometry. Differential proteins found in proteomics was confirmed by RT-PCR and Westhern bloting. QMSC1 cells pretreated with tetramethylpyrazine were irradiated with 20 Gy γradial, irradition inhibited QMSC1 cells growth and tetramethylpyrazine could reverse of this action due to stimulating QMSC1 cells from G1 to S progresssion. Proteomic analytical results showed that 18 protein spots were changed in irradiated QMSC1 cells. The expression level of proteins such as galectin-3, TCTP, p53, Rb were increased, and calmodulin, SDF were decreased in irradiated QMSC1 cells, while tetramethylpyrazine could prevent this change or reverse to some degree. The function of these proteins involves in

hematopoiesis, cell cycle, oxidation, signal transduction, growth factor. This study suggested that stimulating proliferation via tetramethylpyrazine played an important role in the protective effect on irradiated QMSC1 cells.

P320004

Proteomic analysis leads to the identification of hsp90 as a CB2 cannabinoid receptor interacting protein

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CB2 cannabinoid receptor is expressed in the immune system and has been suggested to play an essential role in modulating immune responses. Using an immunoprecipitation and mass spectrometry based proteomic approach, we have identified several candidate proteins that interacting with human CB2 receptor. One of these candidate proteins is hsp90. Immunofluorescence microscopy studies showed that hsp90 and CB2 receptor co-localize with each other. Co-immunoprecipitation experiments demonstrated that Hsp90 is indeed interacting with CB2 receptor. It is known that 2-arachidonoylglycerol (2-AG), an endogenous cannabinoid agonist, causes cell migration. In the current study, knocking down hsp90 with specific short interfering RNAs (siRNAs) in HEK293 cells expressing recombinant human CB2 receptors, as well as in differentiated HL-60 cells expressing native CB2 receptors, markedly reduced 2-AG-induced cell migration. Treatment of cells with geldanamycin, a specific hsp90 inhibitor, also reduced 2-AG-induced cell migration. In conclusion, these data indicate that hsp90 is a CB2 receptor interacting protein that modulates CB2 receptor-mediated cell migration.

Key words: CB2 cannabinoid receptor, proteomics.

P320005

The effect of antenatal glucocorticoid therapy on cardiac function-related proteins in fetal and infant rats

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Antenatal glucocorticoid (GC) therapy has been shown to improve the acute disease in neonate such as infart respiratory distress syndrome (IRDS) and reduce the mortality, though few are known about the effects on cardiac function-related factors in neonate. In the present study, we investigated the effects on cardiac functionrelated factors in GC administered pregnant rats in neonate. Dexamethasone (DEX, 1mg/kg, s.c., for two days) or vehicle was administered to pregnant Wistar rats on the 19th and 21st days of gestation, and 1, 3, and 5day-old neonates were sacrificed. We extracted total proteins of the hearts in neonate and fetal rats, and analyzed the differentiated the proteins by proteomics using technique LC/MS/MS spectrometry. Approximately 10 differentiated spots of proteins all increased with proteome analysis on day 1 after DEX treatment, 5 proteins among them were specified by LC/MS/MS technique as α-enolase, CK-Mtype, β-tubulin, Troponin T and ATP synthase βchain. These results suggest that GC may contribute in increasing cardiac function-related proteins in antenatal therapy.

P320007

Study on Therapeutic effects of Huperzine A on Alzheimer disease using Proteomics

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Objective: To explore the molecular mechanisms of huperzine A (HupA) on aging or Alzheimer disease (AD). Method: The differences of hippocampus proteome among in the two groups of mice, senescence accelerated mice SAM-prone/8 (SAMP8) and treated with huperzine A (SAMP8 + HupA), were analyzed by two dimensional polyacryamide gel electrophoresis (2DE). The proteins were stained with colloidal coomassie blue to produce a high resolution map of the proteome. Results: Compared with SAMP8, 14 proteins expression in hippocampus of HupA + SAMP8 were up-regulated, 14 proteins expressions down-regulated significantly. Using MALDI-TOF-MS, proteins with significant changed were identified by peptide fingerprinting map and the results searched in MASCOT database. The results showed that proteins with changed were associated with mitochondria function, energy metabolism, signal transduction and cytoskeletal protein. Conclusion: The therapeutic effects of HupA on aging or AD are probably exerted via multi-target and multi-path mechanism

Key words: HupA; senescence accelerated mice; hippocampus; proteomics
Acknowledgements: This work is sponsored by the national 973 project (Grant No: 2004CB518907 G1999054401)

P320008

Pharmacoproteomics of Cysteinly Leukotriene 1 Receptor Antagonist in Allergic airway inflammation

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The aim of this study was to investigate global protein profile of bronchoalveolar lavage (BAL) fluid from asthmatic mouse treated with a Cysteinyl leukotriene receptor antagonist MK-571. An asthmatic mouse model was developed using BALB/c mice with OVA immunization followed by OVA aerosol challenge. In treatment group MK-571 was administered intraperitoneally prior every challenge. Control group was given saline vehicle instead. Mice were sacrificed 24 hr post challenge for sample collection. Alleviation of pulmonary eosinophilia as well as serum IgE and IgG1 level was observed in the MK-571-treated group as compared to the saline-treated group. Histological study showed that MK-571 treatment suppressed airway mucus production and inflammatory cell infiltration. BAL fluid protein profile was examined using 2-dimensional gel electrophoresis. Several BAL fluid proteins were significantly reduced by MK-571 treatment. These include lungkine, a chemokine that regulates neutrophil migration; Ym1 and Ym2, members of the chitinase family, which have been shown to be eosinophil chemotactic factors. These protein targets may shed some light on the development of selective inhibitor and biomarker for asthma.

P320009

Effect of chronic morphine exposure on the expression of rat spinal sensory ganglia proteins

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No global protein expression pattern induced by morphine treatment in spinal sensory ganglia has been reported yet. We therefore studied the effect of morphine administration on the level of spinal sensory ganglia proteins. Rats were injected placebo or morphine subcutaneously twice a day for 28 days and sensory spinal ganglia were dissected. The soluble fraction of the spinal ganglia proteins was analyzed by proteomic technologies. Two proteins obviously were altered expression level after morphine administration. They were chosen and identified by database searching of MALDI-TOF MS data, obtained from in-gel tryptic digests of the spots, respectively. They have been identified as aldolase C and proteasome component C8 (PRC8). Subsequently, levels of the two proteins in different regions of rat brain were examined via Western blotting. This report first confirms the effect of morphine on the expression of spinal sensory ganglia proteins and suggests that aldolase C and PRC8 may be related with morphine dependence.

KEY WORDS: Morphine dependence; Mass spectrometry; Aldolase C; Proteasome component C8
Acknowledgment: This study was supported by National "211 Project" in Peking University

P320010

Construction of a two-dimensional gel electrophoresis protein database for the neonatal rat cardiomyocyte

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We have launched a proteomic study of neonatal rat cardiomyocyte, and compiled a profile of proteins expressed in the normal neonatal rat cardiomyocyte by 2-DE and MALDI-TOF MS. In the present study, more than 1000 proteins were separated and displayed from cultured cardiomyocyte. Among those spots, 150 protein spots have so far been identified, and used for the construction of an extensible markup language-based database. On the on-line 2-DE map, the identi-

fied protein spots are hyperlinked to individual protein entries. Further the allidentification information of each protein entry can be obtained through clickable images. This database also possesses the function of high search capacity and links to relevant entries in other on-line databases. In addition, we present protocols describing the sample preparation, 2-DE, MALDI-TOF MS etc, enabling other lab to repeat the results of those experiments.

Keywords: Protein database/neonatal rat cardiomyocyte/2-DE

P320011

Pentadecapeptide BPC157 against muscle crush injury in rat: IP application or cream counteract 6-methylprednisolone-impaired muscle healing

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Stable gastric pentadecapeptide BPC 157 GEPPPGKPADDAGLV, M.W. 1419, used without a carrier, locally or systemically, no toxicity in inflammatory bowel disease (PL-10/PLD116/PL14736 Pliva, Croatia), wound treatment, also heals Achilles tendon or quadriceps muscle after transection, and counteracts the corticosteroid impairment in wounded animal. Therefore, after crush throughout 14 days (rat gastrocnemius muscle complex, impulse force 0.4653 Ns, kinetic energy 0.7217J, force delivered 0.727 Ns/cm^2, not-treated or treated with 6-methylprednisolone 1 mg/kg i.p., once daily), BPC 157 (without a carrier, i.p. (10, 10ng) or locally (1.0 or 0.01 dissolved in distilled water/g commercial neutral cream) as a thin layer, given only immediately after injury (sacrifice at 2h) and/or once daily (finally 24h before sacrifice) improves muscle healing (i) function (walking recovery, motor function index reaches healthy), (ii) microscopy (early increase, then less polymorphonuclears, advanced regenerating myofibres with desmin immunoreactivity and centralized nuclei, larger diameters), (iii) macroscopy (decreased haematoma, edema, hyperaemia, maximum circumference, muscle weight; no post-injury leg contracture).

P320012

Comparative study of the effects of Liuewei and Bawei Dihuang decoction with proteomic techniques

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Liuwei (LW) and Bawei (BW) Dihuang decoction are two classical traditional Chinese medicinal prescriptions. In this study, the effects of LW and BW on the protein profiles in senescence-accelerated mice (SAM) were studied with comparative proteomics techniques. The results showed that compared with that of SAMR1, 49 protein spots were up-regulated and 47 were down-regulated in the serum, 27 were up-regulated and 7 were down-regulated in the hippocampus of SAMP8. LW and BW were found to regulate the abnormal protein expressions of SAMP8 both in serum and hippocampus. There were commonness and differences between the proteins LW and BW affected. Some responded to both LW and BW, some only changed expressions toward LW or BW, and some others showed no responses to both of them. The results suggested that LW and BW may have common and specific reactive proteins, and the specific reactive proteins of LW or BW may be related to their differential pharmacological effects.

Key Words: Comparative proteomics; Liuwei Dihuang decoction; Bawei Dihuang decoction; SAMP8

Acknowledgement: This work was supported by the 973 Project of China (2004CB518907) and the National Natural Science foundation of China (30200367)

P320013

Comparative proteomics on high glucose loaded heart (animal simulation model)

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High blood glucose is the most common problem in diabetic patients. Various cellular responses to this problem were related to oxidative stress-induced cell apoptosis in many kinds of cells. High glucose was supposed to induce generation of reactive oxygen species (ROS) such as superoxide, nitric oxide and peroxynitrite and their derivatives. This ROS accumulation has been accused as major contributor in cell apoptosis and/or possible infarction. Cardiac muscle is one of the most vulnerable tissues that can be impacted by such scenario. This study aimed at disclosing some mysteries related to progression of these events on molecular basis. For doing so, comparative proteomic studies on isolated rat heart that previously perfused for 3 h with high glucose (30 mM) was performed in comparison with control heart (Normal Tyrode perfused). 2-DE proteomic analysis was used to find any proteomic change after glucose loading. MALDI-TOF MS analysis was the 2nd step attempted on the spots that represent the expressed proteins with relative difference (>1.5 fold change) from that in control heart. More confirmation has been done via immunoblotting to assist MS analysis

P320014

Comparative proteomics analysis on the mechanisms of action of Liuwei and Bawei Dihuang decoctions

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The effects of Liuwei (LW) Dihuang and Bawei (BW) Dihuang decoctions on the expression of hypothalamus and pituitary proteins in senescence accelerated mice (SAM) were investigated with comparative proteomics techniques. The results showed that compared with SAMP8, there were 24 up-regulated and 13 down-regulated proteins in hypothalamus and 43 up-regulated and 30 down-regulated proteins in pituitary of LW-treated SAMP8. After treated with BW, there were 29 up-regulated and 20 down-regulated proteins in hypothalamus and 30 up-regulated and 59 down-regulated proteins in pituitary. The results suggested that both LW and BW could regulate the abnormal protein expression of hypothalamus and pituitary in SAMP8. LW and BW had not only common but also specific reactive proteins. These proteins may be the physical bases of their dissimilar pharmacological functions, and also the important protein targets they respectively acted on.

Key Words: Comparative proteomics; Liuwei Dihuang decoction; Bawei Dihuang decoction; Senescence accelerated mice

Acknowledgement: This study was supported by the 973 Project of China (2004CB518907) and the National Natural Science foundation of China (30200367)

P320015

Comparative proteomic study of the effects of Liuwei Dihuang decoction on the hippocampus of senescenceaccelerated mice

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The effects of Liuwei Dihuang decoction (LW) on the expression of hippocampal proteins of senescenceaccelerated mice (SAM) were investigated with comparative proteomics techniques. The results showed that compared with age-matched SAMP8, there were 8 proteins up-regulated and 11 down-regulated in the hippocampus of 6-month-old SAMP8 treated with LW, and there were 6 protein spots up-regulated and 15 down-regulated in 12-month-old SAMP8 treated with LW. Further study found that those differential expressed proteins were closely related with energy metabolism, transcriptional control, mitochondrion function and signal transduction. The results suggested that regulating the protein expression profiles in hippocampus may be one of the underlying mechanisms of its cognitive enhancement of LW.

Keywords: Liuwei Dihuang decoction; Senescence-accelerated mice; Comparative proteomics

Acknowledgement: This study was supported by the 973 Project of China (2004CB518907) and the National Natural Science foundation of China (30200367).

P320016

Proteomic Analysis of Fluconazole Resistance in Laboratory Candida albicans

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In order to develop a more detailed understanding of drug resistance in Candida albicans, comparative proteomic analysis for proteins altered during the development of fluconazole resistance were performed. Quantitative real-time RT-PCR was used to confirm proteomic data. We identified differentially expressed proteins involved in energy metabolisms, cell stress, biosynthesis of macromolecule, and chaperones. Majority of them were found for the first time to be potentially novel fluconazole resistant proteins, e.g., alcohol dehydrogenase, isocitrate de-

hydrogenase, malate synthase, ribosomal protein S5.e, ubiquinol cytochrome-creductase subunit 7, thiol-specific antioxidant-like protein. Measurement of mitochondrial membrane potential and reactive oxygen species provided further confirmation that the metabolism shift and reduced susceptibility to stress damage might contribute to fluconazole resistance in C. albicans.

Key words: Candida albicans/ Fluconazole resistance/ Mass spectrometry/ 2D-PAGE

Acknowledgement: This work was supported by two grants from the National Natural Science Foundation of China (No. 30200012, 30200353).

P320017

Proteomic analysis of striatum mitochondrial proteins in MPTP induced PD mice model.

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Parkinson's disease (PD) is a common age-related neurodegenerative disease. The mechanisms underlying PD are incompletely understood; however, mitochondrial dysfunction is likely to be at least partially responsible. In this study 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a potent mitochondrial toxicant, was used to treat mouse for 7 days. The mitochondrial protein profiles in the striatum were compared between control and MPTP treated mouse. A total of more than 1,000 protein spots have been visualized in the mitochondrial specimens by using proteomics approach. It was found that 11 proteins presenting in the control sample were disappeared in the MPTP treated mouse. This result indicated that these proteins might play important roles in the mitochondrial dysfunction and pathogenesis of PD. Moreover, the significant degradation of movement ability, loss of TH-positive neurons and striatal neurons apoptosis in the MPTP treated mouse were also evaluated by employing the behavioral tests and immunohistochemistry methods.

Key words: Parkinson's disease; proteomic; mitochondria; MPTP

Acknowledgement: This work is supported by the National 973 Fundamental Project of China. NO. 2004CB518906.

P320018

Lipopolysaccharide-stimulated responses in rat aortic endothelial cells by a systems biology approach

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The endothelial cells (ECs) provide an essential defense against pathogens infection. Lipopolysaccharide (LPS) is a critical glycolipid which elicits sepsis or endotoxemia. The aim of the present study is to analyze the late-phase responses of LPS-induced rat aortic ECs by using a systems biology approach, integrating transcriptomics, proteomics, and bioinformatics tools. These high-throughput analyses can provide global changes in the transcriptomic level through a cDNA microarray, as the cellular proteins are identified by 2-DE and MS. The secreted proteins from the ECs are distinguished from the cytokine protein array. Furthermore we design a set of bioinformatic tools to integrate these human databases of the BioCarta, KEGG, and Gene Ontology to analysis the rat data. LPS could promote the phenomena of proliferation, atherogenesis, inflammation, and apoptosis in activated ECs. Interestingly, LPS could also up-regulate the mediators of anti-inflammation, anti-apoptosis, and anti-oxidation to protect themselves. Moreover, the expressions of altered genes, proteins, and pathways can provide further understanding of inflammatory associated responses in ECs.

Key words: Endothelial cells/ Inflammation/ Lipopolysaccharide/ Systems biology

Acknowledgements This work was supported by National Science Council of Taiwan (NSC 93-2320-B-077-008 and NSC 93-2320-B-077-009) and Academia Sinica, Taiwan (AS-94-TP-B10 and 94C008).

P33. Pharmacology of Aging

P330001

Effects of Tetrahydroxystilbene-glucoside on Rat Model of β-amyloid Increased Induced by Hypercholesterolemia

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AIM To investigate the effects of tetrahydroxystilbene-glucoside (TSG) on learning and memory ability, brain β-amyloid (Aβ) content and blood fat in rat model induced by hypercholesterolemia. METHODS The rat model of hypercholesterolemia was induced by feeding high cholesterol forage and TSG was given orally at doses of 30, 60 and 120 mg/kg body wt./day also. Morris Water Maze was tested, the content of β-amyloid was measured by immunohistochemistry and radioimmunoassay methods, the serum cholesterol and low density lipoprotein (LDL-C) were measured by automatic biochemistry analytical methods. RESULTS The learning and memory ability was damaged, the content of Aβ$_{1-40}$ in hippocampus o was increased, and the serum cholesterol and low density lipoprotein level were obviously elevated as well after 10 weeks, TSG could improved these indexes obviously. CONCLUSION TSG possesses obvious action of reducing the content of Aβ$_{1-40}$ in hippocampus, decreasing serum cholesterol and low density lipoprotein, promoting blood circulation and removing blood stasis. These actions may be related to the therapeutic mechanisms of AD.

KEY WORDS: tetrahydroxystilbene-glucoside, β-amyloid, cholesterol

P330002

Gene expression profile in hippocampus of mouse aging model induced by D-galactose

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Rodent chronically treated with D-galactose (D-gal) emerging to be used as an aging model in pharmacological studies. However, the exact mechanism of this model remains unclear. We studied the gene expression profile in hippocampus of mice treated with D-gal. C57 mice were administrated with saline or D-gal for 2, 4 and 8 weeks, followed by learning and memory tests. Then the gene expression in hippocampus was analyzed with cDNA microarray. In comparison of vehicle-treated mice, 8-week D-gal treated mice showed significant spatial learning & memory impairment in Morris water maze; 4 and 8-week D-gal treated mice have significantly lower discrimination index values in object recognition test; 2, 4 and 8-week D-gal treated mice have 10, 14 and 30 genes 2-folds or more down-regulated respectively. These genes are related to ion/protein transport, protein folding and metabolism after 2 or 4-week D-gal treatment, and more genes responsible for protein synthesis, phosphorylation, and signal transduction appeared after 8-week D-gal treatment. This study shows that D-gal induced mouse aging is likely a gradual while complicate process.

Key word: D-galactose; Microarray; Aging model; Mouse

P330004

Effect of APP 17-mer Peptide on Hippocampal Neurodegeneration in Ovariectomized Rats

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The objective of this study was to investigate whether hippocampal neurodegeneration existed in experimental ovariectomized (OVX) rats, and to study the effect of amyloid precursor protein 17-mer peptide(APP 17-mer peptide) on them. The results showed that learning and memory function of OVX rats was damaged, expression of NGF decreased, expression of estrogen receptor-alpha (ER-alpha) increased and mitochondrial swelling occurred in hippocampal neurons. Above changes could be ameliorated by APP17-mer peptide, though the blood estrogen level showed no change. These results indicated that APP 17-mer peptide could ameliorate the neurodegeneration due to estrogen deficiency but the mechanism was not through regulation of estrogen level. Our findings suggest that by activating common intracellular signaling pathways and initiating "cross talk" with neurotrophins, APP 17-mer peptide improves neurodegeneration caused by estrogen deficiency. However, more work will be required to explain the neuroprotection rendered by APP 17-mer peptide in our model.

Key words: ovariectomized rats, neurodegeneration, APP 17-mer peptide

P330005

Effects of combined extracts of Ginseng and Ginkgo Biloba exposure on spatial learning performance and ultrastructure in aged rats

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The aim of this study was to investigate the changes of spatial learning performance and hippocampal neuron ultrastructure in aged rats and the effects of the combination of the extracts of Ginseng and Ginkgo Biloba (NWK), the constitution of extracts for which was derived with orthogonal experiments using normal mice and D-galactose-treated rats. A 90-day NWK administration (62 and 31 mg/kg/day) was performed in a population of 24-month-old Wistar rats. Spatial learning performance was assessed in Morris Water Maze task. Hippocampal neuron ultrastructure was detected with transmission electron microscopy. The escape latencies (in s) and the cumulative distance (in cm) from the platform in the water maze paradigm at both concentrations exposure to NWK were significantly reduced compared to the controls. The morphological and ultrastructural decline of hippocampal neurons was improved at 62-mg/kg dose employed. The results suggest that NWK exert beneficial effects on age-related decline in spatial learning performance, as well as central cholinergic neuron ultrastruture.

P330006

Novel potential antiparkinsonian drug hemantane

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Hemantane [N-(adamant-2-yl) hexamethyenimine hydrochloride] (H) was proved to be effective in various animal models of parkinsonism, including MPTP model. H had a wide spectrum of activity and was more effective than reference drug amantadine. H increased dopamine level, decreased dopamine and serotonin metabolites extracellular levels. The effect of H on the activity of monoamine oxidases (MAO) was investigated in vitro and in vivo. H in vitro acted as a weak competitive inhibitor of MAO-B, partially protected MAO-B against irreversible inhibition by deprenyl. H in vivo while combined with deprenyl caused less pronounced irreversible inhibition of mitochondrial MAO B than deprenyl alone. Thus, protection against MPTP toxicity and the increase of brain dopamine content accompanied by reduction of metabolites may be attributed to MAO B inhibition. Using patch clamp method H was proved to be noncompetitive inhibitor of NMDA channels similar to amantadine. These mechanisms allow to suppose the neuroprotective activity of H. The safety of H was proved in toxicological study. Clinical trials are scheduled.

Key words: antiparkinsonian drugs, adamantanes

P330007

Effect of extract from AV on renal function of early diabetic mice

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Objective: To observe the extract from Chinese medicine AV (AVE) on renal function of early diabetic mice. Methods: The mice were ip injected with alloxan 65 mg·kg^{-1} to induced diabetic model. After 72h, the diabetic mice were treated with AVE 50、100 and 200 mg·kg^{-1} respectively for 2w. The blood was taken by exposing eye ball. The levels of Glu, Trig, Chol, Cre and BUN were determined by Auto-biochemistry Analysis Meter. Advanced glycation end products (AGE) were assayed by AGE-ELISA method. Results: Glu, blood lipids, Cre and BUN increased significantly, meanwhile the number and the size of islet tissues were reduced in alloxan-induced diabetic mice. After treated with AVE, Glu and lipids decreased slightly, while Cre and BUN reduced notablely. The present research also showed that AVE inhibited AGE in vitro significantly. Condution: AVE could antagonise the renal fuction-injured in early diabetic model. This results may be associated with the effect of AVE on inhibiting reaction of nonenyzymatic glycation (NEG) and formation of AGE.

Key Words: AV, renal function, diabetes

P330008

Effects of perirhinal nitric oxide synthase inhibition in the water maze task

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Previous studies indicate that nitric oxide (NO), an endogenous gas generated from L-arginine by nitric oxide synthase (NOS), is involved in synaptic plasticity and memory processes. The present study examined the effects of local inhibition of NOS in the rat perirhinal on performance in the water maze task. Rats with cannulae bilaterally implanted into the perirhinal cortex were trained in the reference memory version of the water maze task for 5 days. The effects of microinfusions of the NOS inhibitor L-NAME (30 μg/side), the inactive isomer D-NAME (30 μg/side), the NO precursor L-Arginine (100 μg/side), L-NAME + L-Arginine, and for comparison, the muscariric receptor blocker scopolamine (3 or 30 μg/side), were then tested. Rats with microinfusions of scopolamine were not significantly impaired in the water maze probe tests. Microinfusions of L-NAME, however, resulted in significant decreases in the percentage time spent in the target quadrant and the number of platform crossings during the probe test. These results show that acute disruption of perirhinal cortex NO disturbs performance of the water maze task, suggesting that NO is involved in memory processes.

Supported by New Zealand Neurological Foundation and University of Otago Research Grant.

P330009

The antifatigue effect of Tushen Yishen Keli

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Objective: To demonstrate the antifatigue effect of Tushen Yishen Keli (TYK). Method: The antifatigue effect of TYK was investigated in swimming model of weight loading mice, hypoxia model of mice in normal pressure, oxidation model of old Wister rats and kidney-YANG asthenia model of mice, respectively. Results: Swimming durations of weight loading mice were prolonged significantly by TYK. The average swimming duration of high dosage group (10 g·kg^{-1}) doubled compared to control. The hypoxia endurance of mice in normal pressure natrica calx container was also enhanced, with livability of TYK gruop prolonged by 82.5%. In the oxidation model of old Wister rats, the content of SOD in akaryocytes and serum testosterone levels of rats in TYK group were elevated. Testicle and accessory sex organ weights as well as serum testosterone levels of renal yang void mice also increased compared to model control group. Conclusion: TYK is a kind of compound preparation of Chinese medicinal herb with the effectiveness of antifatigue, antioxidation and invigorating kidney yang, etc.

key words: Tushen Yishen Keli, antifatigue, antioxygen, invigorate kidney yang

P330010

Study on quality control of Dushenyishen Keli (DK)

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Aim: DK is made up of Eucommia ulmoides Oliv.、Panax ginseng C. A. Mey.、Epimedium brevicornum Maxim、Cynomorium songaricum Rupr.、Rehmannia glutinosa Libosch and Atractylodes macrocephala Koidz. and possesses the function of reinforcing liver and kidney、strengthening bone and musculature、invigorating vital energy and splenic yang. It can be mainly used on effort syndrome、anandria prospermia、sexual function decrescence and so on. establish the standard of drug produce quality control of DK. Methods: deploy TLC to detect the panaxsaponin of Panax ginseng C. A. Mey. and the icariin of Epimedium brevicornum Maxim by comparing different thin layer plate and developing agent and the effect of coloration. Results: the sample shows the same coloration and fluorescence spot on the same positon as control article color spectrum on gel silica G thin layer plate and polyamide film. Conclusion: The method of TLC in this article is of convenience and fort specificity. It can used in quality control of DK.

Key Words: Eucommia ulmoides Oliv; Panax ginseng C. A. Mey. Epimedium brevicornum Maxim

P330011

Distribution and metabolism of Tetrahydroxy-stilbene-glucoside from Polygonum multiflorum in rabbits

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Objective: To investigate the distribution and metabolism of 2,3,5,4'-Tetrahydroxy-stilbene-2-O-β-D-glucoside (TSG), extracted from Polygonum multiflorum, in rabbit plasma and cerebrospinal fluid (CSF) after TSG duodenum perfusion. Methods: TSG was extracted from plasma and CSF of rabbits. After

liquid - liquid extraction, the sample was analyzed by HPLC with SHIMADZU C_{18} column (4.6 mm×150 mm I.D). The mobile phase consisted of acetontrile - water - 1% methenyl acid (15:18:67) at the flow rate of 1.0 mL·min^{-1}, the UV detection wave length was 320 nm. Results: After duodenum perfusion, the time to reach peak concentration of TSG and it's metabolite was 60 min and 210 min in plasma, respectively. TSG was found in CSF 60 min after duodenum perfusion. Conclusion: TSG can cross through the blood - brain barrier and act on the targets in the brain to treat AD.

Key words: Polygounm multiflorum; Alzheimer's disease; Tetrahydroxy - stilbene - glucoside; blood brain barrier; drug metabolism

P330012

Protein Kinase C Epsilon Increases Endothelin Converting Enzyme Activity and Reduces Amyloid Plaque Pathology in Transgenic Mice

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Deposition of plaques containing amyloid beta (A beta) peptides is a neuropathological hallmark of Alzheimer disease (AD). Here we demonstrate that neuronal overexpression of the epsilon isozyme of PKC decreases A beta levels, plaque burden, and plaque - associated neuritic dystrophy and reactive astrocytosis in transgenic mice expressing familial AD - mutant forms of the human amyloid precursor protein (APP). Compared with APP singly transgenic mice, APP/PKCe doubly transgenic mice had decreased A beta levels but showed no evidence for altered cleavage of APP. Instead, PKCe overexpression selectively increased the activity of endothelin converting enzyme (ECE), which degrades A beta. The activities of other A beta - degrading enzymes, insulin degrading enzyme and neprilysin, were unchanged. These results indicate that increased neuronal PKCe activity can promote A beta clearance and reduce AD neuropathology through increased ECE activity.

P330013

Pharmacological Effects of Shen - Wu Capsule on Model Rats of Huntington Disease

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Objective: To observe the pharmacological effects of Shen - wu(SW) capsule on model rats with Huntington Disease(HD). Methods: Rats were treated with 3 - nitropropionic acid to mimic HD. Rats in treating groups were given SW for 25 days. Morris water maze and passive avoidance tests were used to test rats' abilities of learning. Open field test was used to show movement disorder. Radio - ligand test was used to detect bioactivity of choline acetyl transferase(ChAT) in hippocampus. The immunohistochemical staining was used to detect express of BDNF and GDNF in hippocampus. HPLC was used to detect the content of dopamine(DA), dihydroxyphenylacetic acid(DOPAC) and serotonin(5 - HT) in striatum. Results: In Morris water maze, SW could remarkably shorten the swimming time and distance of model rats. In passive avoidance test, latency of SW groups was prolonged markedly. In open field test, SW can improve movement ability of model rats. SW also can improve the content of DA, DOPAC and 5 - HT in striatum, increase expressions of BDNF and GDNF in hippocampus and increase the bioactivity of ChAT. Conclusion: SW can ameliorate the movement disorder of HD model rats and improve the learning and memory ability of HD rats.

P330014

Prolyl - containing dipeptide Noopept - potential therapeutics of Alzheimer disease

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Noopept (GVS - 111, phenylacetyl - prolyl - glycine ethyl ester) was designed as a dipeptide analogue of Piracetam and vasopressin (Seredenin et al., 1995, Patent US, 5.439.930.; Gudasheva et al., 1996). The goal of this study was to evaluate the effect of Noopept (N) in the dementia - related models and to analyze the mechanisms involved. N was revealed to overcome the mnestic deficit caused by long - term scopolamine administration, REM - sleep deprivation, lesion of prefrontal cortex, olfactory bulbectomy. Cholinosensibilizing effect of N, its ability to inhibit glutamate release, to exert the antiapoptotic effect, to increase the neuronal survival under condition of free radical overproduction and Ca - overload, and to produce anti - inflammatory action are testifying to the targeting of this molecule on the important pathogenic mechanisms of neurodegeneration. Basing also on the clinical data on safety and high effectiveness of N in patients with mild cognitive impairment (MMSE score 27 - 28) we came to the conclusion that this systemically active dipeptide can be considered as a promising medicine for multifunctional causal treatment of AD pathology.

Key words: dipeptides, neuroprotection, Alzheimer disease

P330015

Loss of vascular adenosine A1 receptors with age in the rat heart

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This study investigated the effects of age on adenosine A_1 receptor (ADORA$_1$) mediated vascular, inotropic and chronotropic functional responses using a pharmacological approach in Langendorff prepared hearts isolated from immature (6 wks), young (16 wks) and mature (52 wks) rats. The results show a concentration dependent biphasic NECA mediated vasodilator response in hearts from all age groups, with no age related changes. The high affinity site is blocked by DPCPX in immature hearts; evidence that the ADORA$_1$ is involved in NECA mediated vasodilator response in hearts from immature rats but not young and mature rats. In addition, at low concentrations NECA induced a vasoconstrictor response in hearts from animals pre - treated with pertussis toxin (PTX, 48 h 10 mg/kg IP). This response was lost with age. No age - related changes in R - PIA mediated negative inotropic and chronotropic responses were observed. In conclusion, ADORA1 causes vasoconstriction of coronary resistance vessels via a PTX - insensitive pathway and induces vasodilation in hearts from immature rats; responses that decline with age.

Key words: ADORA[1], Age, Vasodilation, Vasoconstriction

Acknowledgement: Appreciation to the Heart Foundation Research Centre, Griffith University for all assistance given.

P330016

Effects of Melatonin on Proliferation and Differentiation of Neural Stem Cells in Rat Ventral Midbrain

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Aim To observe the effects of melatonin (Mel) on proliferation and differentiation of neural stem cells (NSCs) in rat ventral midbrain. Methods Telomerase activity was observed by PCR - ELISA; NSCs proliferation was determined by MTS assay; Differentiation of NSCs was observed by RT - PCR. Results NSCs expression high level of telomerase; By using MTS assay, the values of OD in NSCs were obviously increased under the condition of Mel (0.05、0.1、1、10、100 nM) with basic fibroblast growth factor (bFGF), and decreased significantly after adding MPP+ (1、10、50、100 μM). Mel could return the values of OD incubating 1 h before MPP+ (50 μM) addition; Expression of tyrosine hydroxylase was increased and glial fibrillary acidic protein decreased after adding Mel (1 nM). The expression of neurofilament and choline acetyl transferase was unchanged. Conclusions NSCs show high level of telomerase activity; Mel could promote the proliferation of NSCs and protect NSCs against the oxidative damage of MPP+; It also inhibit the differentiation of NSCs into astrocytes and play the important roles in the early dopaminergic neuron differentiation.

Key Words: melatonin; neural stem cells; proliferation; differentiation

P330017

Neuroprotective effect of Mexidol and Nooglutyl in rats with experimentally produced hemorrhagic and ischemic stroke

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The neuroprotective properties of the positive modulator of AMPA subtype of glutamatergic receptors Nooglutyl (N - (5 - oxynicotinoyl) L - glutamic acid) and antioxidant Mexidol (2 - ethyl - 6 - methyl - 3 - hydroxypyridine succinate) - the agents with nootropic and antihypoxic activities, were studed in the models of intracerebral post - traumatic hematoma (hemorrhagic stroke, HS) and occlusion of the middle cerebral artery (ischemic stroke) in rats. Nooglutyl at a dose of 10 mg/kg diminished HS - induced neurological deficit, movement coordination dis-

turbances, improved the memory in conditioned passive avoidance task and increased the survival in HS model. The volume of ischemic damage caused by the occlusion of distal fragments of the left middle cerebral artery (OMCA) was shown to be 22.51% of ipsilateral hemisphere volume in the saline treated rats. Mexidol at a dose of 50 mg/kg injected i.v. during OMCA diminished the volume of the damage up to 6.5%. These findings suggest the neuroprotective profile of both Mexidol and Nooglutyl action.

Key words: Stroke, Nooglutyl, Mexidol

P330018

Postoperative Nausea/Vomiting (PONV) in the Elderly

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PONV is a common complication in postoperative patients (pts) that can be detrimental to pts and increase the cost of care. Most data in the literature are based upon case histories of adults under age 60. This study is a retrospective survey of the incidence of PONV in pts 60 or older. Of consecutive cases reviewed from Sept 2004 to Feb 2005 1079 (40.4%) were over age 59 (37.6% male, 62.4% female): 3.7% ASA Class I, 54.8% Class II, 35.6% Class III, 5.9% Class IV. The breakdown of pts by method of anesthesia care was: general anesthesia (G) 18.5%; monitored anesthesia care (MAC) 31.3%; topical anesthesia + MAC (TM) 50.1%. Anti-emetic treatment was given intra-operatively to 232/1079 pts (21.5%) based upon history (PONV, motion sickness) or anesthesiologists' discretion: G (168/200), MAC (60/338), TM (4/541). A total of 40 pts required treatment for PONV in the post-anesthesia care unit: G (30/200; 15%), MAC (7/338; 2.1%), TM (3/541; 0.5%). Of these, only 5 (12.5%) were male: G (4/30; 13.3%); MAC (1/7; 14.2%). This study indicates that older pts have the same risk for PONV as do those under 60 years old and confirms reports that the incidence of PONV is significantly higher in females than in males.

P330019

Pharmacokinetics and Pharmacodynamics of Imidapril in Normotensive Elderly

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Object: Imidapril hydrocloride is a prodrug type angiotensin converting enzyme (ACE) inhibitor used in Asian and European countries. ACE inhibitors are reportedly effective for prevention of aspiration pneumonia. Conceivably, it may be used in normotensive patients. We therefore studied safety, pharmacokinetics (PK) and pharmacodynamics of imidapril. Methods: Fourteen normotensive male elderly aged 65 to 80 years were included and 10 of them were administered 2.5, 5, 10 and 20 mg bid for 3 days in dose escalating manner and 4 were administered placebo. Blood samples for imidaprilat, an active metabolite, ACE activity and substance P were collected after the administrations. Results: PK analysis revealed slow increase and slow disappearance of imidaprilat in plasma. Imidaprilat showed a linear PK up to the dose of 10 mg. Supine blood pressure fell by 15 mmHg after the administration of 5 mg, but no further fall was observed in case of 10 or 20 mg. ACE activity decreased dose-dependently, and was well explained by an Emax model. Substance P did not show any change by imidapril. Conclusion: Imidapril was well tolerated and it is considered to be promising in prevention of aspiration pneumonia.

P330020

Unchanged prostate contractility in the aromatase knockout (ArKO) mouse: Comparison between wildtype, heterozygous and mutant mice.

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Changes in the ratio of estrogens to androgens is thought to alter prostate growth and possibly prostate contractility. To further investigate this, isolated organ bath studies using prostates from aromatase knockout (ArKO) mice which were homozygous (Ar-/-) and heterozygous (Ar+/-) for the disrupted aromatase cyp19 gene and wildtype litter mates (Ar+/+), were conducted in Krebs-Henseleit solution at 37°C, bubbled with carbogen, under a resting tension of 0.4-0.7g. Frequency-response curves to electrical field stimulation (1.0 ms pulse duration, 60 V, 0.1-20 Hz) yielded frequency-dependent contractions, while exogenous administration of noradrenaline (10 nM-1 mM) on unstimulated preparations produced concentration-dependant contractions. Prazosin (0.3 mcM) was able to attenuate the responses induced by both noradrenaline and electrical field stimulation in all mice ($P < 0.033$, $n = 4-7$). Dense adrenergic innervation of the prostate was observed in all mice. The results obtained to date suggest that inhibition of aromatase during prostatic development does not alter contractility in mature mice.

Keywords: Aromatase, prostate, knockout mice

P330021

Daily melatonin administration increases the hippocampal MAP2 concentration and the span life of Wistar rats.

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The neuronal structural MAP2 protein associated with cerebral plasticity decreases as a sign of aging. Melatonin (Mel) secretion declines with aging, which could induce neurodegenerative changes observed in old subjects. This study was designed to investigate if the chronic Mel application delays the neuronal degeneration measured through MAP2. Mel (15ug/ml) or vehicle were daily administrated in the drinking water in male eight-monthold Wistar rats (n = 60). Rats were sacrificed at 6 (n = 16) and 12 (n = 16) months of treatment. The remainder rats (n = 28) were maintained until they died. MAP2 determination was made by immunohistochemistry. MAP2 in hippocampal Ca1 and Ca3 was significantly increased in Mel-rats in both treated ages. The span life in the mel treated rats was 20% higher. These data show that exogenous Mel produces a higher MAP2 concentration in the analyzed hippocampal areas which can suggest that this indol could delay the aging in sites involved in memory preserving the neuroplasticity of the brain. Furthermore, Mel significant increases the span life of the Wistar rat.

Key Words: Melatonin, MAP2, Hippocampus, Aging.

P330022

CANNABINOIDS INHIBIT RAT PROSTATE SMOOTH MUSCLE CONTRACTILITY VIA EPITHELIAL CB1 RECEPTORS S Tokanovic, D T

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This study investigated the effect of the synthetic cannabinoid WIN55,212-2 on prostatic smooth muscle contractility. Isolated rat prostates were suspended in 10ml organ baths filled with Krebs-Henseleit solution maintained at 37°C, bubbled with 95% O_2: 5% CO_2. Tissues were stimulated using electrical field stimulation (EFS; 2s train, 0.5ms, 60V, 10Hz, once every minute) and increasing concentrations of WIN55,212-2 (1nM-0.3μM) was tested on the subsequent contractile responses. WIN55,212-2 inhibited EFS ($P < 0.001$) induced contractions in a concentration dependent manner and was blocked by the CB_1 antagonists SR141716 (1μM; $P < 0.001$) and LY320135 (1μM; $P = 0.002$), but not the CB2 antagonist SR144528 (1μM; $P = 0.824$). L-NAME (0.01-1mM) and capsaicin (10μM) had no effect on the inhibition produced by WIN55,212-2 ($P > 0.571$), whereas indomethacin (0.1μM) reversed the effect ($P = 0.041$). These results indicate that WIN55,212-2 inhibits contractions of the rat prostate by a CB1 receptor mechanism, which is dependent on the cyclooxygenase pathway.

Key words: cannabinoids, prostate, smooth muscle, cyclooxygenase

P330023

Expression of Anti-Aging Gene Klotho in Mouse Blood Vessels

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Klotho gene was identified in mice with premature aging. Aorta of those mice manifested undetectable eNOS expression and impaired endothelium-dependent relaxation. Expression of klotho has not been examined in other blood vessels or compared with aorta, in which expression is low. We examined expression of klotho using real-time RT-PCR. Expression of klotho in C57BL/6 mice was low in aorta (7.0 ±2.2 copies/ng RNA; or 4.6e-5 ±1.1e-5 vs. β-actin; mean ±SE, n = 8), carotid artery (2.3 ±0.6; 2.5e-5 ±0.8e-5, n = 4), and coronary artery (9.4 ±7.4; 1.2e-4 ±1.0e-4, n = 4). Levels were dramatically higher in intracranial vessels (1174 ±364; 5.7e-3 ±2.5e-3; n = 7; $P < 0.05$ vs. aorta). Because Klotho protein was reported to upregulate expres-

sion of sod2 (MnSOD), we determined levels of sod1, 2, and 3. In intracranial blood vessels, expression of sod1 and sod2, but not sod3, tended to be higher than in aorta. This finding suggests that Klotho may upregulate antioxidant enzymes in blood vessels. In summary, our findings suggest that intracranial vessels may be an important source for klotho expression, and imply that Klotho may contribute to vasoprotection of cerebral vessels.

Acknowledgment: NIH and VA Medical Service.

P330024

SPONTANEOUS ELECTRICAL WAVEFORMS IN IMMATURE AND OLDER GUINEA-PIG PROSTATES

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Objective: To characterise the spontaneous electrical activity in prostates of immature and older guinea-pigs. Methods: Prostates were removed from guinea-pigs (300-1200g) killed humanely. Electrical activity from the guinea-pig prostate was recorded using intracellular microelectrodes. Results: Four types of electrical activity were recorded in the guinea-pig prostate. In young animals the majority of electrical recordings comprised of slow wave activity (88%) which consisted of a depolarising component with several superimposed nifedipine-sensitive spikes (n=36). Pacemaker activity consisted of a simple waveform of alternating depolarising and repolarising phases and was recorded in 5% of cells; the remaining cells exhibited spike potential discharge (7%). In contrast, the most prevalent electrical activity recorded in the older prostates (56%) was spike potentials (n=22). Slow wave activity was recorded in 28% of cells (n=11), standard transient depolarisations comprised 15% of all electrical recordings and pacemaker potentials were not observed. Conclusion: With age, there is a change in the proportion of cells exhibiting slow wave activity.

Key words: Prostate, electrical activity

Supported by the NH&MRC

P330025

Age-associated decrease in the stimulatory effect of cevimeline on AQP5 levels in the apical plasma membrane of rat parotid glands

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In order to study the mechanisms underlying age-related xerostomia, we investigated age-related changes in the responsiveness of aquaporin-5 (AQP5) in parotid glands. Confocal images revealed that, under unstimulated conditions, AQP5 was located in a diffuse pattern in intracellular structures that likely represent rafts. Ten min after the injection, AQP5 was predominantly located in apical plasma membrane (APM) of interlobular ducts of young but not senescent rats and then 60 min after the injection, there was conversely a diffuse pattern of AQP5. In particular, cevimeline induced a persistent increase in AQP5 levels in the APM in the cells of both young and senescent rats. In some case, however, AQP5 was misrouted to basilar membrane instead of the APM. Thus, cevimeline-induced trafficking of AQP5 to the APM with rafts was decreased in those of senescent rats.

This work was supported in part by a Grant-in-Aid for Scientific Research and Knowledge Cluster Initiative from the Ministry of Education, Science, Sports and Culture of Japan.

P330026

The effects of Liuwei Dihuang decoction on the differential expression genes in the hippocampus of senescence-accelerated mouse

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Liuwei Dihuang decoction (LW), a traditional Chinese medicinal prescription, have been found have the effect of cognitive enhancement. In this study, the effects of LW on the differential gene expression patterns in the hippocampus of 12-month-old male SAMP8 were investigated with cDNA microarray technique. The results showed that LW had significant modulating effects on some of the gene expressions. The expressions of some genes, such as DUSP12, NSF, STUB1, CaMK IIα, AMFR, UQCRFS1 and other 11 novel genes without any functional clues changed significantly. These genes involved in the protein-tyrosine phosphatase family, the AAA gene family, the serine/threonine protein kinases family, ubiquitin ligase, mitochondrial function and so on. Those results suggested that the effects of LW on the cognitive enhancement might be multi-mechanism and the differential expressed genes after the treatment of LW might be the potential gene targets for cognitive enhancing drugs.

Key words: Liuwei Dihuang decoction, senescence-accelerated mouse, cDNA microarray

Acknowledgement: This study was supported by the 973 Project of China (2004CB518907) and the National Natural Science foundation of China (30200367)

P330027

Ubiquitin ligase human Hrd1 facilitates tau degradation

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Abnormal accumulation of hyperphosphorylated tau in intracellular inclusions is a recognized pathological feature of dementias. To explore the pathogenesis of tau deposition, human hrd1 (hHrd1), a ubiquitin ligase with RING finger domain, was used as a candidate supposed to interact with tau in this study. Here we show that hHrd1 expression in the postmortem brain tissue of Alzheimer's disease is inversely correlated with phosphorylated tau recognized by Alz50 antibody. Consistent with this observation, hHrd1 expression is inversely correlated with the the level of tau and hyperphosphorylated tau in 293T cells cotransfected with hHrd1 and tau. Actually, the degradation of tau was enhanced by hHrd1 after cycloheximide, an inhibitor of protein synthesis, was added to the cells stably overexpressing EGFP-tau. Importantly, when MG132 was used to inhibit proteosome, we observed the increase of high molecular weight polyubiquitinated tau when co-transfected with hHrd1, compared with tau alone. Therefore, hHrd1 may play an important role in the regulation of tau degradation, which may be a potential therapeutic target.

Key words: tau; hHrd1; AD; ubiquitination

P330028

Effects of tea polyphenols on the learning and remembrance impediment mice induced by D-galactose

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Objective: To establish the learning and remembrance impediment mice induced by D-galactose and study the improvement effects of tea polyphenol (TP) on the model mice. Methods: D-galactose (120mg/kg) was intraperitoneally injected into mice for 12 weeks. The protective and therapeutic effects of TP were determined by suing water maze test, step down test, step through test and open field test. Results: TP ameliorated the deleterious effects of D-galactose, and thereby improved the animal's learning and memory, prolonged latency time, and the error numbers were significantly reduced, at the same time the autonomic activities were significantly increased. Conclusion: TP can improve the learning and remembrance behavior of mice induced by D-galactose.

Key words: tea polyphenol; D-galactose; learning behaviors

Acknowledgement: We would like to thank the financial support of the Science and Technology Development Fund of Macao (035/2005/A)

P330029

SalB stimulates neurogenesis and angiogenesis in vivo

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The effect of SalB on neurogenesis and angiogenesis was studied in vivo with middle cerebral artery occlusion (MCAO) rats as focal cerebral ischemia model. SalB (1-10mg/kg) was administrated i.p. right after MCAO and consecutively given once daily during the whole experiment period of two weeks. The same time, BrdU was given i.p. every other day. Cell damage was assessed with Nissl stain. Blood brain barrier (BBB) permeability was investigated by fibronectin filtration. Neurogenesis and angiogenesis was represented by new neural cells and endothelia which were recognized with NeuN-BrdU or CD31-BrdU double staining respectively. SalB 5 and 10mg/kg alleviated the neural cell loss and inhibited the fibronectin leakage induced by ischemia. SalB 5 and 10mg/kg signifi-

cantly enhanced the neurogenesis in DG of hippocampus. SalB 5 and 10 mg/kg significantly enhanced the angiogenesis in cortical area of ipslateral side. There are no obvious effect of SalB on Flk and VEGF after MCAO. The time differential of the angiogenesis and neurogenesis remains to be investigated. The results above suggest that SalB could improve neurogenesis and angiogenesis after cerebral ischemia.

P330030

Experimental Study on Prevention and Treatment of Alzheimer's Disease Model Mice with Vitamin E

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Objective: To investigate the prevent and therapeutic effects of vitamin E on alzheimer's disease mice and its mechanism. Methods: Inject D-gal and sodium nitrite to prepare models of alzheimer's disease. Vitamin E was administrated during the period of modeling and after model established separately. Water maze test was performed to evaluate learning and memory ability of the mice. The AchE, superoxide dismutase(SOD) activity, the level of malonaldehyde(MDA) were measured with biochemical method. The expression of β-AP、NF-κB in the brain was measured with the immunohistochemistry method. Results: Compared with model group, those received vitamin E during the period of modeling manifested alleviation of learning and memory capacity($p < 0.01$), enhanced SOD activity and reduced AchE activity, MDA content, the expression of β-AP、NF-κB ($p < 0.01$). But those received vitamin E after model established didn't show any change mentioned above. Conclusions: Vitamin E can prevent the learning and memory ability impairment; the mechanism is probably related to promote the scavenging of the free radicals, reduce AchE activity and the expression of β-AP、NF-κB in the brain.

P330031

A Comparison of Elderly and Adult multiple Organ Dysfunction Syndrome in the Rat Model

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This work was to study the mechanisms of MODSE compared with adult MODS. Elderly and adult rats were ip with zymosan (Zym) to incite MODS. Functional and pathological changes of major tissues, Apoptosis and intracellular Ca^{2+} of alveolar macrophages (AMs), and cytokine levels were studied. Zym treated rats showed dramatic changes in blood gas and biochemical parameters. Obvious pathological lesions in lung, heart, liver, brain, and kidney tissues were found parallel to their functional declination. Remarkable reductions in respiratory, cardiac and renal functions in elderly Zym rats were severer than those in adult rats. AMs from all Zym treated rats showed increased apoptotic rate (AR) and intracellular Ca^{2+}, decreased $\Delta\Psi m$, enhanced supernatart and serum levels of TNF-α and IL-10. The elderly Zym rats clearly had higher AR and serum TNF-α but lower serum IL-10 than the adult Zym rats. This study suggested that Zym induced deterioration changes in major organs irrespective of elderly or adult. However, under the same conditions elderly rats underwent severer damage than adult which are indicative of the possible roles of lung in triggering MODSE.

Key words: MODSE; animal model; rat; zymosan

P330032

Effect of (-) clausenamide on tau hyperphosphorylation induced by Okadaic acid

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Tau hyperphosphorylation leads to neurofibrillary tangles associated with Alzheimer's disease (AD). In this study, we want to detect the effect of (-) clausenamide (clau), a new compound isolated from clausena lanisium lour skells, on tau hyperphosphorytion. Okadaic acid (OA), a specific inhibitor of PP-2A and PP-1, was used to induce tau hyperphosphorylation model in SH-SY5Y cells. Using this model, MTT assay and lactate dehydrogenase (LDH) assay showed (-) clau (10-8 mol/L) decreased the neurotoxicity. Glycogen-synthase kinase-3(GSK-3) is a critical kinase leading to tau hyperphosphorylation. Western blotting experiments showed (-) clau increased the phosphorylation at the 9-Ser and 21-Ser sites of GSK-3 then inhibited its activity but couldn't change its expression. The expression of AT-8 antibody, which reacts specially with phosphorylated Ser199/202 sites of tau, was reduced. From above results, we can conclude (-) clau can improve the viability of SH-SY5Y cells and regulate the activity of GSK-3 then reduce the abnormal tau phosphorylation. In previous studies (-) clau improved cognition and inhibited apoptosis, antagonized β-amyloid induced toxicity, so that (-) clau may be a useful neuroprotective agent for AD.

Key word: AD; tau; hyperphosphorylation

P330033

Bis(7)-tacrine Prevents Focal Cerebral Ischemic Insults More Potently Than Memantine in Middle Cerebral Artery Occlusion Rats

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Bis(7)-tacrine, a novel and promising anti-Alzheimer's dimer derived from tacrine, has been proved to block the NMDA receptor with the similar affinity as memantine. Therefore, we investigated whether bis(7)-tacrine could prevent focal cerebral ischemic insults in middle cerebral artery occlusion rats. Bis(7)-tacrine (0.1-0.2 mg/kg) significantly reduced the neurological deficits including the improvement of neurological score, reduction of infarction and brain edema after 2h occlusion/24h reperfusion. Compared with memantine, bis(7)-tacrine showed approximately 260 times higher neuroprotective activity. Bis(7)-tacrine substantially reduced neurological deficits after focal brain ischemic injury possibly by blockade of NMDA receptor, which might potentially become a potent neuroprotective drug for treatment of stroke.

Key Words bis(7)-tacrine, memantine, NMDA receptor antagonist, stroke
(This work was supported by grants from the Research Grants Committee of Hong Kong (HKUST 6120/02M, 6133/03M, AoE/B15/01))

P34. Pulmonary Pharmacology

P340001

Anthocyanins inhibit airway inflammation and hyperresponsiveness in a murine asthma model

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Asthma is a common chronic inflammatory disease associated with T-helper cell type 2 (Th2) responses such as the production of interleukin-13 (IL-13). Additionally, oxidative stress may play an important role in eosinophilia, mucus hypersecretion, and airway hyperresponsiveness (AHR). It has been reported that anthocyanins, natural pigments in the human diet, have positive effects in various disease models. However, little is known about the effects of anthocyanins in animal asthma models. In the present report, we investigated whether anthocyanins would reduce airway inflammation in a mouse asthma model. Mice were immunized and challenged with ovalbumin (OVA). OVA inhalation elicited inflammatory responses characterized by eosinophilia in bronchoalveolar lavage (BAL) fluid, increase of enhanced pause (Penh), mucus hypersecretion, and an increase in IL-13 mRNA expression in lung tissues. All parameters were attenuated in a dose-dependant manner by administration of anthocyanins. These results demonstrate that anthocyanins may attenuate the development of asthma by downregulating IL-13 mRNA expression. Our findings suggest that anthocyanins may have positive contributions for the prevention of asthma.

P340002

Tumor necrosis factor (TNF)-a and cigarette smoke (CS) synergistically enhances IL-8 production by U937 cells, which is prevented by antioxidants.

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The pathophysiology of CS-induced lung emphysema is complex and involves the attraction and activation of inflammatory cells, like neutrophils. In many reports the crucial role of reactive oxygen species (ROS) and cytokines (TNF-a

and IL-8) have been demonstrated. In the present study, we investigated whether TNF-a and CS alone or in combination induces the production of IL-8 by human lymphoma U937 cells. TNF-a or CS induced a dose-dependent increase in IL-8 production. Interestingly, co-incubation resulted in an enormous synergy in IL-8 production. CS is a source of ROS and therefore we wanted to mimic these effects with SIN-1, a peroxynitrate donor. To further proof the involvement of ROS, the antioxidants N-acetylcysteine and DMSO were used. SIN-1 also induced a dose- and time-dependent increase in IL-8 production, which was again synergistically increased in with TNF-a. Moreover, the synergy could be completely prevented by antioxidants. IL-8 is one of the most important cytokines in COPD, since it attracts and activates neutrophils to release ROS and proteases. A combination therapy directed against TNF-a and ROS might stop the deterioration in lung function in patients with emphysema.

P340003

Effects of the MMP inhibitor GM-6001 on emphysema development, inflammation and MMPs activity induced by cadmium in rat.

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Recently, we developed a cadmium-induced emphysema model in rats sharing some main characteristics of the human chronic obstructive bronchopneumopathy disease. The aim of this study was to assess the effect of a non specific MMP inhibitor on pulmonary emphysema and inflammation in this model.

Rats were exposed with or without GM-6001 and then with CdCl2 for 1h/day, 3 days/week during 5 weeks or vehicle. Immediately or 2 weeks later, BALs were performed on the right lung and cytology, cell count and zymography were performed. The left lung was inflated with formalin and lung emphysema was measured. GM-6001 induced marked anti-inflammatory effects by significantly reducing the numbers of lymphocytes, macrophages and neutrophils in the BAL fluid of treated-rats and significantly reduced emphysema at 5 weeks. GM-6001 significantly reduced MMP-9 activity all along the protocol and MMP-2 activity at 5 weeks in the BAL of treated-rats.

In conclusion, GM-6001 reduces emphysema and inflammation in this model of cadmium-induced emphysema and the results suggest that this protection is mediated by MMP-2 and MMP-9 inhibition.

Keywords: Emphysema, GM-6001, COPD
Grant Number: 021/5112 (RWDGTRE)

P340004

Elucidation of the receptor(s) involved in the modulation of sensory nerve activty evoked by PGE2

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PGE2 is a bronchodilator and anti-inflammatory agent that causes airway irritancy and cough. The aim was to identify the prostanoid receptor(s) (PRs) (EP1-4, DP, FP, IP and TP) involved in the cough response. To this end we attempted to identify the PRs involved in the activation of the sensory nerves triggering the cough response using a range of PR agonists and antagonists on the isolated vagus nerve preparation. Human and guinea pig vagus was de-sheathed, mounted in a 'grease-gap' chamber, exposed to ligands and depolarisation recorded. PGE2 caused a similar depolarisation of human and guinea-pig vagus. Profiling of selective PR agonists demonstrated all ligands to cause depolarisation of the guinea-pig vagus. Pre-treatment with antagonists that blocked EP1, 2, 4 receptors failed to impact on prostanoid induced sensory nerve activation, whereas the TP, EP3 and FP receptor antagonists did have an inhibitory action. These data suggest that PGE2-induced sensory nerve activation is mediated by EP3, IP, TP and/or FP receptors. This data may aid in the development of a selective PR agonist devoid of this side-effect. Lung, Prostanoids, Sensory Nerves Clinical Research Committee, Brompton Hospital

P340005

Reactive oxygen/nitrogen species in airways inflammation

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Although the participant cells and mediators are different, bronchial asthma (BA) and chronic obstructive pulmonary disease (COPD) are both characterized by chronic airway inflammation. Oxidative/nitrosative stress play an important role in the pathophysiology in both diseases. In BA, the production of nitric oxide (NO) is increased probably via the upregulation of inducible NO synthase. It has been reported that the level of exhaled NO is correlated with the severity of airflow limitation, airway hyperresponsiveness or eosinophils infiltration. The anti-inflammatory agent, corticosteroid, which is a key drug for BA, can reduce the NO production as well as airway inflammation and hyperresponsiveness. On the contrary, in COPD airways, the formation of 3-nitrotyrosine rather than NO is much more increased than bronchial asthma. We have found that the several agents including theophylline, corticosteroid and allopurinol can inhibit the oxidative/nitrosative stress. These agents improve the airway inflammation and may prevent the progression of COPD. In this symposium, the importance of oxidative/nitrosative in the airway inflammation and its pharmacotherapeutic modfication will be reviewed.

P340006

DS1 delay lung fibrosis by withstanding HIF-1α

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In this study, we investigated the effect of DS1 on lung fibrosis induced by bleomycin (BLM) in rats, and the possible mechanisms. Following a single intrachacheal instillation of BLM (5 mg/kg) or saline, rats were orally administrated DS1 (2.5 mg/kg body weight) or water once daily for 21 days. DS1 reduced the increases of hydroxyproline content and mRNA expression of collagen I on day 21 after BLM treatment. HE and Masson's trichrome staining also showed that DS1 delayed lung fibrosis induced by BLM. Results of western blotting and RT-PCR revealed that DS1 depressed the high expression of HIF-1α and connective tissue growth factor (CTGF) and the increase transcription of hypoxia-inducible genes of glucose transporter-1, endotheline-1, and vascular endothelial growth factor induced by BLM. In vitro, the significant decline in HIF-1α and CTGF were observed in hypoxic lung fibroblast after treated by DS1 (10 μM) or siRNA of HIF-1α. These results suggested that DS1 significantly delay BLM-induced lung fibrosis by inhibiting accumulation of HIF-1α at least in part.

Key words: lung fibrosis, DS1, hypoxia, HIF-1α

P340007

Intermedin/adrenomedullin-2 (IMD/AM2) dilates the rat pulmonary vascular bed: Dependence on CGRP receptors and NO

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The present study was undertaken to investigate the effects of rat IMD/AM2 (rIMD) in the isolated buffer perfused rat lung (IBPR). When pulmonary vascular tone was increased by U46619, bolus injection of rIMD decreased pulmonary arterial pressure in a dose-dependent manner. Pretreatment with L-NAME and CGRP8-37, unlike meclofenamate and glybenclamide, reduced the pulmonary vasodilator responses to rIMD. rIMD induced cross-tachyphylaxis to the pulmonary vasodlator response to CGRP whereas CGRP did not alter the ability of rIMD to dilate the IBPR. Pulmonary vasodilator responses to repeated injections of rIMD did not undergo tachyphylaxis. The present data suggest activation of CGRP1 receptors and release of nitric oxide mediates the pulmonary vasodilator response to rIMD. The ability of rIMD to induce heterologous desensitization of CGRP1 receptor activation, to retain much of its pulmonary vasodilator activity after inhibition of CGRP1 receptors, and to lack homologous desensitization together suggests the pulmonary vasodilator response to rIMD may depend on other vasodilator mechanisms including receptors in the calcitonin receptor-like receptor family.

P340008

The Inhibitory Effect of Nobiletin on Human non-small Cell Lung Cancer Cell Line A549

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Objective To investigate the inhibitory effect of nobiletin (5,6,7,8,3'4'-hexamethoxyflavone) on A549 cell line and its mechanism. Methods The inhibitory

effect of nobiletin on A549 cells was evaluated by MTT, growth curve, clone-forming assay, microscope, flow cytometric analysis and agarose gel electrophoresis. Results After treated with nobiletin for 24、48、72 hours, MTT assay showed IC_{50} of nobiletin to A549 in 24h、48h and 72h were 38.2 μg/ml, 25.7 μg/ml and 16.7 μg/ml respectively; IC_{50} of nobiletin to A549 cells in clone forming test was 25.9 μg/ml. The dose-effect and time-effect relationship were descibed in the growth curve. The characteristic morphology typical for apoptosis was observed under microscope. The cell cycle was arrested in G_2/M phase, cells in G_0/G_1 phase decreased. The percentage of apoptosis increased. The sub-G_1 peak, DNA ladder typical for apoptosis, Significant raise of bax expression and the ratio of bax/bcl-2 was observed. Conclusions Nobiletin can inhibit the growth of A549 cells in vitro, its mechanism is probably associated with the apoptosis induction.

Key words: Nobiletin; A549 cell line; apoptosis

P340009

COMBINING ATORVASTATINE AND CELECOXIB IN THE TREATMENT OF PULMONARY HYPERTENSION IN THE RAT

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Cyclooxygenase (COX) and HMG-CoA reductase inhibitors by reducing inflammatory processes and cells proliferation might prevent pulmonary hypertension. Methods: Celecoxib (Clb, 7.5 or 25 mg/kg/day), atorvastatine (Ator, 2 or 10 mg/kg/day) or vehicle were given orally, separately or in combination, for 28 days to rats injected or not with monocrotaline (MC, 60 mg/kg intraperitonealy). Results: Treatment by Clb high dose, Ator both doses and combination of both compounds at high-doses prevented the increase in right ventricular hypertrophy of MC rats. We found a beneficial effects of the combination of low-doses Clb and Ator on endothelium-dependent pulmonary artery dilation (Emax = 57 ±6 % vs. 44 ±2 % for MC+Clb7.5+Ator2 and MC, respectively, $p < 0.05$). On the contrary combination of Clb and Ator at high-doses of was associated with a deleterious effect on ACh-induced pulmonary arteries relaxation (Emax = 32 ±6 % vs. 44 ±2 % for MC+Clb25+Ator10 and MC, respectively, $p < 0.05$). Conclusion: This study demonstrates that the use of a lipophilic statin in combination with COX inhibitors can attenuate the development of monocrotaline-induced pulmonary hypertension in the rat.

Key words: statin, COX, Pulmonary hypertension

P340010

Effects of prednisolone on the increased expression of RhoA and CPI-17 in bronchial smooth muscle of airway hyperresponsive rats.

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Airway hyperresponsiveness (AHR) is one of the asthmatic characteristic features. We have demonstrated that Ca^{2+} sensitization is markedly augmented concomitantly with increased expression of RhoA and CPI-17 proteins in bronchial smooth muscle of AHR rats. Inhaled corticosteroids are now the most effective therapy of choice for persistent asthma. Presently, the effects of prednisolone (PRE) on the increased expression of RhoA and CPI-17 in AHR were examined. Male Wistar rats were sensitized with DNP-Asc together with Bordetella pertussis as an adjuvant, and boosted 5 days later. Eight days after the first immunization, the rats were challenged by inhaling DNP-Asc 3 times every 48 hr. During the days 8 to 12, the rats were treated everyday with PRE (10 mg/kg, i.p.). To examine the expression of RhoA and CPI-17 proteins and mRNAs, Western blot and RT-PCR analyses were performed. As a result, both the increased ACh-induced bronchial smooth muscle contraction and expression of RhoA and CPI-17 proteins and mRNAs were significantly inhibited by PRE treatment. Therefore, PRE, at least in a part, seems to inhibit AHR through the inhibition of overexpression of RhoA and CPI-17.

Key words: AHR, RhoA, CPI-17, PRE

P340011

Expression of pro-inflammatory genes (Pro-I) in A2A adenosine receptor (A2A) knockout (KO) mouse model of allergic asthma.

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Adenosine-mediated anti-inflammatory response in the lung involves A2A activation, which may lead to inhibition of Pro-I gene expression. Expression of i-NOS, p65 subunit of NF-kappa B (p65) and A2A genes along with NO and Pro-I cytokines were assessed in A2A KO murine model of asthma. KO and WT mice were sensitized according to our published protocol (Fan and Mustafa. Pulm Pharmacol Ther. 15:147, 2000). A day after last challenge, BALF and lungs were collected for Pro-I gene expression. Ragweed (RW) challenge in sensitized mice increased gene expression of both p65 and i-NOS of WT and KO as compared to the controls ($p < 0.01$). A2A expression was down-regulated by RW challenge in WT sensitized mice as compared to controls with no transcripts being detectable in KO. Pro-I cytokines (IL-2 and IL-4) and NO levels were also increased in KO challenged mice as compared to WT ($p < 0.01$), with KO and WT having greater NO levels than their controls ($p < 0.01$). The data show that A2A down-regulation resulted in higher Pro-I gene expression of p65 leading to increased expression of i-NOS, NO and Pro-I cytokines in the lung, implying a role for A2A in Pro-I gene expression in this model. (Supported by HL-027339)

P340012

The Possible Role of Endogenous Hydrogen Sulfide in Acute Lung Injury Rats Induced by Lipopolysaccharide

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Objective: To explore the changes of hydrogen sulfide (H_2S) in acute lung injury (ALI) induced by lipopolysaccharide (LPS) and the possible relationship with nitric oxide (NO) in rats. Methods: Forty male SD rats were randomly divided into control group, LPS group, LPS + propargylglycine (PPG) group, LPS + NaHS group and LPS + aminoguanidine (AG) group. The contents of H_2S and NO and the activity of H_2S synthase, NOS and iNOS in lung tissue and plasma were detected. Results: The contents of H_2S and NO in lung tissue and plasma in LPS group were higher than control group. Correspondingly, the activity of H_2S synthase and iNOS in lung tissue and plasma were significantly enhanced. Compared with LPS group the contents of NO and the activity of iNOS in lung tissue and plasma were markedly decreased in LPS + PPG group. Inversely, the contents of NO in plasma were increased in LPS + NaHS group. Conclusions: The contents of H_2S and NO were increased after ALI induced by LPS. It could be beneficent for protecting lung tissue in ALI to reduce the level of endogenous H_2S and NO.

Key words: hydrogen sulfide; acute lung injury; nitric oxide

P340013

Inhibition of bronchial smooth muscle hyperresponsiveness by lovastatin in rat allergic asthma

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Our previous studies revealed that a RhoA-mediated Ca^{2+} sensitization of bronchial smooth muscle contraction is markedly augmented in antigen-induced airway hyperresponsiveness (AHR) in rats. The RhoA protein is known to be modulated by posttranslational prenylation, i.e., geranylgeranylation, for its activation. In the present study, the effect of pretreatment with lovastatin, which is one of the statins and an inhibitor of RhoA geranylgeranylation, on the augmented bronchial smooth muscle contraction was investigated in the AHR rats. The bronchial smooth muscle responsiveness to ACh was significantly enhanced in rats that were sensitized and repeatedly challenged with DNP-Ascaris antigen. Systemic treatment with lovastatin (4 mg/kg/day, i.p., for 7 days) markedly and significantly inhibited the in vitro ACh-induced contraction in the AHR rats but did negligibly in control animals. In bronchial smooth muscle of the lovastatin-treated rats, the contents of membrane-translocated active form of RhoA were reduced in both the control and AHR groups. It is thus possible that HMG-CoA reductase inhibitors such as statins may improve AHR in asthmatics.

Key words: asthma, HMG-CoA reductase, geranylgeranylation, RhoA

P340015

Effect of FR167653 on bleomycin induced pulmonary fibrosis in rats

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Aim: To investigate the effect and mechanisms of FR167653 on bleomycin-induced rats pulmonary fibrosis (PF). Methods: PF was induced by intratracheal instillation of bleomycin (5 mg/kg). Then the rats received daily FR167653 (4, 12 and 36 mg/kg, sc) or prednisone (20 mg/kg, ig). Results: Body weight (BW) was reduced while lung indexes and hydroxyproline contents were increased after bleomycin administration. FR167653 and prednisone inhibited bleomycin-induced PF. However, FR167653 (36 mg/kg) did not affect BW. Moreover, FR167653 increased SOD levels while decreased elevated malondialdehyde in lung homogenates. Serum TNF-α and IL-1β also attenuated by FR167653. However, inhibition of epithelial-to-mesenchymal transition (EMT) was partially contributed to the protection of FR167653, but not prednisone, on PF. Conclusion: FR167653 inhibited bleomycin-induced PF, and its effect was associated with anti-free radcals, reduction of proinflmmatory cytokines, and inhibition of EMT.

Acknowledgements: The study was partially funded by the National Natural Science Foundation of China (No. 30400196).

P340016

The Effect of AG on Acute Lung Injury Induced by LPS in Rats

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Objective: To investigate the effects and the mechanisms of inducible NO synthase inhibitor Aminoguanidine (AG) on LPS-induced lung injury.

Methods: Rats were randomly divided into 5 group: group1: control; group2: LPS; group3: AG1(1h+5h); group4: AG2(3h+3h). The rats were injected with either saline or LPS, AG was given 1h or 3h after LPS in group4 and group5, and the rats were killed 6h after saline (control) or LPS injection. Apoptosis, bcl-2 and bax were evaluated by flow cytometry and immunohistochemisty.

Results: Compared with control group, apoptosis of pulmonary cells was significantly increased, bal-2 was decreased and bax was elevated in alveolar and airway epithelial cells in group LPS; AG significantly attenuated LPS-induced pulmonary apoptosis, increased bal-2 and decreased bax; The lung damage was alleviated by AG; The effects were significant in group4 than group5.

Conclusions: It could be concluded that AG has a protective role against LPS-induced lung injury. Upregulating anti-apoptotic protein Bcl-2 and down-regulating proapoptotic protein Bax, through which inhibiting pulmonary apoptosis may be one of the mechanisms.

Key words: Aminoguanidine; Lung injury; Apoptosis

P340017

Pharmacological or genetic deficiency of orexin attenuates hypercapnic chemoreflex that can be restored by supplementation of orexin

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We examined whether the respiratory chemoreceptor reflex in prepro-orexin knockout mice (KO) was blunted or not, and if so, whether supplementation of orexin restore the abnormality. We also studied whether pharmacological blockade of orexin in the wild-type mice (WT) resulted in a similar abnormality. A cannula for intracerebroventricular (icv) injection was implanted to the isoflurane-anesthetized mice together with electrodes for recording electroencephalogram and electromyogram. Ventilation was recorded by whole body plethysmography after recovery period of at least 7 days. After recording baseline breathing for 1 hr, orexin-A, -B, SB-334867 (an orexin receptor antagonist), or vehicle was injected and hypercapnic or hypoxic gas mixture was introduced into the recording chamber for 10 min. Data were examined for only awake periods because sleeping distorts the chemoreflex. Hypercapnic ventilatory responses but not hypoxic responses were attenuated in KO. Similar abnormality was reproduced in WT treated with SB-334867. Icv injection of orexin partially restored the hypercapnic chemoreflex in KO. Our findings suggest that orexin plays a crucial role for CO_2-sensitivity at least during waking periods.

P340019

Protective Effect of Isoliensinine on Paraquat-Induced Acute Lung Injury

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To evaluate protective effect of isoliensinine (IL) on acute lung injury induced by paraquat (PQ), 100 mice were divided into four groups: control (po saline, n=10), IL (n=10), PQ (singly ip 30 mg/kg, n=40), PQ+IL (n=40, PQ: singly ip 30 mg/kg + IL: 20 mg/kg, po, tid)). IL 20 mg/kg (po, tid) treatment started 1 day before PQ and continued. After PQ administration for 8, 24, 48 and 72 h, survival rate, MDA content, SOD level in plasma and bronchoalveolar lavage fluid (BALF) and lung tissue of survival mice were observed by biochemical and pathological measurements. Results show that IL + PQ could slightly increase the survival rate and timedependently suppress the increase of MDA and enhance the content of SOD in plasma and BALF induced by PQ, and the top time-point was 24, 48 h respectively. IL treatment for 8, 24, 48, 72 h could alleviate the degree of lung congestion, leukocytes infiltration and local hemorrhage caused by PQ. IL alone did not affect the mice survival rate, MDA content, SOD level and lung tissue pathological changes. Overall, IL possessed protective effects on paraquat-induced acute lung injury to some extent, maybe related to its antioxidant.

Key words Paraquat, lung injury, Isdiensinine

P340020

Hypoxic inhibition of TASK-1 contributes to the hypoxic depolarisation of rat pulmonary artery myocytes.

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Our objective was to determine whether TASK-1 mediates the hypoxia-induced depolarisation of pulmonary artery myocytes (PAM). Membrane potential responses to changes in oxygen tension in intact small pulmonary arteries were obtained using sharp microelectrodes. Hypoxia induced a two-phase depolarisation in PAM. The initial depolarisation preceded a marked hyperpolarisation followed by a second depolarisation that was present for the duration of the stimulus. Addition of 4-AP in the absence of hypoxia resulted in depolarisation of PAM resting membrane potential, subsequent hypoxic challenge produced a significant further depolarisation. Addition of 4-AP following hypoxic depolarisation resulted in a significant further depolarisation, whereas little further myocyte depolarisation was evident in response to hypoxia after methanandamide (MET) addition (a TASK-1 inhibitor). Exposure to MET generated little further depolarisation following hypoxic depolarisation. These results strongly suggest that TASK-1 is inhibited by hypoxia and contributes to the depolarisation and subsequent contraction of PAM following hypoxia.

Hypoxia, K_2P, TASK-1, K^+-channel
Funded by the British Heart Foundation

P340021

Effect of ketotifen on the bleomycin-induced pulmonary fibrosis in rat

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In the present Study, the effect of ketotifen has been studied on bleomycin-induced pulmonary fibrosis in rats. Positive control group were given single intratracheal bleomycin (7.5 IU/kg). Placebo group received normal saline. Negative control group were given ketotifen (1 mg/kg) daily for two weeks. Groups 4-6: Received oral daily doses of ketotifen (0.05, 0.5 and 1 mg/kg) 5 days before and 2 weeks after bleomycin (7.5 IU/kg) administration. Two weeks after such treatments, animals were killed. Histopathology of positive control group showed infiltration of the inflammatory cells into the alveolar space, increase of alveolar wall thickness associated with pulmonary fibrosis. Ketotifen could reduce the inflammatory reactions and the fibrotic damage in lung tissue with a dose-dependent manner. Hydroxyproline and collagen values in positive control group was significantly higher than negative and saline control groups. In ketotifen-treated groups, such values were significantly less than positive control group. We can suggest that ketotifen can diminish the toxic effect of bleomycin on lung tissue. It may stabilize the mast cell membrane and prevent the release of inflammatory mediators.

P340022

Potential mechanisms of the beneficial effect of chronic naddol treatment in a murine model of asthma

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We have previously shown that chronic treatment with nadolol, a β- adrenoceptor (β- AR) inverse agonist, attenuates bronchoconstriction induced by methacdine in a murine model of asthma (Callaerts et al, PNAS, 2004). This study is aimed at examining potential mechanisms. Radioligand binding assays showed that the decreased β- AR density in lung homogenates of the asthma mice can be rescued by chronic treatment with nadolol or dexamethasone (dex), while co-treatment of dex and nadolol showed no further increase in β- AR density. The increased cellular counts of eosinophils in bronchoalveolar lavage (BALF) of asthma mice were reduced by chronic treatment with either nadolol or dex, but again no synergy was observed. We also measured cytokines in BALF by ELISA and Gi3 protein expression using lung membranes by immunoblotting. IL-10 production was elevated by chronic treatment of naddol, while Gi3 expression was reduced. In conclusion, besides increasing β- AR density, modulation of cytokines and suppression of Gi signaling may also be involved in the possible mechanisms of chronic nadolol treatment.

Key word: β- adrenoceptor, inverse agonist, asthma
Acknowledgement: Sandler Program for Asthma Research

P340023

Effects of propofol on the expression of SP-A and content of ICAM-1 in oleic acid-induced acute lung injury rats

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OBJECTIVE: To investigate the effects of propofol on the expression of surfactant-associated protein A (SP-A) in bronchalveolar lavage fluid (BALF) and the content of intercellular adhesion molecule-1(ICAM-1) in oleic acid-induced acute lung injury (ALI) rats. METHODS: 80 male SD rats were randomly divided into five groups: control group (group I), ALI group (group II), lower, middle, higer of propofol treatment group (group III-V). The lung water content was measured. The lung ultrastructure was detected with electron microscope. The content of ICAM-1 was measured by immunohistochemistry and FCM. The levels of SP-A were determined with Western Blot. RESULTS: In group II, the damages of mitochondrion, rough endoplasmic reticulum and osmiophilic multilamellar body were observed, the damages were lightened in propofol treatment groups. Compared with group I, the content of ICAM-1 was increased while the levels of SP-A in BALF were decreased in group II, and the contents of ICAM-1 attenuated and the levels of SP-A increased in group III-V. CONCLUSION: Administration of propofol could attenuate the ICAM-1 and SP-A, propofol have effects on ALI induced by oleic acid

KEY WORDS Propofol; SP-A; Oleic acid

P340024

Natural product complex CFX attenuates bleomycin- and silica-induced pulmonary fibrosis by regulation of Th1/Th2 polarization in mice and rats

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The tissue immune microenvironment is critical in pathogenesis and development of pulmonary fibrosis. We wonder if nature product CFX, a significant immune modulator, attenuates bleomycin- and silica-induced pulmonary fibrosis in mouse and rats. Animals received daily CFX orally for indicated time. Pulmonary fibrosis was evaluated by histological and pathology iconography. Biochemical and functional changes were determined by PCR, ELISA, immunohistochemistry, or hemodynamic assays. CFX treatment markedly attenuated bleomycin- and silica-induced fibrosis in a dose-dependent manner. CFX significantly reduced content of hydroxyproline, pro-collagen I in lung tissue and levels of AKP in BALF. CFX significantly increased expression of Th1 cytokines, and markedly decreased that of Th2 cytokines and TGFβ1. CFX treatment significantly decreased the right ventricular systolic pressure. Shift of Th1/Th2 balance by CFX was due to CFX-stimulated expression of TLR4 in the lung tissue and innate immune cells. We conclude that anti-fibrosis effect of CFX is due to CFX promoting a shift of Th1/Th2 balance toward Th1 dominant response in the lung tissue.

Key Words: Pulmonary fibrosis, bleomycin, silica, Th1/Th2

P340026

DIFFERENT LYSOPHOSPHATIDIC ACID RECEPTORS MEDIATE THE DIRECT STIMULATION OF PROLIFERATION AND THE SYNERGISM WITH EGF IN AIRWAY SMOOTH MUSCLE CELLS

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Lysophosphatidic acid (LPA) and epidermal growth factor (EGF) both stimulate the proliferation of human airway smooth muscle (HASM) cells, and LPA synergistically enhances the EGF-induced proliferation. Because the direct stimulation by LPA but not the synergism with EGF is blocked by the Gi/o inhibitor pertussis toxin (PTx), we hypothesized that different LPA receptor subtypes would mediate direct stimulation by LPA and its enhancement of EGF stimulation. HASM cells were treated with various agents for 24 hr and proliferation assessed by [^{3}H] thymidine uptake. The LPA1/2 agonist NAEPA and the LPA2/3 agonist OMPT stimulated proliferation on their own and synergized with EGF. For all these agents, the direct stimulation was inhibited by PTx and blocked by a new LPA1/3 antagonist VPC51299, but PTx and VPC51299 did not prevent the enhancement of EGF stimulation. Conversely, the LPA2 agonist FAP12 did not stimulate proliferation on its own but did enhance the EGF stimulation. Together these experiments implicate LPA1/3 in the direct stimulation of proliferation by LPA and LPA2 in the synergism with EGF.

Keywords: LPA, EGF, lung, proliferation. Acknowledgements: Supported by the American Heart Association.

P340027

Tracheal epithelial cell shrinkage induced by hyperosmolar solution.

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Exercise causes airway obstruction in asthmatics, resulting from elevation in the osmolarity of the airway surface liquid. Hypertonic aerosols also elicit obstruction in asthmatics. Exposure of guinea-pig tracheal epithelium (E) to hyperosmolar solution (HS) induces epithelium-derived relaxing factor (EpDRF) release and smooth muscle relaxation; EpDRF regulates airway reactivity. Here we examined whether HS causes shrinkage of E and phosphorylation of p38 and JNK. Suspensions of E cells, prepared by treatment with protease (2%, 1 h), showed beating cilia and excluded trypan. While measuring cell volume using a cell sizer, challenge of the cells with HS [added NaCl, D-mannitol (DM) or urea; 10-120 mosM], resulted in rapid cell shrinkage (up to 25% in 1-5 min) which persisted for 2 h. Raising osmolarity with DM for 15 min caused phosphorylation of p38 and JNK. Our findings indicate that under conditions in which EpDRF is released, HS causes shrinkage of E and protein phosphorylation. The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination of policy.

P340028

Presynaptic 5-hydroxytryptamine (5-HT) receptors modulating noradrenaline (NA) release in rabbit pulmonary artery: functional and molecular studies

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The rablit pulmonary artery (PA) was used to examine whether various 5-HT receptor (R) types modulate NA release also in this blood vessel. PAs preincubated with [^{3}H] NA were superfused in the presence of the alpha2-adrenoceptor blocker rauwolscine and the effects of 5-HTR ligands on the electrically evoked ^{3}H overflow were determined. The 5-HT4R agonist cisapride inhibited ^{3}H overflow (blocked by atropine). The 5-HT1B/1DR agonist 5-carboxamidotryptamine inhibited ^{3}H overflow only in the presence of atropine. The 5-HT4R and 5-HT1B/1DR agonists 5-HT and 5-methoxytryptamine reduced ^{3}H overflow in the absence and presence of atropine (blocked by methiothepin, a non-selective 5-HTR antagonist, in the presence of atropine). In PA 5-HT1BR, 5-HT1DR and 5-HT4R are expressed, the latter being highly homologous to the human one. In conclusion, the cholinergic nerves are endowed with 5-HT4Rs mediating release of acetylcholine which, in turn, activates muscarine Rs on the sympathetic nerves (SN) leading to inhibition of NA release. Blockade of muscarine Rs is necessary to disclose an inhibition of NA release via 5-HT1B/1DRs on the SN. 5-HT4 receptor-5-HT1B/1D receptor-mRNA expression

P340029

Adenosine-1(A1) receptor-mediated protection in ischemic preconditioning in rat isolated lung

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The present study was undertaken to investigate the role of adenosine in IP in the isolated buffer-perfused rat lung (IBPR). In IBPR, 2h of normothermic ischemia significantly decreased phenylephrine (Phe) and KCl-induced pulmonary vasoconstrictor responses when compared to control values. However, one cycle of 5 min of ischemia and reperfusion that were applied prior to 2 h of ischemia, prevented the reduction of receptor-dependent and -independent vasoconstrictor responses. Infusion of adenosine, restored Phe- and KCl-induced vasoconstrictor responses. On the other hand, IP and adenosine prevented pulmonary edema after ischemia. In IP and adenosine groups, malondialdehyde contents of the lung were significantly lower than those in I/R group. Pretreatment with theophylline or DPCPX prior to IP or adenosine abolished the protective effect of IP or pharmacological preconditioning. These results suggest that IP or adenosine prevent the impairment of pulmonary vascular smooth muscle contraction responses in the rat pulmonary vascular bed and activation of adenosine-1 receptors contribute to protective effect of IP or adenosine. This study was supported by a grant from Gazi University (SBE-11/2001-08).

P340030

The MMP inhibitor AS112108 reduces airway inflammation induced by cigarette smoke exposure in mice.

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Gelatinases (MMP-2, MMP-9) and macrophage elastase (MMP-12) are metalloproteinases able to degrade extracellular matrix components including elastin and are suggested to be involved in the airway inflammatory process associated with COPD. The aim of this study was to investigate the effect of the MMP inhibitor AS112108 on the inflammatory process induced by cigarette smoke exposure. C57BL/6 mice were orally administered with AS112108 (10, 30, 100 and 300 mg/kg) then exposed one hour later to the whole smoke of 2 cigarettes (twice a day for 3 days). Cigarette smoke exposure elicited a marked influx of neutrophils in BAL fluid supernatants 24 hours after the last cigarette associated with an increase in gelatinase activity (MMP-9), and in release of IL-6, MIP1αand KC. KC and MIP1αlevels were also increased in lung homogenate. Treatment of mice with AS112108 dose-dependently reduced the increase in neutrophil number in BAL fluids as well as IL-6 and MMP-9 activity. AS112108 also reduced the KC release in the lung parenchyma. These results showed that AS112108 is able to prevent the inflammatory process induced by acute exposure of mice to cigarette smoke.

MMP-12, cigarette smoke, inflammation, COPD

P340031

Muscarinic M2 receptors modulate airway responses to methacholine in a murine model of asthma

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Muscarinic antagonists are second line drugs in patients with asthma, because they are less effective bronchodilators compared with b2 adrenoceptor agonists. Prejunctional inhibitory M2 muscarinic receptors (M2Rs) decrease acetylcholine release and inhibit vagally mediated bronchoconstriction, but activation of postjunctional M2Rs on bronchial smooth muscle cells produce contraction. To evaluate the in vivo role of M2Rs on a model of asthma, M2R knockout (KO) and wild-type (WT) littermates were sensitized and challenged using ovalbumin, and airway response to methacholine was evaluated using the forced oscillation technique. Absence of M2Rs increased maximal airway response to methacholine both in sensitized and nonsensitized animals, but responses were larger in sensitized mice. Measured inflammatory parameters were not different between WT and KO mice. The most important in vivo lung function of M2Rs is to decrease vagally mediated bronchoconstriction. In asthma there is a dysfunction of these receptors and antagonism of M2Rs might enhance constriction. More selective M3R antagonism may improve bronchodilation.

Key words: muscariinic receptors.
Funded by the Sandler Program for Asthma Research

P340033

Pharmacological treatment of Pulmonary Hypertension: mechanism relevance to 5-Hydroxytraptamine, Receptors and Transporters

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There is critical relevance between 5-HT and pulmonary hypertension (PH). Further investigation of receptor and transporter mechanism using chronic "monocrotaline" rats, cultured pulmonary artery smooth muscle cells (PASMC) and liposomal transfection to introduce ERK1/2 ODNs into cultured rat PASMCs shown that selective serotonin reuptake inhibitor fluoxetine and sertraline concentration-dependently inhibited MCT-induced PH in rats and the proliferation of PASMCs induced by 5-HT. 5-HT1B antagonist rather than 5-HT1D antagonist inhibited 5-HT- and 5-HT1B/1D-induced proliferation of PASMC. Meanwhile, antisense ODN to ERK1/2 inhibited 5-HT-induced proliferation of PASMCs. 5-HT1B receptor and 5-HTT mediated mitogenesis of PASMCs by 5-HT and the intracellular signal transduction of 5-HT in PASMCs is dependent on ERKs signal pathway. PH compromised complicated pathology i.e. pulmonary vasoconstriction, vascular remodeling, inflammation and micro-thrombosis, in which multiple factors was involved. 5-HT1B receptor and 5-HTT mechanism are of importance induce PH and both might be novel therapeutic targets.

Key words: Pulmonary Hypertension.
Supported by National Natural Science Foundation, China 30572194

P340034

SSRIs Protect Against Monocrotaline-Induced Pulmonary Hypertension In Rats

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AIM: To investigate the effect of selective serotonin re-uptake inhibitors (SSRIs) sertraline and fluoxetine on monocrotaline (MCT)-induced pulmonary hypertension and its possible mechanisms. Methods: The chronic "inflammatory" pulmonary hypertension model of rat was established by MCT. Pulmonary hemodynamic measurement and lung tissue morphological investigation were conducted. Serotonin transporter (SERT) mRNA was assayed by RT-PCR. The effects of fluoxetine on concentration-response curves of 5-hydroxytramine (5-HT) in pulmonary arteries (PAs) were also studied. Results: Pulmonary artery pressure, right ventricular index, PA wall thickness, the degree of PAs muscularization and the level of SERT mRNA were significantly increased by MCT ($P<0.05$ vs control) and they were decreased by SSRIs ($P<0.05$ vs MCT). In vitro, fluoxetine inhibited PAs contractile response to 5-HT in a dose-dependent manner. Conclusion: SSRIs protect against MCT-induced pulmonary hypertension, which was related to the mechanisms of SERT mRNA reduction and the alleviation of pulmonary vascular tone in rats.

Key Words: SSRI; pulmonary hypertension
Supported by National Natural Science Foundation of China 30572194

P340035

Protective Effects Of Serotonin Transporter Inhibitor In Monocrotaline-Induced Pulmonary Hypertension In Rats

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AIM: To investigate the effect of SSRI fluoxetine on pulmonary hypertension (PH). METHODS: MCT treated rats were used as a model for chronic PH. Fluoxetine started 1 week after MCT injection. Pulmonary arterial pressure was measured. The index of the right ventricular hypertrophy was calculated. RT-PCR to identify mRNA expression of 5-HTT in pulmonary arteries was performed. RESULTS: Chronic PH model in rats induced by MCT was established at the end of 3 weeks and confirmed by a significant increase of mean pulmonary arterial pressure ($P<0.01$) and right ventricular hypertrophy index ($P<0.01$). The expression of 5-HTT mRNA was much higher in MCT rats than in control rats ($P<0.01$) and correlated with the thickness of pulmonary artery medial wall. Fluoxetine treatment prevented right ventricular hypertrophy ($P<0.01$), decreased

pulmonary artery pressure(P < 0.01) and suppressed the 5-HTT increase(P < 0.01). CONCLUSIONS: 5-HTT played a key role in the pathophysiological processes of pulmonary hypertension. Prevention of experimental PH by fluoxetine may provide a potential therapeutic target for this disease.

Key Words; serotonin transporter

Supported by National Natural Science Foundation of China 30572194

P340036

Proliferation Of Pulmonary Artery Smooth Muscle Cells Induced By 5-HT Via 5-HT1B Receptor Mechanism

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OBJECTIVE: To study the 5-HT receptor mechanism of proliferation of pulmonary artery smooth muscle cells (PASMC). METHODS: cultured rat PASMC were evaluated by MTT assay, ^{3}H-TDR incorporation. Proliferation index (PI) and s-phase cell fraction (SPF) was performed by FCM. RESULTS: Both 5-HT and 5-HT1B/1D agonist sumatriptan stimulated proliferation of PASMC. 5-HT1B receptor antagonist SB224289, but not 5-HT1D receptor antagonist BRL1557 concentration-dependently inhibited PASMC proliferation induced by 5-HT. By FCM, the proliferation index (PI) and s-phase cell fraction (SPF) of PASMC stimulated by 5-HT and sumatriptan are significant more than that in control. SB224289 lowered 5-HT-induced increase of PI and SPF. SB224289 inhibited the mitogenesis of 5-HT on PASMC, blocked PASMC from G0G1-phase into Sphase. ^{3}H-TdR incorporation show that SB224289 inhibited the increased ^{3}H-TdR incorporation of PASMC induced by 5-HT. CONCLUSION: 5-HT and Sumatriptan promotes PASMC growth. 5-HT1B receptors play an important roles in 5-HT-induced PASMC proliferation and pulmonary remodeling.

Key words: serotonin, 5-HT1B receptor, smooth muscle cell

Supported: National Natural Science Foundation of China 30572194

P340037

Effect of Fluoxetine on Monocrotaline-induced Pulmonary Hypertension and Pulmonary Vascular Tone in Rats

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AIM: To investigate the effects of fluoxetine on monocrotaline-induced pulmonary hypertension (PH) in rats. METHODS: MCT-treated rats were used as a chronic PHT model. Lung tissue sections were stained with hematoxylin-phloxin-saffron. RT-PCR was performed to measure 5-hydroxytramine transporter (5-HTT) mRNA of pulmonary arteries (PAs). The effects of fluoxetine on concentration-response curves of 5-HT (serotonin) in PAs were also studied. RESULTS: The right heart index was increased in the MCT group, and this was alleviated in the fluoxetine-treated group. The ratio of pulmonary artery (PA) wall thickness to PA radius was increased in the MCT group, and was reduced in fluoxetine-treated group. 5-HTT mRNA levels in PAs in MCT group were increased, and attenuated in fluoxetine-treated group. Fluoxetine also inhibited the contractile response of PAs to 5-HT in a dose-dependent manner. CONCLUSION: Fluoxetine protects against MCT-induced PHT in rats. The mechanisms are related to the decrease of 5-HTT mRNA and the alleviation of vascular tone induced by fluoxetine in rat PAs.

1 Now in Liaoning University

KEY WORDS: Pulmonary Hypertension

upported by Natural Science Foundation of China 30572194

P340038

Collagen-derived peptide: a novel ligand for the chemokine receptors CXCR1 and CXCR2. Possible implication in COPD

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In COPD, neutrophils are a major source for proteases that breakdown collagen leading to pulmonary emphysema. It is has been shown that collagen-derived tripeptide N-acetyl-Pro-Gly-Pro (PGP) has neutrophil chemotactic activities. PGP has structural homology to an important domain on alpha chemokines. In this study we have examined the role of PGP as neutrophil chemo-attractant in COPD. PGP binds CXCR1 and 2 chemokine receptors on human neutrophils causing chemotaxis and superoxide production. PGP is generated in murine airways after LPS exposure and blockade of PGP with mAb reduced this LPS-induced pulmonary neutrophil infiltration. Intra-airway PGP administration results in local neutrophil recruitment and alveolar enlargement in wild type mice, but not in CXCR-/- mice. Finally, PGP is present in substantial concentrations in a majority of BAL samples from COPD patients but not in those from control. In conclusions, PGP's novel activity through chemokine receptors represents a link between extracellular matrix degradation and neutrophil recruitment in the pathology of COPD and peptides like PGP may be biomarkers for disease and novel therapeutic targets.

P340039

Inhibitory effect of somatostatin released by TRPV1 receptor activation on endotoxin-induced airway inflammation and hyperreactivity in mice

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In this study the role of transient receptor potential vanilloid 1 (TRPV1) receptors expressed on capsaicin-sensitive sensory nerves was examined in endotoxin-induced airway inflammation and hyperreactivity in vivo using receptor gene-deleted (TRPV1-/-) mice. Pneumonitis was evoked by intranasal E. Coli lipopolysaccharide (LPS) and Penh, a calculated parameter referring to airway resistance, was measured by whole body plethysmography. Bronchoconstriction was induced by carbachol inhalation 24 h after LPS. Histological scoring and myeloperoxidase (MPO) activity measurement were performed from the lung. Plasma and lung somatostatin (SST) concentrations were determined with RIA. A separate group of TRPV1+/+ mice was treated with the SST receptor antagonist cyclo-somatostatin. Bronchial hyperreactivity, histological changes and MPO activity were significantly greater in TRPV1-/- mice. LPS increased plasma and lung SST in TRPV1+/+, but not in TRPV1-/- mice. Cyclo-somatostatin increased inflammatory parameters and airway hyperresponsiveness. These results provide the first evidence for a novel inhibitory mechanism mediated by SST in the airways.

Grants: OTKA F-046635, T-043467; ETT03543/2003; RET-008/2005

P340040

Role of capsaicin-sensitive afferents in endotoxin-induced inflammation and hyperresponsiveness of the mouse airways

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In this study the role of capsaicin-sensitive sensory nerve terminals in endotoxin-induced airway inflammation and hyperreactivity was studied in vivo in C57/BL6 mice. Pneumonitis was evoked by intranasal E. Coli lipopolysaccharide (LPS) and Penh, a calculated parameter referring to airway resistance, was measured by whole body plethysmography. Bronchoconstriction was induced by carbachol inhalation 24 h after LPS. Histological scoring, measurement of myeloperoxidase activity, substance P (SP), calcitonin gene-related peptide (CGRP) and interleukin-1beta concentrations were performed from the lung. To destroy capsaicin-sensitive afferents resiniferatoxin (RTX) pretreatment was performed. In separate groups, NK1, NK2 or CGRP1 receptor antagonists were administered. LPS increased lung SP and CGRP, which was prevented by RTX pretreatment. Destroying capsaicin-sensitive afferents by RTX-desensitization enhanced the inflammatory parameters, but inhibited hyperreactivity. The CGRP1 receptor antagonist CGRP(8-37) or the combination of NK1/NK2 antagonists (SR140333/SR48968) diminished granulocyte accumulation, the NK2 antagonist inhibited hyperresponsiveness.

Grants: OTKA F-046635, T-043467; RET-008/2005.

P340041

Melatonin prevents neutrophil-mediated oxidative renal injury in E. coli-induced pyelonephritic rats

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Turkiye.
The present study aimed to elucidate the therapeutic effects of melatonin against E. coli - induced renal injury. Wistar albino rats, injected intrarenally with E. Coli, were administered with either saline or melatonin (10 mg/kg/day; intraperitoneally). Twenty - four hours or one week after pyelonephritis induction, rats were decapitated. In kidney samples, malondialdehyde (MDA), glutathione (GSH) levels, myeloperoxidase (MPO) activity and collagen content were measured and histological analyses were made. In the saline - treated pyelonephritis group, a decrease in renal GSH along with increases in MDA level, MPO activity, and collagen content were observed ($p < 0.05$ - 0.001), while serum TNF - α, lactate dehydrogenase, BUN and creatinine levels were elevated as compared to control. However, melatonin treatment reversed all these biochemical indices ($p < 0.05$), as well as renal injury observed histologically. The protective effects of melatonin may be due to its ability to inhibit neutrophil infiltration and to balance oxidant - antioxidant status, suggesting a future role for melatonin in acute pyelonephritis treatment.
Key words: Pyelonephritis; glutathione; myeloperoxidase; TNF - α

P340042

Mechanism of Triterpene Acids of Loquat. Leaf in Chronic Bronchitis Therapy

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Objective To investigate the probable mechanism of TAL on CB therapy. Methods CB model was established by BCG + LPS injection and the in vitro and in vivo experiments were used to investigate the effect of TAL on iNOS expression and activity, NO concentration in supernatant of AM and HO - 1 mRNA expression in AM of CB rats and to visit the effect of NO on HO - 1 mRNA expression. The relationship between MAPK and iNOS mRNA expression was also investigated. Results TAL could significantly inhibited the increased NO concentration, iNOS expression and activity and HO - 1 mRNA expression in AM of CB rats. In vivo test we found that SB203580(10uM) and TAL could significantly inhibit iNOS mRNA expression in AM. L - Arg(10^{-6}、10^{-5}、10^{-4}、10^{-3}、10^{-2} mol/L) notably increased HO - 1 mRNA expression in AM, while excessive L - NAME could reverse the effect of L - Arg. Conclusion These data indcate that TAL highly decreased the excessive iNOS expression and NO induction in AM of CB rats and inhibited the HO - 1 mRNA expression in a NO - dependent mechanism. The effect of TAL on iNOS expression in AM might be related to its inhibition of p38 MAPK signal transduction pathway.
Key words: chronic bronchitis; alveolar macrophage; iNOS; heme oxygenase - 1; MAPK signal transduction pathway
* National Natural Science of China No. 30371766 and No. 30572355

P340043

Role of Rho - kinase in endothelin - 1 - induced phosphorylation of CPI - 17 in rat bronchial smooth muscle

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It has been reported that CPI - 17 (PKC - potentiated inhibitory protein for heterotrimeric myosin light chain phosphatase of 17 kDa) is phosphorylated by excitatory agonists in smooth muscle contraction. However, endothelin - 1 (ET - 1) - mediated regulation of CPI - 17 in bronchial smooth muscle has not been documented. We therefore investigated whether phosphorylation of CPI - 17 is induced by ET - 1 in rat bronchial smooth muscle. Moreover, the role of Rho - kinase was investigated in phosphorylation of CPI - 17 induced by ET - 1 in rat bronchial smooth muscle. The ET - 1 - induced contraction was attenuated by Y - 27632 (a Rho - kinase inhibitor, 10^{-6} M). ET - 1 induced a phosphorylation of CPI - 17 and myosin light chain; these phosphorylation responses were significantly inhibited by Y - 27632 (10^{-6} M). These findings suggest that the activation of Rho - kinase is involved in force development and CPI - 17 phosphorylation induced by ET - 1 stimulation in rat bronchial smooth muscle. Thus, cross - talk of RhoA/Rho - kinase and CPI - 17 pathways is considered to play an important role in the ET - 1 - induced Ca^{2+} sensitization of bronchial smooth muscle contraction.
Key words: PKC, bronchial smooth muscle, endothelin - 1, CPI - 17

P340044

Glycosaminoglycan synthesis by airway smooth muscle cells is differentially modulated after treatment with beta2 agonists and corticosteroids

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Asthma involves alterations of extracellular matrix molecules, such as glycosaminoglycans (GAGs), in the airways. We studied the effect of beta2 adrenergic agonists and corticosteroids on the synthesis of GAGs employing primary human airway smooth muscle cells (ASMC), established from lung tissue biopsies of asthmatics (aASMC), and healthy (hASMC) individuals. ASMC were treated with salmeterol, formoterol, budesonide or fluticasone. Total GAG synthesis was assessed by incorporation of tritiated - glucosamine. GAGs were isolated and purified from supernatants and cell layers by ethanol precipitation after pronase, DNAse and alkali treatment. The relative amount of hyaluronic acid (HA) was estimated by ELISA. Corticosteroids (but not beta2 agonists) inhibited glucosamine incorporation in cell layers and supernatants of aASMC to a higher extent as compared to hASMC. However, the relative amount of HA was significantly increased in cell layers of aASMC after treatment with beta2 agonists, corticosteroids or their combination. This effect was less pronounced in hASMC. The results indicate that aASMC and hASMC differentially respond to drugs used in the treatment of asthma with respect to matrix formation.

P340045

TGF - beta1 mediates hyaluronic acid homeostasis in human primary vascular smooth muscle cells

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Hyaluronic acid (HA) is a key glycosaminoglycan (GAG) mediating vascular smooth muscle (VSMC) proliferation and migration. We investigated the effect of TGF - beta1, on HA turnover in primary human VSMC obtained from the pulmonary artery. Cells were incubated with TGF - beta1 for 6, 12 and 24 h. Total GAGs synthesis was assessed by tritiated - glucosamine incorporation and the secretion of HA by ELISA. mRNA levels of HA synthases (HAS), hyaluronidases (HYAL) and the HA receptor CD44 were estimated by RT - PCR. TGFbeta1 significantly increased tritiated - glucosamine incorporation into GAGs secreted or deposited in the cell layers. Pharmacological inhibition of the kinase activity of TGF - beta receptor type I by SB431542 and of the p38 kinase pathways by SB203580 abolished the TGF - beta1 effect. Furthermore, TGF stimulated in a dose - and time dependent manner the secretion of HA by VSMC. RT - PCR analysis revealed that TGF - beta1 significantly increased, in a dose - dependent manner, mRNA levels of HYAL 1, 2 and 3, CD44 and HAS 2, whereas it inhibited gene expression of HAS 3. Our results suggest that TGF - beta1 regulates the homeostasis of HA in VSMC which may be important in lung pathology.

P340046

Effect of Flunisolide and Nitric Oxide (NO) - Releasing Flunisolide on Silicosis in Mice

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Inhalation of crystalline silica dusts leads to silicosis, a chronic fibrotic lung pathological process. We have here compared the curative effect of flunisolide (FLU) and NO - flunisolide (NO - FLU) on silicosis in mice. Intranasal injection of silica into Swiss - Webster mice led to an increase in the number leucocytes in BAL, mainly neutrophils and mononuclear cells, from 7 to 28 days. Zymograms of BAL showed the presence of active forms of gelatinases, which paralleled with a marked lung leucocyte influx at 7 days and numerous granulomas at day 14, mostly with peribronchial distribution. The fibrotic response progressed and a collagenous framework was observed in the center of the granulomas much later. Intranasal administration of FLU or NO - FLU for 1 - wk period at earlier times and from days 7 - 14 post - challenge, significantly inhibited both leukocyte infiltration and gelatinase secretion in the BAL, although failed when given from days 21 - 28. Doses required of NO - FLU were lower than those of FLU. Our

data indicate that FLU as well as NO-FLU do constitute a promising therapy for silicosis if given at earlier times after silica challenge.

Key words: Silicosis - inflammation - glucocorticoids

P340047

Effects of quercetin on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) metabolizing enzymes in rat lung

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Flavonoids possess extensive biological activities, but not all of which are beneficial. NNK is the most potent and abundant carcinogen in tobacco and tobacco smoke. The objective of this study was to evaluate the effects of quercetin (3,5,7,3',4'-pentahydroxyflavone) on NNK metabolizing enzymes in rat lung. After animals treated with quercetin 2 mg/kg (daily intake) and 80 mg/kg (nutrition supplement) i.p. ×4d, CYP1A1 and AhR mRNA expression in lung was not changed, while CYP2B1 was significantly increased. However, pentoxyresorufin O-dealkylation activity, linked to CYP2B isoforms activity, remained unchanged. Both of CYP1A1 and CYP2B1 are responsible for NNK metabolic activation. And 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and UGT2B1 catalyze NNK detoxification pathway. Quercetin significantly increased 11β-HSD1 mRNA expression, but not UGT2B1. Additionally the mRNA expression of O^6-methylguanine-DNA methyltransferase (O^6-MGMT), removing NNK induced DNA adduct O^6-methylguanine, was greatly inhibited by quercetin. Thus quercetin had few effects on NNK metabolic activation, and activated NNK detoxification pathway, which suggest that preventive effect of quercetin on NNK iuduced pulmonary toxicity. The significance of O^6-MGMT mRNA expression inhibition by quercetin is under further investigation.

KEY WORDS quercetin; NNK; lung

P340048

The effect and mechanism of sumatriptan-induced proliferation of pulmonary smooth musdes cells

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AIM To study the effect of sumatriptan on the pulmonary smooth muscle cells proliferation, and investigate the mechanism of extracelluar signal pathway of sumatriptan. METHODS The effects of sumatriptan and ODNs to ERK1/2 MAPK (extracelluar signal-regulated kinase/mitogen-activated protein kinases) on the proliferation of pulmonary artery smooth musle cells (PASMCs) were measured by cell counting and evaluated by cell cycle analysis, microculture tetrazolium (MTT) assay and Flow cytometry (FCM), respectively. RESULTS Liposomes mediated the transfection of ODNs into PASMCs with high efficiency. MTT assay showed ASODN inhibited the proliferation of PASMCs induced by sumatriptan (1μmol/L) in vitro from 164.7% ±6.7% to 76.7% ±0.2% ($P<0.01$). Flow cytometric analysis showed that the increase of sumatriptan induced S-phase fraction (SPF) was significantly inhibited by antisense ODN with SPF from 11.7% ±0.3% to 3.3% ±0.3%, and proliferation index (PI) from 27.3% ±0.3% to 22.0% ±0.6% ($P<0.01$) respectively.

CONCLUSION The proliferation of pulmonary smooth muscles cells induced by sumatriptan is dependent on ERK1/2 MAPK signal pathway.

P340049

Modulation of oxidants signaling as a new therapeutic approach of obstructive pulmonary diseases

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Chronic obstructive pulmonary disease (COPD) is a major public health problem that is related to cigarette smoke exposure. COPD is characterized by non reversible airflow obstruction, secondary to airways and lung parenchyma inflammation and remodeling. An increased airway smooth muscle mass and mucus hypersecretion are characteristic features of airways remodeling whereas a proteases/antiproteases imbalance is characteristic of lung remodeling (also know as emphysema).

Heme oxygenase (HO) and NADPH oxidase (NOX) are anti and pro-oxidant proteins respectively, that are involved in the control of smooth muscle proliferation, mucus protein expression and proteases/antiproteases balance, via oxidants signaling and mitogen activated protein kinases. We have shown that a decreased HO expression, secondary to a promoter polymoprhism in HO-1 gene, is associated with an accelerated decline in lung function in smokers, and that experimental up regulation of HO and down regulation of NOX proteins prevent airway and lung remodeling after cigarette smoke exposure in vivo and in vitro. Therefore, modulation of oxidants signaling by acting on HO and/or NOX could be proposed as new therapeutic approachs of COPD.

P340050

Effect of AEF999 in oleic acid-induced lung injury

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OBJECTIVE: This study was designed to document the effects of AEF999 on acute respiratory distress syndrome (ARDS) in rats induced by olieic acid.

METHODS: Acute respiratory distress syndrome (ARDS) in rats was induced by olieic acid. We focused on the biochemical detection of malondialdehyde (MDA), myelope-roxidase (MPO), catalase, total antioxidart capacity (TAOC) of the serum, determination of. Lung wet/dry ratio and lung/body ratio, lung tissue Na^+, K^+-ATPase determination and histopathological evaluation.

RESULTS: AEF999 significantly improved the OA-induced histological changes. The OA induced increase of lung/body ratio, the reduction of TAOC、CAT、Na,K-ATPase,the increase of MPO、MDA were significantly improved in group administrated AEF999 (1-2 mg/kg). And AEF999 are more effective than Ambroxol Hydrochloride, especially in antioxidant and reduced edema in lung.

KEY WORDS: AEF999, ARDS, antioxidant, anti-inflammatory

P340051

SSRIs Protect Against Monocrotaline-Induced Pulmonary Hypertension In Rats

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AIM: To investigate the effect of selective serotonin re-uptake inhibitors (SSRIs) sertraline and fluoxetine on monocrotaline (MCT)-induced pulmonary hypertension and its possible mechanisms. Methods: The chronic "inflammatory" pulmonary hypertension model of rat was established by MCT. Pulmonary hemodynamic measurement and lung tissue morphological investigation were conducted. Serotonin transporter (SERT) mRNA was assayed by RT-PCR. The effects of fluoxetine on concentration-response curves of 5-hydroxytramine (5-HT) in pulmonary arteries (PAs) were also studied. Results: Pulmonary artery pressure, right ventricular index, PA wall thickness, the degree of PAs muscularization and the level of SERT mRNA were significantly increased by MCT ($P<0.05$ vs control) and they were decreased by SSRIs ($P<0.05$ vs MCT). In vitro, fluoxetine inhibited PAs contractile response to 5-HT in a dose-dependent manner. Conclusion: SSRIs protect against MCT-induced pulmonary hypertension, which was related to the mechanisms of SERT mRNA reduction and the alleviation of pulmonary vascular tone in rats.

Key Words: SSRI; pulmonary hypertension
Supported by National Natural Science Foundation of China 30572194

P340052

Expression of urocortin in rat lung and its effect on pulmonary vascular permeability

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The aim of this study was to investigate the expression profile of Urocortin (UCN) in rat lung and the effect of UCN on lung vascular permeability. The expression of UCN mRNA was detected by RT-PCR. UCN peptide was measured by immunohistochemistry and western blot analysis. We found that both UCN mRNA and peptide were obviously expressed in rat lung. We also found that rats receiving an inhalation aerosol of UCN had a significant elevation of lung vascular permeability by evans blue (EB) technique. Enhanced pulmonary vascular permeability induced by UCN was markedly inhibited by pretreatment with nonselective peptide CRH receptors antagonist astressin, mast cell stabilizer cromolyn and histamine-1 (H1) receptor antagonist azelastine respectively. Taken together, in the present study we firstly demonstrated that UCN was expressed in rat lung and it contributes to an increase in lung vascular permeability through activation of CRH receptors. Mast cells and histamine may be involved in this effect of UCN.

Keywords: urocortin, rat, lung, pulmonary vascular permeability

Acknowledgments: This work was supported by National Natural Science Foundation of China (No. 30572185).

P340053

Upregulation of PDE4 activity and expression in lung of asthmatic rats*

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Object: To investigate the changes of phosphodiesterase (PDE) activity and mRNA expression of PDE4 subtype in the lung of asthmatic rat model and to explore the possible regulative effect of interleukin-4 (IL-4).

Methods: Asthmatic rats were induced by ovalbumin (OVA) sensitized and repeated OVA challenged. cAMP-PDE activities, IL-4 levels and PDE4A, 4B, 4C, 4D mRNA expression in lung tissues were determined. Dynamic lung compliance (Cdyn) and pulmonary resistance (RL) of pulmonary function were determined by using a single chamber whole body plethysmograph.

Results: cAMP-PDE activities and IL-4 level were increased in the lung homogenate of asthmatic rat, and cAMP-PDE activities in the asthmatic rat were statistically correlated with the increased IL-4 level. mRNA expression of PDE4A, 4C, 4D were also increased in the lung of asthmatic rat., in particular PDE4C. OVA sensitized and challenged significant decreased Cdyn and increased RL.

Conclusion: Coincidental enhanced cAMP-PDE activity and mRNA expression of phosphodiesterase 4 subtype in the lung of ovalbumin-sensitized and challenged SD rats. The increased IL-4 levels in the lung might be responsible for the elevated PDE activity.

Key words: Phosphodiesterase-4; cAMP; asthma; rat

P340054

Expression of PEPT2 mRNA in the Lung of Rat with Bleomycin-induced Pulmonary Fibrosis

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Peptide transporter 2 (PEPT2) expressed mainly in lung is an integral membrane protein. PEPT2 can transport both peptides and peptidomimetic drugs and becomes a target for a rational drug design for a new generation of respiratory drugs. We examined whether PEPT2 mRNA expression levels were changed in lung of rat with bleomycin-induced pulmonary fibrosis. SD rats were treated intratracheally with bleomycin (5 mg/kg) and were killed on 7, 14 and 28 days, respectively. Control rats were untreated. The lung samples were processed for light microscopy and the method of sample alkali hydrolization determined the hydroxyprdine concentration. The expression levels of PEPT2 mRNA were evaluated by semiquantitative RT-PCR. Hydroxyproline levels markly increased on 14 and 28 days of bleomycin-treated rats ($P < 0.01$). HE staining showed typical pathological changes of pulmonary fibrosis, GS staining showed collagenous fiber proliferated. Semiquantitative RT-PCR results showed there was no significant change in PEPT2 mRNA levels in the lung of bleomycin-treated rats. We conclude that PEPT2 mRNA levels do not change in the lung of rat with bleomycin-induced pulmonary fibrosis.

P340055

Role of hypoxia in bleomycin induced pulmonary fibrosis in rat

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To investigate the potential role of hypoxia in pulmonary fibrogenesis, a time course study was carried out to depict the changes of HIF-1α expression following bleomycin (BLM) intratracheal instillation in rats. Results showed increased HIF-1α expression occurred in the very early stage in this model, as indicated by western blotting and immunohistochemistry, which was accompanied by increase transcription of hypoxia-responsive genes. These findings occurred before any traditional evidence of interstitial injury like presence of myofibroblasts and collagen accumulation. Moreover, the HIF-1α protein level persisted high until interstitial fibrosis developed. In addition, valsartan, an angiotensin II type I receptor blocker, attenuated BLM-induced pulmonary fibrosis obviously. Meanwhile, the HIF-1α and hypoxia-responsive genes expression were decreased in valsartan-treated animals. These results suggested that hypoxic milieu in the lung is relevant to the interstitial damage. The activation of local rennin-angiotensin system may be, in part, involved as well.

Key words: pulmonary fibrosis; hypoxia; hypoxia-inducible factor-1α; bleomycin

P340056

Acetylchdine-induced phosphorylation and membrane translocation of CPI-17 in bronchial smooth muscle of rats

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A translocation of PKC from cytosol to plasma membrane has been reported as an association with agonist-induced Ca^{2+} sensitization in smooth muscle contraction. It is thus possible that a downstream target of PKC, CPI-17 (PKC-potentiated inhibitory protein for heterotrimeric myosin light chain phosphatase of 17 kDa), might also be translocated to plasma membrane when activated. To confirm this hypothesis, cytosolic and membrane fractions of CPI-17 were measured in acetylcholine (ACh)- and high K^+-stimulated bronchial smooth muscles of rats. An active form of CPI-17 (phosphorylated CPI-17) was also measured in both the fractions. Immunoblot analyses demonstrated a translocation of CPI-17 from cytosolic to membrane fraction by ACh, but not high K^+ depolarization. Interestingly, phosphorylated CPI-17 was detected only in membrane fractions in the ACh-stimulated tissues. However, in the high K^+-stimulated tissues, phosphorylated CPI-17 was not detected in both the membrane and cytosolic fractions. In conclusion, we for the first time suggested that CPI-17 is translocated and phosphorylated by ACh, but not high K^+ depolarization, in rat bronchial smooth muscle.

Key words: PKC, bronchial smooth muscle, acetylcholine, CPI-17

P340057

Effect of cigarette smoke exposure in vivo on bronchial smooth muscle contractility in vitro **in rats**

Norihide Matsuuchi[1], Yoshihiko Chiba[1], Masahiko Murata[1], Hroko Ushikubo[1], Yuji Yoshikawa[2], Akiyoshi Saitoh[2], Hroyasu Sakai[1], Junzo Kamei[2] and Miwa Misawa[1]1Dept. of Pharmacol., [2]Dept. of Pathophysiol. and Therap., Sch. of Pharmacy, Hoshi Univ., 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, JAPAN.

Cigarette smoking is a risk factor for the development of chronic obstructive pulmonary disease (COPD) and airway hyperresponsiveness. The effect of cigarette smoking on the contractility of airway smooth muscle is however unclear. The present study was performed to determine the responsiveness of bronchial smooth muscle (BSM) isolated from rats that were exposed (for 2 h/d. every day, 2 wk) to mainstream cigarette smoke in vivo. The responsiveness of intact BSM isolated from cigarette smoke-exposed rats to ACh, but not to high K^+-depolarization, was significantly augmented when compared with that from air-exposed control group. In permeabilized BSM strips, the ACh-induced Ca^{2+} sensitization of contraction was significantly augmented in rats exposed to cigarette smoke, although the contraction induced by Ca^{2+} was within control level. Immunoblot analyses revealed an increased expression of RhoA protein in the BSM of rats that were exposed to cigarette smoke. Taken together, these findings suggest that the augmented agonist-induced RhoA-mediated Ca^{2+} sensitization may be responsible for the enhanced bronchial smooth muscle contraction induced by cigarette smoking.

P340058

CpG OLIGODEOXYNUCLEOTIDES INHIBIT HUMAN EOSINOPHIL APOPTOSIS

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Oligodeoxynucleotide (ODN) sequences containing unmethylated cytidine phosphate guanosine (CpG) motifs prevalent in bacterial DNA attenuate allergic lung inflammation in experimental models of asthma. On the other hand, bacterial respiratory tract infections exacerbate asthma in humans. Our aim was to investigate the effect of CpG ODNs on constitutive and glucocorticoid-induced apoptosis and cytokine-afforded survival of human eosinophils in vitro. Eosinophil apoptosis was determined by flow cytometric analysis of relative DNA content, by Annexin-V labelling and morphological analysis. CpG ODNs were found to inhibit con-

stitutive eosinophil apoptosis and to further enhance granulocyte macrophage-colony stimulating factor (GM-CSF)-induced eosinophil survival. In contrast, CpG ODNs did not inhibit apoptosis in the presence of a glucocorticoid. Non-CpG ODNs occasionally acted in a similar manner to CpG ODNs. Our results may partially explain the exacerbation of eosinophilic lung inflammation during respiratory tract infection.

Keywords: Asthma, Eosinophils, Apoptosis, CpG oligodeoxynucleotides

P340059

The neuro-endocrine-immuno regulation of 5-lipoxygenase in the asthmatic model of rats

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Aim: To explore the neuro-endocrine-immunoregulation of 5-lipoxygenase (5-LO) in ovalbumin-induced rat asthmatic model.

Methods: Aerosol antigen-induced rat asthmatic model and pulmonary function and brain histology were investigated by molecular biological, Immunological and physiological methods.

Results: Antigen challenge induced a significant inflammation and increase of Th2 cytokines in lung. 5-LO metabolites such as LTB4 and LTC_4 in lung and cerebral cortical homogenates from the asthmatic model rats were markedly higher than that of control. The expression of 5-LO and LTA_4-H mRNA, and 5-LO protein in lung and cerebral tissue were also higher. 5-LO positive cells in lung are infiltrated inflammatory cells and airway epithelial cells. 5-LO is expressed by cerebral cortex neurons and glial cell in the brain, and thalamus and hypothalamus are most strongly expressed. Pretreatment of LTB4 (icv) prevented against the antigen-induced decrease of lung function.

Conclusion: These results indcated that 5-LO metabolites may cause neuro-endocrine-immuno modulation in rat asthmatic model.

Acknowledgements: The study was funded by the National Natural Science Foundation of China (No. 30371314).

P340061

Translation of beta 2-adrenoceptor pharmacology between guinea pig, canine and human in vitro.

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In the present study, we have compared the potency of isoprenaline, formoterol and salbutamol in isolated guinea pig, canine and human airway smooth muscle preparations contracted by electrical field stimulation (EFS). These data were compared to the elevation of cAMP by either human or canine recombinant beta 2 adrenoceptor (rB2AR) expressed in a CHO cell line. All 3 compounds caused concentration-dependent inhibition of the EFS response in isolated tissues from all 3 species, and increased cAMP levels in the rB2AR cell lines. The rank order of potency (formoterol > isoprenaline > salbutamol, n.E. 3) in guinea pig trachea was similar to the human bronchus. Interestingly, the rank order in the canine bronchus and rB2AR (human) was formoterol > isoprenaline = salbutamol (n = n.E. 3). These data highlight the significance of investigative translational pharmacology for drug discovery. We conclude that the guinea pig airways are a more appropriate translational model for native human beta 2-adrenoceptor pharmacology than the canine.

P340062

Effects of Phosphodiesterase Inhibitors on Pulmonary Hypertension in Rats

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We evaluated the beneficial effects of zaprinast, dipyridamole, and cilostamide, for the treatment of monocrotaline-induced pulmonary hypertension in rats.

Material and Methods: After a single intraperitoneal injection of 60 mg/kg monocrotaline, albino rats were divided into five groups. Vehicle-treated rats (control, n = 8) and monocrotaline-treated rats (n = 32) were fed a commercial diet. Dipyridamole (5 mg/kg/day), zaprinast (5 mg/kg/day), or cilostamide (5 mg/kg/day) were injected intraperitoneally to monocrotaline-treated rats for 21 days. Hemodynamic studies were performed on anesthetized animals. Arterial blood pressure, right ventricular pressure were recorded for 10 minutes. Finally 1 ml blood samples were collected for determining of nitric oxide, after sacrification and right ventricles were weighed.

Results: Right ventricle pressures and weights were significantly high in monocrotaline-treated rats. Phosphodiesterase inhibitors especially zaprinast had beneficial effect against pulmonary hypertension in regard to hemodynamic and biochemical measurements. These results suggest that phosphodiesterase inhibitors will be useful for the treatment of pulmonary hypertension and nitric oxide production may play a role in their beneficial effects.

Key Words: Pulmonary Hypertension, PDE inhibitors.
This study was supported by Eskisehir Osmangazi University Research Foundation.

P340063

Comparison of the Effect of Lung Preservation Solutions on the EDHF-Mediated Endothelial Function in Small Porcine Pulmonary Arteries

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We studied the effect of Perfadex and Celsior solutions (for lung preservation) on the endothelium-derived hyperpolarizing factor (EDHF)-mediated function in small porcine pulmonary arteries. The EDHF-mediated relaxation was induced by bradykinin (BK, 10^{-10} ~ $10^{-6.5}$ log M) in the presence of inhibitors of nitric oxide and prostacyclin before and after incubation in Perfadex (Group Ia), Celsior (Ib), or Krebs (Ic) at 4 °C for 4 hours (n = 8). The EDHF-mediated hyperpolarization of smooth muscle cells was measured after 4-h cold storage in Perfadex (IIa, n = 5), Celsior (IIb, n = 4), or Krebs (IIc, n = 6), followed by washout within 45 min. After storage, BK-induced, EDHF-mediated function markedly decreased in Ib (59.7 ±7.7 % vs. 37.3 ±7.2 %) and IIb (4.5 ±0.2 vs. 6.6 ±0.1 mV) ($P < 0.05$), but not in Ia (72.4 ±4.8 % vs. 61.2 ± 3.9 %), IIa (6.5 ±0.3 vs. 6.6 ±0.1 mV), and Ic (66.2 ±6.1 % vs. 61.8 ± 2.6 %). We concluded that compared to Celsior, Perfadex better preserves endothelial function related to EDHF in small porcine pulmonary arteries.

EDHF; Endothelium; Lung preservation
Supported by Research Grant Council of Hong Kong (Projects CUHK4127/01M and CUHK4383/03M) and St. Vincent Medical Foundation, Portland, OR, U.S.A.

P340065

Anti-asthma effects of perilla seed oil in the guinea pigs in vitro and in vivo

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Aim: To investigate the anti-asthma effects of perilla seed oil in vitro and in vivo in sensitized guinea pigs.

Methods: Aerosolized antigen caused an immediate bronchoconstriction in the sensitezed guinea pigs.

Results: Perilla seed oil showed a dose-dependent inhibition of lung resistance increases and dynamic lung compliance decreases. Perilla seed oil at doses of 0.5 to 2 g/kg dose-dependently inhibited total leukocyte, mononuclears, eosinophils and neutrophils infiltration caused by inhalating antigen. Pretreated with different concentration of perilla seed oil (5 ~ 500 μg/ml) inhibited SRS-A release from the sensitized lung tissues of guinea pig induced by antigen challenge. Pretreated with different concentration of perilla seed oil inhibited leukotriene D4 release from the lung tissue of nonsensitized guinea pig stimulated by A23187 in concentration-dependent manner.

Conclusion: These results indicated perilla seed oil may improve lung function by suppressing LT production and is an effective approach to improve allergic diseases such as asthma through control of eicosanoid production.

P340066

Change in the expression of matrix metalloproteinase-12 in airways of rats with allergic bronchial asthma

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Recent genetic studies revealed that matrix metalloproteinase-12 (MMP-12) might be one of the asthmatic candidate genes, but the detailed role of MMP-12 in asthma hasn't been clear. Here, the change in the expression of MMP-12 in airways of rats with allergic bronchial asthma was investigated. Rats were sensitized and repeatedly challenged with DNP-Ascaris antigen. The airway tissues

were taken at 1, 3, 6, 12 and 24 hours after the last challenge. The mRNA and protein expressions were detected by RT-PCR and western blot analysis, respectively. The mRNA expression of MMP-12 was significantly increased in the airway tissues of rats with allergic bronchial asthma. The protein expression of proenzyme of MMP-12 (54kD) was not changed in the airway tissues of rats with asthma. Surprisingly, the intermediate form of MMP-12 (45kD) was significantly decreased in airway tissues of rats with asthma when compared with normal rats. It is thus possible that the regulation of MMP-12 expression in the airways of allergic bronchial asthma might be complex. The discrepancy between the expressions of mRNA and protein of MMP-12 should be resolved.

Key words: Bronchial asthma, MMP-12, Airway tissue, Rat

P340068

Interleukin-13 induces upregulation of RhoA protein in human and mouse bronchial smooth muscles

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Allergic bronchial asthma is characterized by an eosinophilic airway inflammation with marked airway hyperresponsiveness (AHR) and upregulation of T helper (Th) 2 cytokines. Interleukin-13 (IL-13), one of the Th2 cytokines, is now proposed as a central mediator of AHR induction. In the present study, the effects of IL-13 on the expression of RhoA, a major protein responsible for Ca^{2+} sensitization of smooth muscle contraction, in bronchial smooth muscles (BSM) were investigated. Intranasal administration of IL-13 (1 ug in 20 uL of PBS) to naive mice caused BSM hyperresponsiveness with an upregulation of RhoA protein. Similarly, in tissue culture of mouse BSM, the RhoA expression and BSM contractility were significantly augmented by treatment with IL-13 for 12 hr (100 ng/mL). In cultured human BSM cells, treatment with IL-13 also caused an upregulation of RhoA protein. The upregulation was inhibited by co-incubation with leflunomide, an inhibitor of STAT6 activation. These findings suggest that IL-13 induces an upregulation of RhoA protein via STAT6, resulting in an augmented BSM contractility, that is AHR.

Key words: airway hyperresponsiveness; Ca^{2+} sensitization; RhoA; IL-13

P340069

PKC isoforms involved in ACh-induced contraction of rat bronchial smooth muscle

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Recent studies revealed that PKC/CPI-17 pathway might play an important role in the agonist-induced contraction of rat bronchial smooth muscle (BSM). The physiological role of PKC isoforms in agonist-induced BSM contraction is however unknown. The purpose of the current study was to determine the role of PKC isoforms in acetylcholine (ACh)-induced BSM contraction in rats. The expression of PKC isoforms in rat BSM was determined by RT-PCR and Western blot. In addition, the effects of three PKC inhibitors, GF1092603X, G?6976 and rottlerin, on the ACh-induced BSM contraction were examined. In RT-PCR analyses, mRNAs of all PKC isoforms were clearly detected in rat bronchial smooth muscle. GF109203X (inhibitor of PKCα, β, γ, δ and ε) significantly inhibited the ACh-induced BSM contraction, although G6976 (PKCα, β and γ inhibitor) and rottlerin (PKCδ and inhibitor) had no effect. In the immunoblot analyses, GF1092603X-sensitive PKC isoform proteins were expressed in the rat bronchial smooth muscle. Taken together, these findings suggest that PKCε might be involved in the ACh-induced BSM contraction in rats.

Key words: PKC isoforms, bronchial smooth muscle, acetylcholine, contraction

P340070

Expression of PEPT2 mRNA in the Lung of Rat with LPS-Induced Acute Lung Injury

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Peptide transporter 2 (PEPT2) expressed mainly in kidney and lung is an integral membrane protein. PEPT2 can transport both peptides and peptidomimetic drugs and becomes a target for a rational drug design for a new generation of respiratory drugs. We examined whether PEPT2 mRNA expression levels were changed in acute lung injury rat lung. SD rats were randomly devided into five groups: control, saline, LPS 2, 4 and 8 h. Control rats did not receive any treatment. LPS-treated rats received intratracheally 0.5 mg/kg of LPS, and were killed after 2, 4 and 8 h. Lung histopathology, lung W/D ratios, lung MPO activity and total protein concentration were measured. The expression levels of PEPT2 mRNA were evaluated by semiquantitative RT-PCR. Typical pathological changes of ALI in the lung were observed in LPS-treated rats. We found that the lung W/D ratios, MPO activity and total protein concentration increased significantly in LPS-treated rats after 2, 4 and 8h. ($P < 0.01$). Semiquantitative RT-PCR results showed there was no significant change in PEPT2 mRNA levels in the lung of LPS-treated rats. We conclude that PEPT2 mRNA levels do not change in the lung of rat with LPS-induced acute lung injury.

P340071

Inhibitory effects of Neferine on bleomycin-induced pulmonary fibrosis in mice

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To evaluate the antifibrosis of neferine (NEF, purity 95%), an bisbenzylisoquinline alkaloid extracted from the seed embryo of Nelumbo nucifera Gaertn, 120 mice were randomized into 10 groups as Sal(saline)-Sal, BL(bleomycin)-Sal, BL-NEF(each for 7d), Sal-Sal, BL-Sal, BL-NEF(for 14d), Sal-Sal, BL-Sal, BL-NEF and BL-pirfenidone (for 21d). Bleomycin (0.1 mg) or saline (0.05 ml) was singlely applied intratracheally, and saline, NEF (20 mg/kg, tid) or pirfenidone (100 mg/kg, tid) was administered orally. Animals were sacrificed 7, 14 or 21 days after intratracheal treatment. Lung hydroxyproline content, Lung tissue superoxidae dismutase (SOD) content and malondialdehyde (MDA) level were determined by biochemical measuremetns. Lung tissue structures were observed with HE stain. Results show that pirfenidone could inhibit the formation of lung fibrosis. Similarly, NEF could suppress the increase of hydroxyproline content and abated the lung histological injury time-dependently. NEF could enhance the SOD content and decrease the MDA level. Overall, these data supported an antifibrotic effect against bleomycin-induced pulmonary fibrosis in mice.

Key words: bleomycin, pulmonary fibrosis, neferine

P340072

Ginsenosides Reduce the Adherence of *Staphylococcus* aureus into Rat Pulmonary Epithelial Cells

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The aim of this study was to introduce the novel effect of ginsenosides extracted from ginseng on the in vitro invasion of Staphylococcus aureus into rat pulmonary epithelial cells. It's effect on S. aureus adherence to the host cells and fibronectin protein was also examined. Reverse transcription polymerase chain reaction was used to demonstrate the expression change of related genes. Addition of ginsenosides could reduce the bacterial number inside the cells significantly and the adherent activity of S. aureus by downregulating the fibronectin binding proteins and fnbA gene expression. Global regulator sarA might also be involved. The results suggested for the first time that ginsenosides had novel active targets besides immune systems and highlighted its potential as an adjuvant to antibiotics to the treatment of persistent and chronic S. aureus infections.

Key Words: Ginsenosides; Staphylococcus aureus; Rat Epithelial cells; fnbA
Acknowledgents: The study was supported by: Scientific Research and Development Program Tsinghua University (A2002162). Beijing Science; Technology Program (No. Z0004105040311); The Science and Technology Grant Tsinghua University (No. 03fd28).

P340073

c-Src mediates thrombin-induced NF-κB activation and IL-8/CXCL8 expression in lung epithelial cells

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In this study, we examined the regulation of NF-κB activation and IL-8/CX-

CL8 expression by thrombin in human lung epithelial cells. Thrombin cause a concentration- dependent increase in IL- 8/ CXCL8 release in human lung epithelial cells. Thrombin - induced IL - 8/ CXCL8 release was attenuated by PPACK, U73122, and Ro - 32 - 0432. The thrombin - mediated increase in the activity of IL - 8/ CXCL8 - luciferase was also inhibited by the c - Src DN. Thrombin caused a time - dependent increase in phosphorylation of c - Src at Tyr416 and c - Src activity, which was attenuated by Ro - 32 - 0432. The thrombin- induced IL - 8/ CXCL8 - luciferase activity was attenuated by cell transfected with κB site mutation of the IL - 8/ CXCL8 construct. Pretreatment of A549 cells with Ro - 32 - 4032 and c - Src DN inhibited thrombin - induced IKKα/ β activity, κB - luciferase activity, and NF- κB - specific DNA - protein complex formation. Further studies revealed that thrombin induced PKCα, c - Src, and IKKα/ β complex formation. These results for the first time show that thrombin activates the PI - PLC/ PKCα/c - Src/ IKKα/ β signaling pathway to induce NF - κB activation, which in turn induces IL - 8/ CXCL8 expression and release in human lung epithelial cells.

Key word: thrombin, IL - 8/ CXCL8, c - Src, IKKα/ β, NF - κB, lung epithelial cells

P340076

Inhibitory effects of local anesthetics on contractions of pregnant rat myometrium

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Object: To study the inhibitory effects of lidocaine, ropivacaine, bupivacaine, and tetracaine on contractions of isolated pregnant rat myometrium.

Methods: Full - thick myometrial strips were exposed to those local anesthetics with cumulative doses, recorded amplitude and frequency of the myometrium contractility.

Results: Four local anesthetics all caused a dose - dependent inhibition of contractility of pregnant uterine. On amplitude of myometrium, the beginning inhibitory concentration of lidocaine, ropivacaine, bupivacaine and tetracaine was from 3×10^{-5}, 3×10^{-5}, 10^{-5} and 3×10^{-7} mol/ L, respectively. But when the concentration reached 10^{-4} mol/ L, the amplitude of myometrium contractions was about 62 %, 53 %, 32 % and 8. 8 % of baseline, respectively. On the frequency, except lidocaine, the inlibitory concentration of ropivacaine, bupivacaine and tetracaine was 3×10^{-4}, 3×10^{-5} and 10^{-5} mol/ L, respectively.

Conclusion: These results suggest that the local anesthetics may inhibit myometrial contractions of pregnant rat in a dose - dependent manner. The rank of the potency was: tetracaine > bupivacaine > ropivacaine > lidocaine.

Key words: local anesthetics; myometrium; rat; in vitro

P340077

Altered ryanodine receptor functions of cultured airway smooth muscle cells in asthmatic guinea pig

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The functional changes of Ca^{2+} - induced Ca^{2+} release channels of airway smooth muscle cells (ASMCs) were investigated in asthmatic guinea pig. $[Ca^{2+}]i$ was measured with a fluorescent Ca^{2+} indicator (Flou- 3/ AM). In extracellular Ca^{2+} - free condition, Ryanodine, 50 μM to 200 μM, induced $[Ca^{2+}]i$ increase in primary cultured ASMCs in control and asthmatic groups, with the more significant increase of $[Ca^{2+}]i$ in asthmatic group($P < 0.01$). Ryanodine (50 μM) induced $[Ca^{2+}]i$ increase in primary cultured ASMCs of asthmatic group was higher than subcultured cells($P < 0.01$), while in 100 μM and 200 μM ryanodine, the $[Ca^{2+}]i$ increase in primary cultured ASMCs of asthmatic group and subcultured was not significant different ($P > 0.05$). In extracellular Ca^{2+} - free condtion, although histamine (100 μM) increased $[Ca^{2+}]i$ in primary cultured ASMCs in control and asthmatic groups, the increase was not significant different between the two groups ($P > 0.05$). Conclusion Ryanodine receptor, but not IP3 receptor of ASMCs of asthmatic guinea pig showed hypersensitivity. Under specified condition, the characteristics of ryanodine receptor still retain in subcultured ASMCs of asthmatic guinea pig.

Key words:, $[Ca^{2+}]i$; ryanodine receptor.; asthma; airway smooth muscle cell

P340078

Changes of calponin and TGF - β1 in pulmonary artery smooth muscle cells of pulmonary artery hypertension rats induced by MCT

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Objective To explore the changes of calponin and transforming growth factor β1 (TGF - β1) in pulmonary artery smooth muscle cells (PAMC) of pulmonary artery hypertension (PAH) rats induced by monocrotaline (MCT). Methods PAH rat model was established by abdominal injection of MCT (60 mg/ kg). Immunnohistochemistry, western blot were used to detect the expression of calponin and TGF - β1. Results In immunohistochemmistry test, calponin expression was located in cytoplasm of PAMC. Its expression markly decreased in PAH group. Average optical density was 129. 5 ±22. 64 in control group whereas 55. 22 ± 17. 13 in PAH group ($p < 0.05$). TGF - β1 expression was located in cytoplasm of PAMC and tunica adventitia. Its expression markly increased in PAH group. Average optical density was 28. 83 ±12. 49 in control group whereas 69. 65 ± 19. 38 in PAH group ($p < 0.05$). In western blot test, calponin to β- actin signal ratio decreased from 149. 67 ±10. 12 % in control group to 41. 5 ±15. 5 % in PAH group ($p < 0.05$). TGF - β1 to β- actin signal ratio increased from 41. 67 ±3. 06 % in control group to 130. 25 ±14. 95 % in PAH group ($p < 0.05$). Conclusions Calponin and TGF - β1 might take part in the formation of PAH.

Key word: calponin; transforming growth factor β1; pulmonary artery hypertension;

P340081

Inhibitory Fibrotic Effect of Captopril and Enalapril on Paraquat - Induced Lung Fibrosis in Rats.

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Although, many treatments of pulmonary fibrosis have been investigated, but poor therapeutic options available and often are untreatable. In this study, we examined the effects of two groups of angiotensin - converting enzyme inhibitors, captopril and enalapril, on pulmonary fibrosis induced with paraquat in rats. Furthermore, paraquat, a bipyridil contact herbicide, can use as a model to study the lung fibrosis. In this study, male albino wistar rats weighing 150 - 300 g were used and divided in eight groups (n = 3 - 5 each). group 1, received tap water; group 2, received captopril (10 mg/ kg/ 24h; po); group3, received enalapril (5 mg/ kg/ 24h; po); group4, received single does of paraquat (20 mg/ kg) intraperitoneally; group 5, treatment group of captopril; group 6, pretreatment group of captopril; group 7, treatment group of enalapril; group 8, pretreatment group of enalapril. After 21 days of treatment, right lungs homogenized and the levels of hydroxyproline, glutathione, and lipid peroxidation were determined. Also sections from left lungs stained for light microscopic to qualitative the fibrosis (Masson trichorome staining). Result demonstrated that captopril and enalapril improved pulmonary fibrosis as shown by lung histopathology, as well as by a decreased lung content of hydroxyproline ($p < 0.001$). Our study suggest that antifibrotic effect of this drugs may be related to inhibition of angiotensin II not through their antioxidant action.

Key words: pulmonary fibrosis; captopril; enalapril; paraquat

P35. Receptor Structure and Pharmacology

P350001

Cholesterol depletion reduces serotonin binding and signalling via human 5 - HT7(a) receptors in HeLa cells

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Lipids, including cholesterol, are critical components of plasma membranes where they are enriched in micro domains, lipid rafts, which organize and concentrate proteins involved in signal transduction. The present study examined the effects of cholesterol depletion on human 5 - HT7a receptor signaling in stably transfected HeLa cells. Saturation binding experiments showed that inhibition of cholesterol synthesis by combined treatment with mevastatin, fumonisin B1 and mevalonate

caused a significant reduction of tritium-labeled serotonin ([3H]5-HT) binding to 5-HT7a receptors, an effect that could be reversed by adding back cholesterol. Similar effects were found after treatment with methyl-β-cyclodextrin. None of the treatments had any effect on the potency of [3H]5-HT binding to 5-HT7a receptors or on the ability of 5-Metoxytryptamine to displace bound [3H]5-HT. Serotonin caused a strong induction of Ser63-ATF-1 and Ser133-CREB phosphorylation that were significantly counteracted by cholesterol synthesis inhibition. The study demonstrates that cholesterol depletion alters the binding properties of [3H]5-HT to 5-HT7a receptors and 5-HT7a mediated intracellular signaling.

Key words: 5-HT, lipid raft

P350002

Distribution of equilibrative nucleoside transporter subtype 1 in human brain

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Equilibrative nucleoside transporters (ENTs) are important for (a) nucleoside salvage for DNA, RNA and ATP synthesis, (b) cellular uptake and release of the signaling molecule adenosine, and (c) cellular uptake of chemotherapeutic nucleosides. The present study characterized the distribution of ENT1 in human brain. Sections of cerebral cortex, corpus callosum, basal ganglia, hippocampus, midbrain, cerebellum, and choroid plexus were obtained from individuals 23 weeks gestation (fetus), newborn, 2 months, 6 years, 40 years and 75 years of age. Immunohistochemistry was performed using a monoclonal antibody directed against amino acids 254-271 of the human ENT1 sequence. A low level of expression was evident in all tissue sections. The most intensely labeled cells were the basal epithelial cells of the choroid plexus and endothelial cells. The external granule layer of the cerebellum was strongly labeled in fetal and newborn brain. These findings suggest that ENT1 is important for facilitating nucleoside permeation of the blood-CSF and blood-brain barriers and may also be important for nucleotide synthesis or adenosine signaling in proliferating neuronal precursors.

Supported by CIHR

P350003

Differential modulation of G protein-coupled receptors (GPCR) by singlet oxygen

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We have examined in isolated rat pancreatic acinar cells and hepatocytes and other non-excitable cells the effect of singlet oxygen generated by a plasma membrane-localized photodynamic drug sulphonated aluminium phthalocyanine (SALPC), and found that the specific and direct target molecule of SALPC photodynamic action was the cell surface receptors, but not relevant G proteins, phospholipase C, inositol trisphosphate receptors or other signalling molecules. The effect of photodynamic action was different depending on the type of G proteincoupled receptors (GPCR) examined. Singlet oxygen specifically activated CCK1 cholecystokinin receptors, having no effect on M3 muscarinic receptors, but desensitized the V1 vasopressin and alpha1 adrenergic receptors, with the V1 receptor being more sensitive to singlet oxygen than the alpha1 receptor. Taken into the fact that singlet oxygen may be generated endogenously and released into the extracellular space by certain peroxidases (myeloperoxidase, lactoperoxidase, etc.), by infiltrating neutrophils during inflammation, and by other processes, there may exist in vivo an endogenous singlet oxygen receptor pharmacology that has not been discovered before.

P350004

An excess of prostanoid EP3 receptors decreases hetero-oligomerization with thromboxane A2 receptors

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The prostaglandin E2 receptor subtype EP3 and thromboxane A2 (TP) receptors mediate synergistic vasoconstrictor responses. N-terminally-tagged human TP (HA-hTPalpha) and human EP3 (2myc-hEP3-I) receptors were prepared with Renilla luciferase (Rluc) or Green Fluorescence Protein (GFP2) at the C-termini in order to assess oligomerization by the generation of a Bioluminescence Resonance Energy Transfer (BRET) signal. BRETmax values were 0.37 +/- 0.06 and 0.16 +/- 0.00 and BRET50 values were 1.54 +/- 0.61 and 0.9 +/- 0.21 in living HEK293 cells transfected with hTP or hEP3 receptors, respectively (mean +/- SEM, n = 3). The hEP3-Rluc receptors were also able to oligomerize with hTP-GFP2 receptors, producing BRETmax and BRET50 values of 0.09 +/- 0.02 and 1.20 +/- 0.33, respectively. However, when the hEP3-GFP2 cDNA was in excess of hTP-Rluc cDNA, Bret2 signals decreased. In conclusion, hTP and hEP3 receptors constitutively form homooligomers, but the formation of hetero-oligomers between these prostanoid receptors is attenuated when hEP3 receptors are in excess of hTP receptors.

Research supported by a Direct Grant for Research (2004.1.027)

P350005

Real-time observation of step-by-step transportation of alpha1A-adrenergic receptors stimulated by agonist in living cells

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To investigate the information about the dynamics of alpha1A-AR movement stimulated by agonist phenylephrine (PE), we have made efficient use of high tempo-resolution wide field fluorescence imaging techniques to explore the route and mechanism for the internalization of alpha1A-ARs in living human embryonic kidney 293A cells (HEK293A) in real time. We labeled alpha1A-ARs using Cy3-conjugated IgG (Cy3-IgG), and recorded the trajectory of their transport process in response to PE in the living HEK293A cells. 25 ms exposure time of stack frames was chosen, and the pixel array of each diffraction limited spot was fitted to a two-dimensional Gaussian peak to increase spatial precision. Analysis of alpha1A-AR trajectories in cells in response to PE stimulation provides information about the mechanism and dynamic properties of receptor transport. A directed movement of alpha1A-ARs on microfilaments with an average step of 32 nm was detected by us. It suggests that alpha1A-AR may transport by myosin along actin filaments in a hand-over-hand manner in living cells. Our current work provides several new insights into the mechanism and dynamic properties of receptor transport.

P350006

Beta-blockers show partial inverse agonism to a novel constitutively active mutant of β1-adrenoceptor

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Constitutively active mutants of GPCRs are found naturally in disease states and have stimulated research for naturally occurring GPCR mutant in humans. We provide a new mutant in β1-adrenergic receptor (β1-AR) by point mutations which can constitutively activate β1-AR. D104 β1-AR in the 2nd transmembrane was replaced with alanine.

The mutant β1-AR was created by site-directed mutagenesis kit using primers containing desired mutants and later expressed in HEK-293 cells by using a lipofection transfection kit.

The D104A β1-AR cells displayed high level of constitutive activity with respect to wild-type ($P < 0.05$ %). The constitutive activity of the mutant was confirmed by the finding that the enhanced activity is dependent on the high level of receptor expression. Some beta-blockers show partial inverse agonism to this increased basal activity.

The results of this study might have interesting implications for future studies aiming at elucidating the activation process of the β1-AR as well as mechanism of action of beta-blockers.

Key words: β1-adrenoceptor, constitutive activity, inverse agonism

This research was supported by a grant from the Promotion and Mutual Aid Corporation for Private Schools of Japan.

P350007

Tandemly arranged ligand binding sites in melanocortin 4 receptors

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The comparative analysis of the binding of the peptide analogue [125I]NDP-MSH, and the low molecular weight radionucleid [125I]THIQ to melanocortin 4 receptor (MC4R) revealed that the binding proceeds consecutively to two tandemly arranged interconnected binding sites. When bound to the MC4R, [125I]

NDP MSH can be released from only one of the sites and the second molecule remains practically irreversibly bound to the receptor. The fast dissociation of bound [125I] THIQ was slowed down by the addition of NDP - MSH, confirming the presence of two interconnected MC4R sites. The complex mechanism of the ligand binding to MC4Rs caused the situation where the apparent potencies of the same ligand determined in displacement experiments differed more than three orders of magnitude, depending on the experimental conditions and the radioligand used. We present a minimal model for ligand binding to MC4R - dimers, where binding sites are tandemly arranged and mutually dependent on each other.

Supported by ESF (6492), EMES (0182734), SVR (04X - 05957).

P350008

EXPERIMENTAL RADIOIMMUNOTHERAPY OF A XENOGRAFTED HUMAN EPIDERMOID CARCINOMA USING 188Re - LABELED MONOCLONAL ANTIBODY TO EPIDERMAL GROWTH FACTOR RECEPTOR (h - R3).

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The humanized anti - epidermal growth factor receptor (EGF - R) monoclonal antibody (MAb), h - R3, labeled with Rhenium 188 administered intratumorally may have potential for the treatment of patients bearing high grade tumors of neuroephitelial origin in CNS. In an effort to enhance the efficacy of radioimmunotherapy (RAIT), we evaluate the combinated treatment of ^{188}Re - h - R3 and the naked h - R3 in nude mice bearing subcutaneously the human squamous cell carcinoma A431 cells. Group 1 was treated with a single intravenous (i. v.) administration of 150 µCi of ^{188}Re –labeled 1 mg h - R3 and 6 (i. v.) administration of 1 mg of h - R3 every 48 hours. Group 2 was treated with 7 i. v. administration of 1 mg of h - R3 each 48 hours and group 3 was treated with 7 i. v. administration of PBS. Animals were weighted and tumors were measured with a vernier caliper. Hematological, biochemical and anatomo - pathological study was carry out to all animals. The combined treatment and the unlabeled monoclonal antibody did not show any toxic effects on mice corporal weights and elicited a significantly reduction of tumor size regarding to the control group. Platelets, leukocytes and hemoglobin peripherial values as well as the bone marrow studies did not show toxicological effects. Hepatic and renal function did not show any alteration according to the creatinine, aspartate aminotransferrase, alanino aminotransferrase values. A similar reduction of the overall microvascular density and an elevated apoptotic index in the remaining tumors were observed in the treated groups with RAIT and h - R3 and the group treated with h - R3 alone. h - R3 MAb proved to be effective in mice with a xenotransplanted squamous cell carcinoma, the combinate h - R3 + RAIT treatment at the administered doses did not improve the results.

Key words: toxicity; monoclonal antibody; cancer treatment; rhenium188 - h - R3

P350009

Synthesis and release of calcitonin gene - related peptide is regulated by vanilloid receptor 1 in endothelial cells

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Objective: To explore the in situ synthesis and release of calcitonin gene - related peptide (CGRP) in endothelial cells and the regulatory effects of vanilloid receptor - 1 (VR1). Methods: Human umbilical vein endothelial cells (HUVECs) were treated with capsaicin or heat stress. The level of CGRP mRNA was detected by RT - PCR, and protein level was measured by both radioimmunoassay and immunofluorosense. Results: The expression of CGRP mRNA, both a - and b - subtype, could be detected in HUVECs. Acute treatment with capsaicin significantly increased the level of CGRP in a concentration - dependent manner in the culture medium, and upregulated the expressions of both a - and b - CGRP mRNA in endothelial cells, and the effects of capsaicin were abolished by pretreatment with capsazepine, a competitive antagonist of VR1. Treatment with hyperthermia (43 °C, 30 min) also increased the expression of both alpha - and beta - CGRP mRNA. Conclusion: There is the expression of CGRP mRNA in HUVECs, both alpha - and beta - subtype, and the synthesis and release of CGRP in HUVECs is regulated by VR1.

Key words: Calcitonin gene - related peptide; Vanilloid receptor 1; Endothelial cells

supported by grant from NSFC No. 30430740

P350010

ROLE OF ARRESTIN - DEPENDENT SIGNALLING IN CELLULAR PROLIFERATION AND APOPTOSIS

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Traditionally recognised as modulators of G protein - coupled receptor (GPCR) desensitisation and internalisation, arrestins have more recently been shown to act as scaffolds for various intracellular proteins, including ERK1/2. However, the physiological role of arrestin - mediated signalling remains elusive. In the present study, we aimed to investigate whether arrestins are required for Angiotensin II (AngII) - stimulated cellular proliferation and apoptosis. Using murine embryonic fibroblasts (MEFs) lacking beta - arrestins 1 and 2 (2KO) as a model, we made a retrovirus of the AngII type 1 receptor (AT1R) and infected both 2KO and wildtype control MEFs to generate stable cell lines. AngII stimulation caused AT1R internalisation in wildtype MEFs, but not 2KOs, confirming appropriate expression and regulation of the receptor. We examined proliferation via 3H - thymidine incorporation and changes in cell number, while apoptosis was measured by annexin - V staining and activation of caspases 3 and 7. Finally, we used the arrestin - selective AngII analogue, Sar1Ile4Ile8 AngII, to examine arrestin signalling in cardiomyocytes. This study should provide important insights into the potential physiological role of arrestin - mediated signalling from GPCRs.

Key words: arrestin, angiotensin II, G protein - coupled receptors, proliferation

P350011

ADRENERGIC RECEPTOR MEDIATED TEMPERATURE EFFECTS OF MDMA

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Alpha2A - adrenergic receptor (AR) knockout (2A - KO) alters the temperature response to MDMA in mice from monophasic hyperthermia to biphasic hypothermia then hyperthermia (Bexis & Docherty, 2005). In rats, the hyperthermia to MDMA is attenuated by prazosin plus the beta3 - AR antagonist SR59230A (SR) in combination (Sprague et al., 2004). We studied these compounds in mice. 2A - KO and C57 - BL/6 WT mice were implanted under ether anaesthesia with temperature probes (DSI) in the abdomen, and after 14 days, temperature was recorded by telemetry. In WT mice, prazosin (0.1 mg kg^{-1}) or SR (5 mg kg^{-1}), or the combination, altered the response to MDMA (20 mg kg^{-1}) from a monophasic hyperthermia to a biphasic hypothermia then hyperthermia. In 2A - KO mice, MDMA produced biphasic responses, and following prazosin and SR, MDMA produced a greater initial hypothermia than in the absence of antagonist. However, in in vitro studies, SR showed relatively high potency at alpha1D - and, to a lesser extent, alpha1A - AR (pKB, 6.83 ±0.13 and 5.43 ±0.12, respectively, n = 4 - 5). In conclusion, alpha1 - , alpha2 - , and possibly beta3 - AR may be involved in hyperthermic actions of MDMA in mice.

MDMA, adrenergic receptors, temperature

P350012

Agonists increase and antagonists decrease F - loop mobility suggesting its involvement in the nicotinic receptor activation network

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Several studies suggest that initiation of the activation signal or wave of the nicotinic acetylcholine receptor starts at the C - loop covering the agonist binding pocket of the receptor. This signal is transmitted to the transmembrane gate via the beta - strands linked to the C loop. To test this activation mechanism by monitoring ligand - induced changes in alpha - carbon backbone flexibility in relevant regions in the acetylcholine binding protein from Lymnaea, a soluble nAChR extracellular domain homolog, we monitored the time - resolved decay of fluorescence anisotropy from a sulfhydryl - reactive fluorescein derivative stably conjugated to 5 individually engineered cysteines and one naturally occurring cysteine. In the absence of ligands, these sites on the C - loop, beta - strands extending from the C - loop, the F - loop, and the beta7 strand near the binding pocket revealed vastly different mobilities. The C - loop sites (C188 and D194C) showed the least

segmental mobility, and the beta9 strand (T177C) the greatest mobility. Agonist and antagonist - induced influences on segmental mobility correlated more closely with the F - loop site (Y164C) than the C - loop.

Support : R37 - GM18360 and a PhRMA pre - doctoral fellowship.

P350013

Mapping Structural Dynamics of Acetylcholine Binding Protein (AChBP) by Hydrogen/Deuterium Exchange Mass Spectrometry

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AChBP serves as a high resolution structural template for the ligand binding domains of the nicotinic acetylcholine receptor. To examine how changes in protein dynamics and flexibility contribute to ligand dependent activation of the nicotinic receptor, we have employed mass spectrometry to probe changes in hydrogen/deuterium exchange in AChBP in the presence and absence of different classes of ligands. These include nicotinic agonists (epibatidine and lobeline), partial agonists, alkaloid antagonists, short and long peptidic antagonists (alpha - conotoxins and alpha - neurotoxins), and non - competitive ligands (galanthamine). In the apo - protein, two regions facing the active site at the subunit interface, loop C (175 - 193) and loop F (164 - 171), adopt highly flexible conformations. The various ligands all protect loop C to varying extents and with distinctive exchange kinetics. The partial agonist, an anabaseine derivative, and small alkaloid antagonist, methyllycaconitine, also simultaneously protect residues on loop F (164 - 171), on the complementary subunit interface. These data underscore the selective influence on dynamic state of AChBP by pharmacologically different classes of nicotinic ligands.

P350014

Conformational States of AChBP revealed by X - ray crystal Structures of bound nAChR Agonists, Antagonists and Non - competitive ligands.

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We use the acetylcholine binding protein (AChBP) from mollusks as a soluble surrogate for the extracellular ligand binding domain of nicotinic acetylcholine receptors (nAChR). Ligand binding in the nAChR extracellular domain induces conformational states that allosterically open an ion channel. nAChR states have not been studied at atomic resolution. We have solved X - ray crystal structures of receptor agonists, antagonists and non - competitive ligand bound to AChBP. These structures reveal large conformation changes in the ligand binding pocket and distinct interface binding surfaces. Conformational changes in loop C reveal a general mechanism for agonism and partial agonism. X - ray structures of non - competitive receptor ligands galanthamine, cocaine, and thienyl - cylohexylpiperidine (TCP) in complex with AChBP reveal valuable information for accurately describing non - competitive receptor modulation. A crystal structure of apo AChBP is presented and compared to that of bound agonists, lobeline and epibatidine, and antagonists, alpha - conotoxin ImI and methyllycaconitine.

P350016

G - protein coupled receptor dimers, homomers and heteromers

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G - protein coupled receptors have long been considered to be monomeric membrane proteins. While numerous recent studies have indicated that GPCRs can form multimeric complexes, the functional and pharmacological consequences of this phenomenon have remained elusive. With the discovery that the functional GABAB receptor is an obligate heterodimer, and the use of energy transfer technologies, it is now accepted that GPCRs can form heteromultimers. In some cases, specific properties of such heteromers not shared by their respective homomers have been reported. Although in most cases these properties have only been observed in heterologous expression systems, there are a few reports describing data consistent with such heteromultimeric GPCR complexes also existing in native tissues. The present presentation will illustrate well - documented examples of such native multimeric complexes, lists a number of recommendations for recognition and acceptance of such multimeric receptors, and finally defines a minimal rule for their nomenclature.

P350017

Biosynthesis and NMR Analysis of a 75 - Residue Fragment of Cannabis sativa G - Protein Coupled Receptor

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The cannabinoid (CB) receptor subtype CB2 is classified as a member of the G - protein Coupled Receptor (GPCR) family and has been an important drug discovery target for numerous of potential therapeutic applications, in particular for immune treatment. CB2 is predominantly expressed in the peripheral immune system, and is likely involved in cell signal transduction in immune system. The CB2 segment, reported to be functionally important for communicating with G proteins and signal transduction, was cloned, overexpressed and double - isotopically labeled in the 15N/13C - enriched M9 media. Advanced 2D/3D NMR experiments were carried out using a 800 MHz NMR spectrometer to analyse structure of the CB2 fragment in membrane mimic environment. Our NMR data indicated predominantly two a - helical transmembrane domains in this segment. In addition, our NMR data revealed that TM2 region has a relative rigid helical structure whereas TM1 region exhibits certain degrees of conformation exchange with relative higher mobility in our current NMR experimental condition. The NMR - determined helix structures were then incorporated into the homology - constructed CB2 model in aid of receptor - based drug design.

P350018

Potential contribution of GABA rho subunits to ionotropic GABA receptors in mouse cerebellar Purkinje cells

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This study investigated potential subtypes of ionotropic GABA receptors in cerebellar Purkinje cells (PCs). We compared responses to agents selective for either GABA - C or GABA - A receptors; we also determined the distribution of GABA rho subunits (which, when present as homomers, form GABA - C receptors) in the cerebellum. We used patch clamp electrophysiology to record whole - cell currents from PCs in mouse cerebellum slices. We identified a population of ionotropic GABA receptors with an atypical, mixed pharmacology, displaying characteristics of GABA - A and GABA - C receptors. Thus, currents activated by the GABA - C - preferring agonist CACA were sensitivity to the selective GABA - C antagonist TPMPA, but were also affected by the GABA - A selective agents bicuculline and pentobarbital. Moreover, synaptic transmission, mediated by endogenous GABA release, was reduced by both TPMPA and bicuculline. Immunohistochemistry suggested that GABA rho subunits are expressed predominantly in PC somatodendritic/proximal dendritic compartments with a lower level in distal dendrites. Together, these data suggest that rho subunits may contribute to ionotropic GABA receptors in PCs.

Work supported by The Wellcome Trust.

P350019

BODIPY - labeled free fatty acid: a fluorescent probe for studying free fatty acid - sensitive cell surface receptor

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Several orphan G protein - coupled receptors, including GPR40, have recently been shown to be responsive to fatty acids. In this study, a fluorescent analog of free fatty acid (C1 - BODIPY - C12) was characterized for its ability to act as a suitable fluorescent probe for the GPR40 receptor. Human GPR40 was integrated into HEK - 293 cells and expressed with the Tet - on inducible system to control the expression levels of exogenous protein. Flow cytometry analysis showed that C1 - BODIPY - C12 binding is saturable and GPR40 - specific. C1 - BODIPY - C12 displayed submicromolar affinity for the GPR40 receptor, which corresponds well with the intracellular Ca^{2+} response previously reported. The results describe a BODIPY - labeled ligand for the GPR40 receptor and the use of the ligand as a fluorescent probe for the GPR40 receptor. Thus, C1 - BODIPY - C12 is a fluorescent probe that is useful for the study of the binding and functional characteristics of the free fatty acid receptor.

P350020

Synthesis and Biological Evaluation of Simple Methyllycaconitine Analogues on Nicotinic Acetylcholine Receptors

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Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels with potential therapeutic applications for the treatment of neurodegenerative diseases such as - Alzheimer's disease, Parkinson's disease and schizophrenia. Methyllycaconitine 1 (MLA, K_B = 237 ±79 pM) is a selective and potent α7 nicotinic acetylcholine receptor (nAChR) antagonist and a prime candidate for exploring nAChRs.

Simple analogues of MLA were synthesised and evaluated via two-electrode voltage-clamp technique on rat neuronal homomeric α7, and heteromeric α4β2, and α6α3β2 nAChRs expressed in Xenopus oocytes.

The most potent analogue 2 (K_B = 6.0 ±1.5 μM) evaluated was an antagonist with mixed effects at the different receptor subtypes. This was also true for related analogues. The results obtained in this study have demonstrated that MLA analogues are not highly selective for the α7 nAChR subtype and can be used to help define the structural activity relationships of MLA analogues at the nAChRs. Understanding between MLA ligands and the nAChRs interaction provides potential lead compounds for the treatment of the neurological diseases mentioned.

Key words: Methyllycaconitine, nicotinic acetylcholine receptor

P350021

A Novel Splice Variant of the Equilibrative Nucleoside Transporter 1 (ENT1)

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We have identified an alternative splice variant of the mouse nucleoside transporter, mENT1, which results from the exclusion of exon 11, and leads to a truncated ENT1 protein missing the last three transmembrane domains (mENT1Δ11). mENT1Δ11 transcript was found, by PCR, to be differentially distributed among mouse tissues relative to full length mENT1. PK15 NTD (nucleoside transport deficient) cells were stably transfected with pcDNA3.1-mENT1 or -mENT1Δ11 and were assessed for nucleoside transport. PK15-mENT1Δ11 cells bound the ENT1 probe [^{3}H] nitrobenzylthioinosine (NBMPR) with high affinity (KD 0.11 nM) and had enhanced accumulation of the purine [^{3}H] 2-chloroadenosine (KM 64 μM) as well as the pyrimidine [^{3}H] uridine. Like the full-length transporter, PK15-mENT1Δ11 was inhibited by dipyridamole, dilazep, NBMPR and draflazine. These data suggest that the last three transmembrane domains of ENT1 are not necessary for transport activity. The expression of mENT1Δ11 truncated variant may be important in the regulation of cell nucleoside transport capacity.

Key words: adenosine, transporter, alternative splicing

This research was funded by Natural Science & Engineering Research Council of Canada.

P350022

Differential binding profiles of M1 muscarinic ligands competing against ectopic and classical muscarinic radioligands

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Endogenous ligands bind and activate G-protein coupled receptors by interacting with several residues within transmembrane domains of these receptors. ACADIA identified a family of ligands that bind and activate M1 muscarinic receptor by interacting with an "ectopic" site (Spalding et al. 2002 and accompanying poster by Risso Bradley et al.). This "ectopic" site is different from the "orthosteric" binding site interacting with the endogenous ligand. To further define this "ectopic" site, we have radiolabeled an "ectopic" muscarinic agonist. The binding profiles of various muscarinic agonists and antagonists were described using this "ectopic" agonist. For comparison, competition binding assays were also carried out with the conventional muscarinic radioligands, [3H]-NMS and [3H]-pirenzepine. Our results demonstrate that muscarinic ligands have different binding profiles against the conventional muscarinic radioligands compared to the "ectopic" radioligand in both native and recombinant M1 receptors. These results suggest that the binding studies using this "ectopic' radioligand can be explored for design of subtype selective M1 agonists.

P350023

Site-directed mutations of the muscarinic M1 receptor reveal that structurally diverse agonists have distinct mechanisms of receptor activation

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To characterize the molecular nature of ligand interactions with the muscarinic M1 receptor we examined the activation characteristics of a number of diverse agonists at two mutated variants of the M1 receptor (Y381A and W101A). We found that structurally distinct M1 receptor agonists have markedly different activation profiles at these receptor variants. These mutations substantially reduced the activity of carbachol and related ligands, whereas, the activity of AC-42 and N-desmethylclozapine was maintained or enhanced. Specifically, AC-42 and related analogs demonstrated increased potency at the W101A mutant but were not markedly affected by the Y381A mutation. Conversely, the maximal responses to clozapine and related compounds were increased at both mutations, most notably at the Y381A mutant. These mutations reveal at least three distinct modes of interaction of agonists with the muscarinic M1 receptor, confirming and extending the findings of Spalding et al., 2002, Sur et al. 2003, Lameh et al., (this meeting) and Langmead et al.,. 2005. These mutations, by their enhanced sensitivity to novel agonists have utility in drug-discovery.

P350024

EXPRESSION AND CELLULAR DISTRIBUTION OF MUSCARINIC RECEPTOR SUBTYPES IN HUMAN COLON

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Acetylcholine is the major neurotransmitter in intestine. In human and animal intestinal smooth muscle, binding and functional studies show the most abundant muscarinic receptor subtype is M2, but contraction is mediated via M3 receptor. We used RT-PCR and immunohistochemistry to study muscarinic receptor subtypes in human sigmoid colon. M1, M2 and M3 receptor mRNAs were densely expressed in taenia coli (TC), circular muscle with longitudinal muscle (CM.LM) and mucosa, with no regional differences. M5 mRNA expression was 3-fold higher in mucosa than in TC or CM.LM ($p < 0.05$). M4 mRNA expression was very weak in all regions. Strong M1 immunoreactivity (IR) was present on many myenteric and submucosal nerve cell bodies and on endothelium of submucosal vessels. M2IR occurred on smooth muscle and on nerve fibres in CM, LM and enteric ganglia. M3IR was widespread on CM and LM, and present on myenteric nerve cell bodies and mucosal cells. The results support a role for M1, M2 and M3 in neurotransmission, M2 and M3 in direct contraction of muscle and M3 and M5 in mucosal function in human colon.

Key words: human colon; muscarinic receptors; enteric nervous system

Support: NHMRC of Australia, MRS

P350025

BINDING CHARACTERISTICS OF CLINICALLY EFFECTIVE MUSCARINIC RECEPTOR ANTAGONISTS IN HUMAN BLADDER DETRUSOR AND UROTHELIUM

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Muscarinic receptor (MR) antagonists used to treat overactive bladder may act on urothelium as well as on detrusor muscle. We used the MR ligand [3H] quinuclidinyl benzylate (QNB) to examine the binding characteristics of MR antagonists, in membranes from mucosa (urothelium + lamina propria) and detrusor (n = 8). Membranes were incubated with relevant MR antagonists and 200 pM [3H] QNB for 2 h at 37 °C. All antagonists displayed high affinity competition for [3H] QNB binding, with fesoterodine ≥ trospium > oxybutynin > darifenacin in

detrusor , compared to trospium > fesoterodine > oxybutynin > darifenacin in mucosa. Darifenacin and fesoterodine displayed 2site binding. Darifenacin bound to detrusor with higher affinity than to mucosa (P < 0. 0001) , trospium bound to mucosa with higher affinity (P < 0. 0001) , and fesoterodine showed equal affinity. These results support the hypothesis that MR in the mucosa (probably , M2 receptors on the urothelium) may represent a novel site of action for MR antagonists.

Key words : human bladder ; muscarinic receptors ; urothelium
Support : Australasian Urological Foundation , Dr R Pfleger

P350026

Functional Role of b - Adrenoceptor Subtypes and cAMP Phosphodiesterases in Catecholamine Mediated Responses in a Prostate Cancer Cell Line (LNCaP)

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Aluciferase activity reporter gene assay (6 CRE - LUC) was used to measure drug - induced cAMP changes in LNCaP cells. The rank order of agonist catecholamine potency (-) - isoproterenol (ISO) > (-) - epinephrine (EPI) > > (-) - norepinephrine ; and (ISO) responses were blocked by (S) - (-) - propranolol (PROP) (KB = 0. 12 nM) and ICI 118 ,551 (KB = 0. 13 nM). Isomers showed a high stereoselectivity : [(-) > > (+)] of EPI and soterenol. Saturation assay and [^{3}H] - CGP12177 radioligand displacement showed a receptor density of 81. 2 f moles/ mg protein and Ki values for (S) - (-) - (PROP) , ICI 118 ,551 and ICI 89 ,406 were 0. 27 , 0. 50 and 114 nM, respectively. Preincubation with a series of PDE inhibitor [IBMX, > papaverine (PAP) , rac - rolipram, diazepam, cilostamide , MM- IBMX and dipiridamole] gave increases in cAMP responses to (-) - ISO. PDE inhibitors potency , at 10 µM, were : racrolipram > PAP > dipiridamole = IBMX > diazepam > cilostamide = MM- IBMX. Rolipram isomers , potency was : (R) - rolipram > rac - rolipram > (S) - rolipram. These results suggest : (1) a homogenous b2 - adrenoceptor population exists (2) PDE4 plays an important role in controlling catecholamine - induced Camp levels in these cells.

Supported by THE NCNPR and A USDA , ARS GRANT.

P350027

Investigating inter - species variation in adrenoceptor pharmacology : alpha 2 - adrenoceptor characterisation in isolated rat , dog and human vas deferens

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Understanding species differences in pharmacological responses is important in the translation of pre - clinical animal data to man. The aim of the present study was to compare responses to a range of alpha 2 - adrenoceptor agonists in isolated , electrically field stimulated (EFS) rat , dog and human vas deferens (VD) preparations , mounted in tissue baths (n ≥3). As expected , UK14304 caused an inhibition of EFS neuronally - mediated contractile responses in rat and human VD (mean pEC50 ±s. e. m. 8. 6 ±0. 3 and 7. 7 ±0. 1) but interestingly potentiated EFS responses in dog VD (mean pEC50 ±s. e. m. 6. 7 ±0. 1). However , in all three species responses to UK14304 were competitively antagonised by yohimbine (mean pKb rat 8. 2 , human 8. 1 , dog 8. 6). Guanfacine and clonidine also caused inhibition of EFS responses in rat VD (mean pEC50 ±s. e. m. 8. 2 ±0. 1 and 8. 5 ±0. 2) but , in contrast , potentiated EFS responses in both dog (mean pEC50 ±s. e. m. 6. 0 ±0. 3 and 5. 6 ±0. 1) and human VD (mean pEC50 ± s. e. m. 5. 6 ±0. 1 and 5. 9 ±0. 2). These data demonstrate species differences in pharmacological responses to adrenoceptor agents and the importance of human tissue data and translational models to better explain in - vivo findings.

Key words - vas deferens , alpha2 - adrenoceptor

P350028

Regulation of calcitonin gene - related peptide expression by vanilloid receptor - 1 receptor in dosal root ganglion cells

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Objective : Calcitonin gene - related peptide (CGRP) , the most potent vasodilator neuropeptide, is mainly synthesized and released from dorsal root ganglion (DRG) neuron cells , and vanilloid receptor - 1 (VR1) has been shown to be closely related to CGRP release. We investigated whether activation of VR1 can also induce CGRP synthesis in DRG cells. Methods : Primary DRG cells were cultured from neonatal rats and treated with capsaicin or rutaecarpine (RUT) for 24 hours. CGRP concentration in the culture medium was determined by radioimmunoassay , and mRNA level was determined by RT - PCR. Results : Treatment with the high dose of capsaicin or RUT induced a 10 - fold increase in CGRP content in the medium and significantly upregulated the mRNA level of CGRP in DRG cells. Pretreatment with capsazepine , an antagonist of VR1 receptor , significantly decreased the upregulation of CGRP expression by capsaicin or RUT. Conclusion : Capsaicin and RUT can increase expression of CGRP in DRG cells through VR1 activation pathway , which may contribute to the therapeutic effects of those compounds.

Key words : Calcitonin gene - related peptide; Vanilloid receptor; Dorsal root ganglion

P350029

Differential vascular reactivity of isolated abdominal aorta of control and knockout B1 receptor mice

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Vascular reactivity to bradykinin (BK) was assessed in wild type (WT) control and kinin B1 receptor knockout (KOB1) mice. Aortic rings were suspended in organ chambers for recording isometric tension development in response to BK. From the peptide - induced contractions the values of pD2 : 6. 8 &# 21025 ; 0. 1(WT) and 7. 1 &# 21025 ; 0. 3 (KOB1) and of Emax (%) : 41 &# 21025 ; 0. 6 (WT) and 24 &# 21025 ; 1. 8 (KOB1) were obtained. Angiotensin I - converting enzyme (ACE) inhibitor , enalaprilate potentiated BK - induced effect in aorta from WT but not KOB1 mice. The finding that the potency was inaltered whereas the efficacy was drastically reduced in aorta from KOB1 mice suggested that the lack of B1 receptor favoured the homodimerization of B2 receptor , known to cause its activation and desensitization. The lack of potentiating effect of enalaprilate on BK - induced efffect in KOB1 suggests an interaction between kinin receptors , ACE and ACE inhibitor. It is concluded that the disruption of the B1 receptor gene affected the B2 receptor system.

Key words : bradykinin , kinin receptors , knockout mice , enalaprilate.
Acknowledgements : This work was supported by CNPq and FAPESP.

P350030

Potential vascular alpha1adrenoceptor blocking properties of an array of 5HT receptor ligands in the rat.

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This study investigated the potential ability of some 5 - hydroxytryptamine (5HT) receptor ligands to interact with vascular alpha1adrenoceptors in pithed rats. These ligands included: methiothepin , methysergide , metergoline , WAY100635 , buspirone , ipsapirone , 8 - OH- DPAT, GR127935 , ketanserin , ritanserin , spiperone , pizotifen , granisetron , metoclopramide , tropisetron , ergotamine , clozapine , LY215840 and mesulergine. Hence , the increases in diastolic blood pressure produced by phenylephrine were analysed before and after the above antagonists or saline. Thus , the phenylephrine induced vasopressor responses were dose dependently antagonised with the following apparent rank order of potency by : methiothepin > ketanserin > clozapine ≥lisuride > > buspirone. In contrast , the other compounds were devoid of any blocking effect on the responses to phenylephrine. These results show that methiothepin , ketanserin , clozapine , lisuride and buspirone can block α1adrenoceptors in the rat systemic vasculature (as compared with the antagonism produced by prazosin).

Key words : α1 - Adrenoceptors , Blood pressure , 5HTligands.
Acknowledgements : We thank CONACyT (Mexico) for their support.

P350031

Mechanisms of extracellular signal - regulated kinase (ERK) 1/2 phosphorylation following activation of relaxin family peptide receptor 3 (RXFP3) by human relaxin - 3 (H3 relaxin).

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The relaxin family peptide receptor 3 (RXFP3) is the cognate receptor for H3 relaxin with highest expression in the paraventricular nucleus of the hypothalamus and the supraoptic nucleus that may have potential for development as a target for novel anti - anxiety drugs. This study examines the mechanism of ERK1/ 2 acti-

vation in CHO- K1 (CHO- RXFP3) and HEK293 (HEK- RXFP3) cells stably expressing human RXFP3 receptors. Direct assay of pERK (Surefire , TGR Biosciences) showed that ERK1/2 is rapidly and transiently activated following stimulation of RXFP3 by H3 relaxin. Inhibition of ERK1/2 phosphorylation was observed when cells were pre - treated with the inhibitors pertussis toxin (Gi/o) , U0126 or PD98059 (MEK) , Ro - 31 - 8220 or chelerythrine (PKC) , sucrose or methyl - β- cylcodextren (internalisation) (all n = 6). LY294002 or wortmannin (PI - 3 - kinase) and PP1 or PP2 (src) reduced ERK1/2 phosphorylation by ~ 50 % (all n = 6). AG1478 (EGFR) decreased ERK1/2 phosphorylation by ~ 40 % in HEK- RXFP3 cells in response to H3 relaxin (n = 6). This study suggests that ERK1/2 activation in response to RXFP3 activation involves a Gi/o protein , activation of PKC and PI - 3 - kinase , with EGFR transactivation contributing to this pathway in HEK- RXFP3 cells.

Key words : relaxin - 3 , G- protein coupled receptor , signal transduction

Acknowledgments : The authors wish to thank Dr John Wade for relaxin peptides and grant support from ARC linkage grant LP 0560620

P350032

The role of the α1 - adrenergic receptor subtypes in embryonic implantation in the rat

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Objective : Our studies focused on the possible role of α1 - adrenerg receptors (α1 - ARs) in the rat embryonic implantation. Methods : The α_1 - AR subtypes mRNAs and proteins expressions , and pharmacological reactivity were measured by reverse transcription - polymerase chain reaction and Western blotting , and isolated organ bath, respectively. Results : The presence of all α1 - AR subtypes (α_{1A} - , α_{1B} - α_{1D}) were proved with a predominance of α_{1A} - ARs. The maximum expressions of α_{1A}- ARs were attained on day of implantation. The selective α_{1A} - AR antagonist 5 - methylurapidil inhibited the uterine contraction in a dose - dependent manner. The numbers of embryonic implantation sites were decreased (approx. 75 %) in α_{1A} - AR knock - down transformed rats (using antisense oligonucleotids). Conclusion : The α_{1A} - AR dominance has a crucial role in the embryonic implantation of the rat. Further studies are needed to evaluate this role as a new therapeutic possibility in pregnancy maintenance.

Key words : implantation, α1 - adrenergic receptor , antisense oligonuclectids , rat

P350033

Selective up - regulation of the beta - cell specific Zinc - Transporter 8 (ZnT - 8) by GLP - 1 in INS - 1E cells.

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Zn is necessary to form Zn - insulin crystals in secretory vesicles. Moreover , after glucose stimulation , Zn is secreted together with insulin. We previously identified a pancreas - specific Zn transporter , ZnT - 8. Here we describe its localization in human pancreas , its influence on insulin secretion and whether the transporter is regulated by GLP - 1.

In human pancreas ZnT - 8 protein was localized in islets exclusively. Moreover , ZnT - 8 was co - localized with insulin in islet beta cells. We next found that overexpression of ZnT - 8 in the rat beta cell model , INS - 1E cells , significantly increased insulin secretion in a glucose - dependent manner. In addition , we found that the expression of granule - localized ZnT - 8 can be selectively manipulated by GLP - 1 , since no other ZnTs were regulated by GLP - 1.

We conclude that the zinc transporter ZnT - 8 is specific for pancreatic beta cells , and that it may play an important role in regulating insulin synthesis/ secretion. Our data imply that an increased need for zinc during storage in secretory granules is met by an increase of ZnT - 8 expression.

Key words : ZnT8 , insulin , GLP - 1

P350034

Actions of NK_1 Receptor Antagonists on [Sar^9 $Met(O_2)$ 11] substance P - induced Contractions of *Suncus murinus* (House Musk Shrew) Isolated Ileum

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In the present studies , we investigated the contractile action of the tachykinin NK_1 receptor agonist [Sar^9 $Met(O_2)_{11}$] substance P on the ileum of Suncus murinus to enable an assay to estimate the in - vitro potency of NK_1 receptor antagonists CP - 99 ,994 , R116301 and R115614. Briefly , the ileum was removed and placed in organ bath with Kreb 's solution. Cumulative dose response curves were constructed by adding [Sar^9 $Met(O_2)_{11}$] substance P (1 nM - 1 μM) in the absence and presence of the antagonists (0 - 100 nM) and responses were normalized against the contractions induced by 120 mM KCl. [Sar^9 $Met(O_2)_{11}$] substance P induced contractions of the ileum with a pEC50 value of 8.1 ±0.1. CP - 99 ,994 competitively antagonized the action of [Sar^9 $Met(O_2)_{11}$] substance P with a pA2 value of 7.26 ±0.25. R116301 and R115614 caused insurmountable antagonism yielding apparent pKB values of 7.8 and 7.3 respectively. The relative potency of the antagonists was similar to their activities to prevent cisplatin - induced emesis confirming the importance of the NK1 receptors in the emetic reflex of this species.

Key words : NK1 receptor antagonist , Suncus murinus , ileum

P350035

Different roles of α2 - adrenergic receptor subtypes in the pregnant uterine contractility in the rat

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Objective : The aim was to investigate the possible role of α_2 - adrenergic receptor (α_2 - AR) subtypes in pregnant rat myometrium contractility. Methods : The mRNA and protein expressions of α_2 - AR subtypes from last day and hormonally prematured pregnant uteri were detected by reverse transcription - polymerase chain reaction and Western blotting , respectively. The myometrial contractions were stimulated by noradrenalin (NA) or clonidine (CL). The α_2 - AR subtypes were blocked by non - selective (yohimbine) or subtype selective α_2 - AR antagonists (BRL 44408 , ARC 239 and spiroxatrine for α_{2A} - AR , α_{2B}/C - AR and α_{2C} - AR , respectively). Results : All subtypes of α_2 - ARs were detected with the dominance of α_{2B} - AR. Yohimbine and ARC 239 blocked , BRL 44408 and spiroxatrine enhanced , while BRL 44408 and spiroxatrine together extremely increased the NA or CL stimulated contractions. Conclusion : Myometrial α_{2A} - and α_{2C} - ARs mediate relaxation while α_{2B} - AR mediates contraction in the pregnant uterus. The development of subtype selective α_{2B} - AR antagonists or α_{2A}/C - AR agonists may have therapeutic importance in uterine relaxation.

Key words : α_2 - adrenergic receptor , pregnancy , premature labour , rat

P350036

Functional characterization of α_1 - adrenoceptors in aorta media layer: changes with aging and hypertension

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α_1 - Adrenoceptors occur in arteries. Most studies do not exclude the adventitia layer , this could overestimate adrenoceptors amount in complete arteries.

Adventitia - and endothelium - free aortic rings from spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats , 6 and 12 months old , were exposed to phenylephrine (PHE) and to prazosin (α1 - antagonist) , the α_{1A} - antagonist RS 100329 (5 - methyl - 3 - [3 - [4 - [2 - (2 ,2 ,2 , - trifluoroethoxy) phenyl] - 1 - piperazinyl] propyl] - 2 ,4 - (1 H) - pyrimidinedione) , and the α_{1D} - antagonist BMY7378 (8 - [2 - [4 - (2 - methoxyphenyl) - 1 - piperazinyl] ethyl] - 8 - azaspiro[4.5] decane - 7 ,9 - dione).

pD_2 values to PHE were similar between strains and age. Prazosin showed high affinity while RS100329 showed no different affinity in 6 months WKY and SHR, but increased in 12 months old SHR. BMY7378 pA_2 was similar in 6 months WKY and SHR, and increased in 12 months old animals. α_{1D} - Adrenoceptors mediate contraction in rat aorta media layer. Functional expression in aorta is modulated by aging in WKY and SHR for both alpha1D - and α_{1A} - subtypes.

Key Words : α1 - adrenoceptors , aortic media , aging , hypertension.

Conacyt grant 47481 , Fundacion Miguel Alemán and PAPIIT IN322005. JHGZ Conacyt fellow 175141

P350037

Identification of a domain in the $GABA_A$ receptor δ subunit (S238 - L277) that confers high agonist sensitivity.

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GABA, the major inhibitory neurotransmitter in the mammalian brain , exerts most of its effects by acting at $GABA_A$ receptors. It has been suggested that the extrasynaptic α4β3δ subtype is activated by the low concentrations of GABA that overspill from the synapse. Using voltage clamp techniques , we show that the recombinant α4β3δ receptor expressed in Xenopus oocytes is activated by 6 - to 25 - fold lower concentrations of agonists (GABA, THIP, muscimol) than those that activate the putative synaptic receptor , α4β3γ2. Structural determinants underlying the functional differences between these receptors have been probed by co - expressing chimeric δ γ2 subunits with the α4 and β3 subunits. A structural domain lying between residues Ser238 and Leu277 (a segment that incorporates the M1 and M2 domains) of the δ- subunit is shown to play an important role in determining its higher sensitivity to agonists. However , the effects of the competitive antagonists are not significantly altered by incorporation of the δ γ2 chimeric subunits , suggesting that the observed differences are agonist - dependent and are likely to involve changes in the transduction mechanism that links agonist binding to channel activation.

Key words : $GABA_A$ receptor ; δ subunit ; δ γ2 chimeras.

This work is supported by CIHR and UCB Pharma.

P350038

GHHPH motif in histidine rich glycoprotein indicates anti - angiogenic effect under neutral condition.

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Histidine rich glycoprotein (HRG) is relatively abundant plasma glycoprotein with an unusual high histidine contents , and has four functional domains ; Cys1 , Cys2 , His - Pro rich and C - term domain. From in vitro studies , HRG was revealed to interact with a number of body constituents including heparin , heparan sulfate , metal ions and other plasma components. Hence , it has been proposed as a modulator of coagulation/fibrinolysis or angiogenesis , although its exact function remains to be clarified. In Matrigel plug assay , recombinant His - Pro rich domain , particularly GHHPH motif , exerted significant anti - angiogenic effect against bFGF and heparin - induced angiogenesis. Its anti - angiogenic activity didn't result from the adsorption of heparin by His - Pro rich domain , because the binding between both was observed only under acidic condition , and this plug assay was routinely performed at neutral pH. These findings indicated that His - Pro rich domain may interact with the other unknown factors in angiogenic process. Thus , pull down assay using His - Pro rich domain or GHHPH motif as affinity ligand to find unknown factor are currently in the works.

P350039

Constitutive Homodimerization of Angiotensin II Receptor AT_2

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The objective of this study was to determine whether and how constitutively active AT_2 forms constitutive homodimer. Methods : Western blot, confocal microscopy , bimolecular fluorescence complementation (BiFC) and endoplasmic reticulum (ER) trapping were applied to detect the homodimerization in AT_2 - expressing CHO cells. Results : The constitutively active wild - type AT_2 homodimerized in the absence of Ang II. The magnitude of homodimerization increased ~50 % in the presence of AngII. AT_2 - specific antagonists PD123319 and CGP42112A failed to inhibit the homodimerization. The constitutive homodimerization was independent of extracellular disulfide bond formation and G protein activation as detected with C35A - C290A mutant and inactive mutant D141A - R142L, respectively. A Gly mutation at Asn^{127} and an Asn mutation at Ser^{311} that disrupted the constitutive activity blocked the constitutive homodimerization. ER trapping showed that AT_2 homodimerized prior to its plasma membrane delivery. Further studies identified structural domains critical for AT_2 homodimerization. Conclusion : The results show that AT_2 undergoes constitutive homodimerization.

Key words : AT_2, dimerization , BiFC;

supported by NIH grant (HL065492) to YHF

P350040

Upregulated expression of endothelium - derived calcitonin gene - related peptide in phenol - induced hypertensive rats : role of alpha2 - adrenoreceptor

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Objective : To investigate the role of alpha2 - adrenoreceptor in the expression of endothelium - derived CGRP in phenol - induced hypertensive rats. Methods : Neurogenic hypertensive rats were induced by an injection of phenol in the left kidney. Protein expression and mRNA level of CGRP in the artery endothelium were measured by immunohistochemistry and in situ hybridization , respectively. Human umbilical vein endothelial cells (HUVECs) were treated with clonidine , a selective agonist of alpha2 - adrenoreceptor. Level of CGRP mRNA in HUVECs was detected by RT - PCR. Results : Both mRNA and protein level of CGRP in artery endothelium were upregulated in the phenol - induced hypertensive rats. Treatment with clonidine significently increased the expression of CGRP mRNA in HUVECs. Conclusion : Alpha2 - adrenoreceptor may be involved in the upregulated expression of endothelium - derived CGRP in phenol - induced hypertensive rats.

Keywords : Endothelial cells ; Calcitonin gene - related peptide ; Hypertension ; Alpha2 - adrenoreceptor

P350041

The role of the C - terminal tail of the human β_2 - adrenoceptor in stimulation of glucose uptake in CHO cells.

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Glucose homeostasis is maintained by insulin , which stimulates glucose uptake in adipose tissue and skeletal muscle. GPCRs increase glucose uptake , utilizing components of the insulin pathway. Thus , activation of β_2 - AR increases glucose uptake in L6 muscle cells by activation of cAMP, PI3K and Gi. The β_2 - AR C - terminal tail contains PKA and GRK phosphorylation sites , which when phosphorylated cause desensitization of the receptor , coupling to Gi , and activation of PI3K and MAPK. In this study we transfected CHO cells with human β_2 - AR (wild type (WT) or truncated at amino acids 344 or 349) and measured glucose uptake and cAMP accumulation. In CHO cells expressing WT β_2 - AR, glucose uptake was increased by isoprenaline , insulin and 8 - bromo - cAMP. In cells expressing truncated receptors (349 or 344) insulin and 8 - bromo - cAMP increased glucose uptake to the same degree as in the wild type β_2 - AR - CHO cells , but isoprenaline - stimulated glucose uptake was significantly reduced , suggesting that C - terminal PKA and GRK phosphorylation sites are important for β_2 - AR stimulated glucose uptake. cAMP responses to forskolin or isoprenaline were not significantly changed between the wild type and truncated receptors.

Keywords : glucose uptake , β_2 - adrenoceptor , signal transduction

Acknowledgments : The authors wish to acknowledge support from NH&MRC grant 236884 and from the the Tage Erlanders Gästprofessur (RJS)

P350042

Pharmacological characterization of novel ligands for CB1 and CB2 cannabinoid receptors

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Novel derivatives of tetrahydrocannabinol were synthesized and evaluated for CB1/ CB2 receptor affinity and activity. Affinities were evaluated by radioligand binding studies using cortical membranes (CB1 receptors) , membranes from cells expressing the hCB2 and [^{3}H] CPP 55 ,940 as the radioligand. [^{35}S] GTPgS binding assays assessed the activity of the ligands. Twelve new agents (DPGs) were tested and selectivity , for both CB1/ CB2 receptors with high , intermediate and low affinities , was established. DPG4 [Ki (nM) 0.26 CB1 ; 0.12 CB2] and DPG5 [Ki (nM) 470 CB1 ; 3.54 CB2) were chosen for further activity studies. DPG4 increased basal [^{35}S] GTPgS binding [EC_{50} 1.65x10^{-6} M] , DPG5 had no effect and WIN55 ,212 - 2 displayed an EC_{50} value of 1.29x10^{-6} M. DPG4 and

DPG5 increased GTPgS activity in hCB2(Sf9cells) membranes [EC_{50} $2.0x10^{-4}$ M and $1.0x10^{-4}$ M, respectively]. WIN55,212-2 displayed an EC_{50} value of $3.0x10^{-4}$ M. Behavioral paradigms support a CB1 agonist nature for DPG-4 for. Structure-activity relationship studies are in progress to assess the selective CB1/CB2 DPG agents with promising therapeutic value.

Key words: cannabinoids, CB1/CB2, GTPgS binding. Supported by a GSRT-EU grant YB60

P350043

Binding characteristics of theinorphine to doned μ-, δ- and κ- opioid receptors stably expressed in CHO cell *

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AIM: we mainly describe the binding of theinorphine to opioid receptors, and its effects on guanosine-5'-O-(3-[^{35}S]thio)triphosphate ([^{35}S] GTPγS) binding mediated by μ-, δ- and κ- opioid receptors. METHODS: CHO cells transfected with opioid receptors were used for receptor binding and [^{35}S] GTPγS binding. And [^{3}H]-naloxone displacement assay in rat brain membrane was also used. RESULTS: The IC_{50} and Ki of theinorphine against the binding of [^{3}H] diprenorphine to receptors were lower than that of morphine but similar to those of buprenorpline. There was no obvious difference among the Ki of theinorpline against the binding of [^{3}H] DHA to receptors. And the maximal stimulation (%) of theinorpline to receptors was lower than that of morphine. The EC_{50} of theinorphine-stimulated [^{35}S] GTPγS binding to receptors was lower than that of morphine. CONCLUSION: Theinorphine exhibited higher affinities for receptors than that of morphine, but showed no selectivity to receptors. In the [^{35}S] GTPγS binding assay, theinorpline displayed higher stimulation efficacy than that of morphine at the same concentration. The order of theinorphine-stimulated [^{35}S] GTPγS binding to receptors was μ> κ> δ

Key words: theinorphine, [^{35}S] GTPγS, [^{3}H]-naloxone, [^{3}H] DHA

P350044

Differential G protein coupling of the relaxin family peptide receptors RXFP1 and RXFP2 is due to differences in the C-terminal tail

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Relaxin is a two-chain hormone, structurally similar to insulin, that mediates pleiotropic effects in various physiological systems. The recent discovery of the human gene 2 (H2) relaxin receptor, RXFP1, (Hsu et al. Science, 295, 671, 2002) and the related insulin-like peptide 3 (INSL3) receptor, RXFP2 (Kumagai et al. JBC, 277, 31283, 2002), identified two G protein-coupled receptors that cause cAMP accumulation. We recently identified differential G-protein coupling of these receptors to cAMP: both receptors can couple to Gs and Go_B, which increase and decrease cAMP accumulation respectively; but only RXFP1 recruits G_{i3} with time to further increase cAMP via a Gβγ-PI3K-PKCζ pathway (Halls et al. Mol Pharmacol, submitted, 2006). This study examined the mechanism of differential G protein coupling using an α-screen cAMP accumulation assay. C-terminal tail truncates were generated for both RXFP1 (tRXFP1-703) and RXFP2 (tRXFP2-712). cAMP accumulation characteristics of tRXFP2-712 did not differ from RXFP2. However, tRXFP1-703 lost the ability to couple to G_{i3} and stimulate the PI3K-PKCζ pathway, instead becoming 'RXFP2-like'. Differences in cAMP accumulation therefore stem from the C-terminal tail, which may contain required phosphorylation sites or G_{i3}-coupling motifs.

Key words: relaxin, G-protein coupled receptor, signal transduction

Acknowledgments: The authors wish to thank Dr John Wade for relaxin peptides and grant support from ARC linkage grant LP0560620

P350045

Agonist-Induced Homodimerization of Angiotensin II Receptor AT_1

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The objective of this study was to understand how AT_1 homodimerizes. Methods: Western blot, confocal microscopy, bimolecular fluorescence complementation (BiFC) and endoplasmic reticulum (ER) trapping were applied to detect the homodimerization in CHO cells expressing AT1. Results: AT1 homodimerized in the absence of angiotensin II (AngII). The magnitude of homodimerization tripled in the presence of AngII but not antagonist Losartan and Candesartan. The homodimerization was independent of G protein activation as detected with inactive mutant D125A-R126L and AngII analog [$Sar^1Ile^4Ile^8$] Ang II. Constitutively active mutant N111G induced no constitutive homodimerization. A Cys mutation at Asp^{74} that prevents AT1 from conformational change failed to generate fluorescence in BiFC assay. Consistently, ER trapping assay denied the possibility that AT1 might homodimerize prior to its plasma membrane delivery. Further studies identified the structural determinants critical for AT_1 homodimerization. Conclusion: The results show that AT_1 homodimerization is dependent on agonist-induced conformational change of the receptor.

Key words: AT_1, dimerization, BiFC;
supported by NIH grant (HL065492) to YHF

P350046

The contribution of ryanodine receptor type 2 to E-C coupling and the regulation of resting tone in urinary bladder myocytes

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In urinary bladder smooth muscle (UBSM) of the mice, ryanodine receptors (RyR) are essential molecules for excitation-contraction (E-C) coupling triggered by a single action potential (Morimura et al., AJP, 2005). Although RyR2 is thought to be general Ca^{2+}-induced Ca^{2+} release (CICR) channel, both RyR2 and 3 are expressed in UBSM. The contribution of RyR2 to E-C coupling and resting tone was examined using UBSMs from RyR2 heterozygous KO mice (*RyR2*$^{+/-}$), in which RyR2 mRNA expression decreased. Other RyR subtypes, Ca^{2+} activated K+ channel, and IP3R mRNA were not changed in $RyR2^{+/-}$. The elevation of $[Ca^{2+}]i$ and BK channel current upon depolarization was smaller in $RyR2^{+/-}$. The force development by direct electrical stimulation was also smaller in $RyR2^{+/-}$. In resting conditions, Ca^{2+} sparks activated BK channels to elicit spontaneous transient outward currents (STOCs). The frequency of STOCs was reduced in $RyR2^{+/-}$. These results suggest that RyR2 play a central role in Ca^{2+} mobilization during E-C coupling and in the regulation of resting tone in UBSMs.

P350048

The Measurement of the Interaction Forces between μ-Opiate Receptor and β-Endophine in the Membranes of Living Cells in Physical Solution*

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ABSTRACT The cloned cells with high expression of μ-opiate receptors obtained by gene transfection (μ-66 cell) were immobilized onto the bottom of 9500J3 AFM fluid cell filled with PBS. The ligand β-endophine was covalently tethered onto the surface of AFM tip. The force spectrum between the tip with β-endorphine and the surface of the cells were recorded. Specific μ-receptor antagonist, naloxone, was used to recognize the specific force peaks in the spectrum. The forces between the μ-opiate receptors and β-endorphine on the tip were obtained by the measurement of the special peaks. The sum adhesion between μ-receptors and β-endophine was 365.9 ± 194.0 pN and that between single receptor-ligand pairs was 33 ± 1 pN. These results show that AFM force spectrum can be successfully used to the studies of receptor-ligand interactions on living cells in physical solutions.

* Supported by National Science Foundation of China, 90406024-7.

P350049

THE DOWN-REGULATION OF PROSTAGLANDIN EP3 RECEPTOR SUBTYPE DURING NEURONAL DIFFERENTIATION

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The expression of COX-1 increases in rat pheochromocytoma (PC12) cells following differentiation by nerve growth factor (NGF). Therefore, the aim of this study was to determine if NGF affected the expression of prostaglandin E_2 (PGE_2) receptor subtypes in PC12 cells. We tested the effects of PGE_2 (EP1-4 agonist), sulprostone (EP3/1 agonist), ONO-DI-004 (EP1 agonist), ONO-AE1-259 (EP2 agonist), ONO-AE-248 (EP3 agonist) and ONO-AE1-329 (EP4 agonist). PC12 cells were culture for 32 h (±50 ng/ml NGF) and [^{3}H]cAMP and [^{3}H]inositol phosphate ([^{3}H]IP) production was assayed in response to 1 μM agonists (±1 μM forskolin). None of the agonists tested affected [^{3}H]IP production. PGE_2 and ONO-AE1-259 increased [^{3}H]cAMP in nondifferentiated cells and ONO-AE1-329 was active in NGF-treated cells ($P<0.05$). PGE_2, sulprostone and ONO-AE-248 inhibited forskolin-stimulated [^{3}H]cAMP in undifferentiated cells ($P<0.01$) and this response was attenuated in NGF-treated cells ($P<0.05$ for PGE_2 and ONO-AE-248). In conclusion, the predominant effect of NGF on PC12 cells is to down-regulate the Gi-coupled EP3 receptor subtype.

This work was supported by a grant from the RGC of the HKSAR (HKUST3/03C).

P350050

Coupling of agonist binding to effector domain activation in metabotropic glutamate-like receptors

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Many membrane receptors are made of a ligand binding domain and an effector domain mediating intracellular signaling. This is the case for the metabotropic glutamate (mGlu)-like G-protein coupled receptors. How ligand binding leads to the active conformation of the effector domain in such receptors is largely unknown. Here we used an evolutionary trace analysis and mutagenesis to identify critical residues involved in the coupling (allosteric communication) between the Venus Flytrap (VFT) ligand binding domain and the heptahelical Gprotein activating domain of the mGlu-like receptors. We show that a conserved interdomain disulfide bridge is required for this allosteric interaction. Taking into account that these receptors are homodimers, this finding provides an important new information explaining how the different conformations of the dimer of VFTs lead to different signaling of such dimeric receptors.

P36. Signal Transduction

P360001

Novel alpha1-adrenergic receptor signaling pathways: regulation of interleukin 6, growth factor and extracellular matrix (ECM) protein expression

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We employed oligonuclectide microarray technology to explore the effects of both short (1h) and long-term (18h) activation of alpha1A-AR on gene expression alterations in rat fibroblasts. Diverse gene expression alterations included genes relating to inflammatory responses, cell growth, cell adhesion, cell cycle and cardiac hypertrophy. The most notable changes included the dramatic up-regulation of gene expression for the proinflammatory cytokine interleukin 6 (IL-6), secreted growth factors, and extracellular matrix (ECM) proteins. RT-PCR studies confirmed that PKC was a critical regulator of alpha1A-AR mediated gene expression alterations and secreted IL-6 and FGF7 also contributed to some of these alterations. Immunochemistry results confirmed the expression change of several ECM genes such as Syndecan 4, CD44 and tenascin C. Our results suggest novel alpha1A-AR signaling pathways that regulate the expression of interleukin 6, growth factors and ECM proteins.

Key words: alpha1-adrenergic receptors, IL-6, growth factors and extracellular matrix protein.

P360002

The mechanism of acetylcholine-induced asynchronous calcium waves and tonic contraction in the porcine tracheal muscle bundle

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In this study, we characterized the mechanism of ACh-induced ACW in intact porcine tracheal muscle bundle. Inhibition of the receptor-operated channels/store-operated channels (ROC/SOC) by SKF96365 abolished the nifedipine-resistant component of ACh-induced ACW and contraction. Blockade of Na^+-Ca^{2+} exchange (NCX) with KB-R7943 or 2',4'-dichlorobenzamil or extracellular Na+ removal also inhibited the nifedipineresistant component of ACh-induced ACW and contraction. Inhibition of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase by cyclopiazonic acid abolished the ongoing ACW. Inhibition of IP3-sensitive receptor by 2-APB or xestospongin C did not affect ACh-induced ACW and contraction. However, caffeine or ryanodine prohibited ACh-induced ACW. Furthermore, procaine or tetracaine prevented the generation and abolished the ongoing ACh-induced ACW and contraction. Collectively, these results indicate that the AChinduced ACW in porcine tracheal muscle are produced by repetitive sarcoplasmic reticulum Ca^{2+} release through ryanodine-sensitive receptor and plasmalemmal Ca^{2+} entry involving the reverse-mode NCX, the ROC/SOC and the L-type VGCC is required to refill the SR to support the ACW.

P360003

Elucidating the Role of Pyridinium bis-Retinoid (A2E) in Retinal Pigment Epithelium (RPE) cell damages

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The lipofuscin fluorophore A2E, a pyridinium bis-retinoid, is known to be an initiator of blue-light-induced apoptosis in retinal pigment epithelial cells (RPE). The aim of this study is to gain insight into the mechanisms which underlie A2E-mediated damage to the RPE. A2E and a spectrum of A2E derivatives with groups carrying specific functionalities were synthesized to provide a better tool for following A2E modification under blue light. In addition, A2E was loaded into RPE cell lines for bio-analytical and biochemistry studies, including assessment of mitogen-activated protein kinase (MAPK) signal transduction changes by Western blot analysis. A2E-like derivatives under blue light irradiation were found to be suitable for bioanalytical research involving mass spectrometry studies. Intracellular signaling (MAPK) in RPE cells following exposure to A2E was detected, indicating the involvement of a MAP-kinase pathway. Investigating A2E-like compound modification under blue light and tracing some of the MAP-kinase intracellular changes enable us to obtain a better understanding of the factors mediating damage and/or taking part in cell rescue in retinal diseases.

P360004

Phosphoinositide 3-kinase/Akt activates nitric oxide synthase II/peroxynitrite at rostral ventrolateral medulla during mevinphos intoxication

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The organophosphate poison mevinphos (Mev) induces cardiovascular toxicity via nitric oxide (NO) produced by NO synthase II (NOS II) in the rostral ventrolateral medulla (RVLM), the origin of sympathetic neurogenic vasomotor tone. We investigated the regulatory role of phosphoinositide 3-kinase (PI3K)/Akt signaling in this process. In Sprague-Dawley rats anesthetized with propofol, microinjection bilaterally of Mev into the RVLM induced an increase (Phase I) followed by a decrease (Phase II) in sympathetic vasomotor tone, alongside a progressive increase in Akt phosphorylation at Thr308 and Ser473, nuclear translocation of phospho-Akt, and NOS II or nitrotyrosine (an experimental marker for peroxynitrite) level in the ventrolateral medulla. Co-microinjection bilaterally of PI3K inhibitors (Wortmannin or LY294002) into the RVLM significantly potentiated and prolonged the increased vasomotor activities during Phase I Mev intoxication, and blunted the augmented expression of phospho-Akt, NOS II or nitrotyrosine in the ventrolateral medulla. We conclude that PI3K/Akt signaling is upstream to NOS II/peroxynitrite expression in the RVLM during Mev intoxication.

Key words: mevinphos, NOS II, PI3K/Akt

P360005

Mechanism of Induction of Pancreatic Acinar Cell Apoptosis by Hydrogen Sulfide

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The present study investigates the mechanism of mouse pancreatic acinar cell apoptosis induced by H_2S in an in vitro system, using isolated pancreatic acini. Treatment of pancreatic acini with 10 microliter NaHS (a donor of H_2S) for 3 hours caused phosphatidylserine externalization as shown by annexin V binding, an indicator of early stage apoptosis. This treatment also resulted in the activation of the caspase cascade and major changes at the mitochondrial level. Caspase 3, 8 and 9 activities were stimulated by H_2S treatment. Inhibition of caspase 3, 8 and 9 significantly attenuated H_2S - induced phosphatidylserine externalization as shown by reduced annexin V staining. The mitochondrial membrane potential was loss in H2S treated acini as evidenced by fluorescence microscopy and quantitative analysis. Furthermore, the treatment of acini with H_2S caused the release of cytochrome C by the mitochondria. These results demonstrate the induction of pancreatic acinar cell apoptosis in vitro by H_2S and the primary role in the mitochondrial pathway of apoptosis in this induction.

P360006

The possible involvement of nitric oxide signaling in glycogenolytic response to glucagon and adrenergic agonists in hepatocyte culture

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In this work, we sought to investigate whether NO is produced during glucagon - or adrenoceptor agonist-induced glycogenolysis in rat hepatocytes in cultures. Isolated rat hepatocyte culture (glycogen rich) was used. NO production (NO_2^-) was assessed under the effect of adrenergic agonists, glucagon, and adrenergic agonist/antagonist pairs, nitric oxide synthase (NOS) inhibitors. (iNOS) mRNA was examined by RT - PCR. Glycogenolysis Glucose and NO_2^- released by glycogen - rich hepatocytes was increased as a result of glucagon, epinephrine or phenyephrine treatments. The increase in glucose and NO_2^- released by epinephrine or phenyephrine was blocked by prazocin pretreatment and by NOS inhibitors aminoguanidine and N - nitro - Larginine methyl ester. iNOS gene expression was upregulated by both glucagon and epinephrine. We conclude that glycogenolysis occur through adrenoceptor or glucagon receptor stimulation signaling cascade may involve NO production downstream of receptor - cAMP pathways in hepatocyte culture.

Key words: Nitric oxide, glycogenolysis
Supported by grant IGA MZ NL/7418 - 3

P360007

Signaling mechanisms of leucine - stimulated DNA synthesis and proliferation in primary cultures of adult rat hepatocytes.

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We investigated the effects of branched - chain amino acids on DNA synthesis and proliferation in primary cultures of adult rat hepatocytes. Isolated hepatocytes were cultured in serum - free Ham's nutrient mixture (F - 10). Of the branched - chain amino acids, only leucine induced hepatocyte DNA synthesis and proliferation in a time - and dose - dependent manner. The addition of valine or isoleucine on its own had no significant effects on the hepatocyte DNA synthesis and proliferation. When combined, isoleucine competitively antagonized leucine-stimulated hepatocyte mitogenesis. U73122, genistein, wortmannin, PD98059 and rapamycin inhibited the ability of leucine to stimulate the hepatocyte DNA synthesis and proliferation, suggesting that phospholipase C, tyrosine kinase, phosphatidylinositol 3 - kinase, MAP kinase, and p70 S6 kinase are involved in leucine signaling. The results suggest that leucine stimulates hepatocyte DNA synthesis and proliferation through a putative leucine receptor to induce tyrosine kinase/MAP kinase activity and other downstream growth - related signal transducers.

P360008

Polypeptide from Chlamys farreri protects HaCaT cells from UVA - induced apoptosis through p38 MAPKs and caspase - 3

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Previous studies have shown that Polypeptide from Chlamys farreri (PCF) is an inhibitor on UVA - induced apoptosis in HaCaT cells. In this study we further investigated whether PCF could protect HaCaT cells from UVA - induced apoptosis by affecting p38 MAPK pathway and activation of caspase - 3. Using DNA fragmentation assay, we found that PCF significantly protected against UVA - induced apoptosis, and p38 inhibitor SB203580 or caspase - 3 inhibitor Ac - DEVD - CHO enhanced the cytoprotective action of PCF. As determined by Western blot analysis, PCF inhibited UVA - induced phosphorylation of p38 MAPKs. UVA-induced activation of caspase - 3 was inhibited by PCF dose - dependently as assayed by flow cytometry. These results indicated that PCF protects HaCaT cells from UVA - induced apoptosis through inhibition of p38 MAPKs and caspase - 3. In addition, SB203580 pretreatment could prevent activation of caspase - 3. Therefore, inhibitory effect of PCF on activation of caspase - 3 may partly attributes to inhibition of p38 MAPKs.

Key words: Polypeptide from Chlamys farreri (PCF); UVA; p38 MAPK; caspase - 3
Acknowledgement: This work was supported by the National Natural Science Foundation of China (No. 30471458)

P360009

Basic Fibroblast Growth Factor Enhances Fibronectin Expression via the PLC - r2/PKCa/c - Src/NFkB Pathway in Osteoblasts

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Fibronectin (Fn) is involved in early stages of bone formation and basic fibroblast growth factor (bFGF) is an important factor regulating osteogenesis. Here we investigated the signaling pathways involved in bFGF - induced NF - kB activation and Fn expression in osteoblasts. The Ca^{2+} chelater (BAPTA - AM), PI - PLC inhibitor (U73122), PKC inhibitor (GF109203X), Src inhibitor (PP2) or NF - kB inhibitor (PDTC) attenuated the bFGF-induced Fn expression. bFGF - induced increase in Fn - luciferase activity was inhibited by cells transfected with the kB binding site deleted Fn construct. Stimulation of cells with bFGF activated IKKa/b activity, IkBa phosphorylation, IkBa degradation, p65 and p50 translocation from the cytosol to the nucleus, the formation of an NF - kB - specific DNA - protein complex, and kB - luciferase activity. bFGF - mediated increase in IKKa/b activity and DNA - binding activity was inhibited by U73122, GF109203X or PP2 and dominant negative mutants of PLCr2, PKCa and c - Src. Our results suggest that bFGF increased Fn expression in rat osteoblasts via the PLCr2/PKCa/c - Src/NF - kB signaling pathway.

Key words: bFGF; Fibronectin; Osteoblast; NFkB
Acknowledgement: This work was supported by grants from NSC

P360010

Study the expression of BRCA1 protein and the mechanism of silence in the sporadic breast carcinoma

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Purpose: To study the mechanism of BRCA1 gene silence and the role of BRCA1 gene in the carcinogenesis of sporadic breast carcinoma. Methods: using immunohistochemistry (IHC) and methylation specific PCR (MSP) assay the expression and methylation status of BRCA1 gene. Detect copy number of BRCA1 and CEP17/Cell by fluorescence in situ hybridization. Results: The rate of loss expression of BRCA1 protein was 39.62 % (42/106) in sporadic breast cancer. 15 cases in 106 sporadic breast cancer patients (14.15 %) were detected hypermethylation of BRCA1 gene, and all of them low express BRCA1 protein. The Mean copy number of BRCA1 in methylated cases (mean = 1.19) was lower than in unmethylated cases (mean = 1.96) ($P < 0.01$). The mean copy number of CEP17 in the methylated cases (mean = 2.16) was also lower than in the unmethylated cases (mean = 2.91) ($P < 0.01$). The BRCA1/CEP17 ratio in the methylated cases (mean = 0.55) was slightly lower than in the unmethylated cases (mean = 0.68). Conclusion: Hypermethylation and loss copy relate to silence of the BRCA1 gene in human sporadic breast cancer, and BRCA1 gene relates to carcinogenesis of sporadic breast carcinoma.

key words: BRCA1; breast cacinoma; MSP; FISH

P360011

Involvement of the signal - transducing function of Na+, K+ - ATPase in the mechanism of the positive inotropic effect of cardiac glycosides

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Aim To investigate whether the signal - transducing function of Na^+, K^+ - AT-

Pase involves in the mechanism of the positive inotropic effect of cardiac glycosides. Methods The chronic congestive heart failure model was produced in guinea pig by a procedure that descenting aorta was constricted. Left ventricular myocytes were enzymatically isolated. The cardiac myocytes from both normal and failure hearts were preincubated by PD98059 (MAPK inhibitor), genistein and PP2 (Src inhibitors) respectively, then the contractile and calcium transient of a single myocyte induced by strophanthidin (Str, 25 μm) were assessed simultaneously. Results The increases of contractile and calcium transient induced by Str of normal or failure cardiac myocyte were decreased through preincubating with genistein, PP2 and PD98059. Conclusions The signal-transducing function of Na^+, K^+-ATPase involves in the positive inotropic effect of cardiac glycosides in normal and failure heart.

Key words: Chronic heart failure; Contractile; Calcium transient; Strophanidin

P360012

Sphingolipids regulate cytosolic phospholipase A2 (cPLA2) activity

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cPLA2 hydrolyzes membrane glycerophospholipids containing arachidonic acid (AA) and has a pivotal role in the initiation of inflammatory responses because its activation is the rate-limiting step of eicosanoid biosynthesis. We previously reported that sphingosine inhibited cPLA2 activation by suppressing translocation of this enzyme from the cytosol to the membrane. Thus, shingolipids may be key modulators of cPLA2 activity. We, therefore, examined the effect of ceramide on AA release. Pretreatment with C2 ceramide decreased platelet-activating factor (PAF)-induced AA release, whereas increased A23187-induced AA release in CHO cells. Pretreatment with C2 ceramide also decreased lysophosphatidic acid (LPA)-induced AA release. C6 and C8 ceramides have similar effects as well as C2 ceramide. PAF and LPA receptors are Gq type of G protein coupled receptors. Therefore it is possible that ceramides may block cPLA2 activity located downstream of Gq signaling. In combination with our previous study, these results suggest that sphingolipid metabolism has a key role in the regulation of cPLA2 and is a potential target for new therapeutics for inflammatory diseases. (Key words; cPLA2, ceramide, sphingosine)

P360013

Polypeptides from Chlamys farreri protect HaCaT cell from apoptosis by JNK signaling pathway

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Objective: To study the protection of polypeptides from Chlamys farreri (PCF) on HaCaT cells damaged by UVA + UVB in vitro. Methods: Apoptosis rates of HaCaT cells and the activation of caspases were measured by flow cytometry. Western Blot analysis was performed to investigate the phosphorylation of JNK. Results: UVA + UVB irradiation can induce HaCaT cells to apoptosis, and the decrease in apoptosis was observed in UVA + UVB irradiated HaCaT cells treated with PCF previously. JNK was persistently activated by dual specific phosphorylation in apoptotic HaCaT cells. The caspase inhibitor zVAD can block the apoptosis, but not the phosphorylation of JNK. PCF can decrease both the phosphorylation of JNK and the activation of caspase3, 8 and 9. Conclusions: UVA + UVB irradiation can induce the activation of JNK, which further cause activation of the caspases cascade via possible apoptotic pathways and lead to apoptosis of HaCaT cells. PCF protected HaCaT cells apoptosis damage by UVA + UVB via interfering with JNK signaling pathway.

Key words: JNK; UV; HaCaT; apoptosis

Acknowledgement: This work was funded by the National science Natural Foundation of China (No. 39970638).

P360014

Amphetamine modulates the spontaneously generated action potentials in central snail neurons

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Effects of amphetamine (Amp) on the spontaneously generated action potential were studied electrophysiologically in the right parietal ganglia RP 1 and 4 neurons of the African snail, Achatina fulica Ferussac. Bursting firing of action potentials (BoP) were reversibly observed after extra-cellular application of dorl-Amp or intraneuronal injection of Amp. Ratiometric confocal Ca^{2+} measurements revealed that intracellular calcium content was increased in Amp treated neuron. The BoP was decreased after intracellular injection of high magnesium ion or EGTA or extracellular application of KT-5720, H89 (protein kinase A inhibitor). Two electrodes voltage clamped studies revealed that amphetamine decreased the fast Na^+, Ca^{2+} and IA currents of the neuron. It also decreased the steady-state K^+ current and elicited a negative slope resistance (NSR) in the steady-state I-V curve. Forskolin (adenylyl cyclase activator) and vinpocetine, EHNA, milrinone, rolipram or caffeine (phosphodiesterase inhibitors) did, while sildenafil (viagra) did not facilitate the BoP. It is concluded that Amp elicited BoP through intracellular calcium ion, potassium channels and cyclic AMP messenger system

P360015

CHX Inhibits Apoptosis Induced by Itself through the PI3K/Akt Pathway in U937 Cell Line

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Cycloheximide (CHX) is a typical protein synthesis inhibitor of eukaryotes. It has been already used in curing cancer. CHX has two main effects on cell apoptosis: one is to trigger cell apoptosis with the selection of cell types, the other is to promote or inhibit cell apoptosis induced by various stimuli. In U937 cell line, CHX can induce cell apoptosis, but this inducing process can be self-resisted. This kind of resistance is highly related to cell adhesion. Further studies suggest that PI3K may participate in the downstream regulation of this effect; since the PI3K inhibitor wortmannin and LY294002 can sharply increase the proportion of cell apoptosis while the PKC inhibitor GF109203X has little impact on CHX induced cell apoptosis. PI3K pathway is a typical cell survival pathway. It can pass cell survival signals and results in cell living. The downstream pathway of PI3K is mainly processed by PKB/Akt. CHX may perform the drug-resistance mentioned above by activating this pathway.

Key words: Cycloheximide, Apoptosis, Self-resistance, PI3K

P360016

Stimulation of ionotropic GABAc receptors leading to PKA activation

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Previously we showed cyto-protective effects of GABAc receptor stimulation involving PKA activation. Further study showed a complex composed of GABAc receptor rho subunit, AKAP220 and PKA. Presently, we investigate how signaling is transduced and whether AKAP220 is essential for this signal from GABAc receptor to PKA. Stimulation of GABAc receptor with its agonist, CACA, increased phosphorylation level of a PKA substrate at about 135 kDa detected by Western blotting in primary cultured rat hippocampal neurons. This increase in phosphorylation of PKA substrate was suppressed by pre-incubation with GABAc receptor antagonist, TPMPA, as well as PKA inhibitor, H89 or KT5720. Pre-treatment of cells with antisense oligonucleotides against AKAP220 showed decreased AKAP220 protein expression simultaneously with a less phosphorylated PKA substrates responding to CACA stimulation. These data suggest that stimulation of GABAc receptors with CACA activates PKA, which may be mediated by AKAP220. Thus, cyto-protective effects of GABAc receptor stimulation appeared to be mediated by activation of scaffold protein (AKAP220)-associated PKA, probably resulting in phosphorylation of cyto-protective molecules.

P360017

Arrestin serves as a molecular switch, linking endogenous alpha2-adrenergic receptor to Src-dependent but not Src-independent ERK activation

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In this paper we address whether arrestin plays a role in determining the route of alpha2AR-evoked ERK signaling activation, taking advantage of endogenous expression of the alpha2AAR subtype in mouse embryonic fibroblasts (MEFs) and the availability of MEFs without arrestin expression (derived from Arr2,3-/- mice). Our data demonstrate that endogenous alpha2AAR evokes ERK phosphorylation through Src-dependent and Src-independent pathway, both of which

are G protein - dependent and converge to the Ras - Raf - MEK pathway. Arrestin is essential to recruit Src to this process , as Src is not required in alpha2 AAR- mediated ERK signaling in Arr2 ,3 - / - MEFs. Although alpha2 - agonists have similar potencies in stimulating Src - dependent and - independent ERK phosphorylation in WT and Arr2 ,3 - / - cells , respectively , the Src - independent alpha2 AAR mediated ERK activation has a longer duration and phospho - ERK is more rapidly translocated into nuclei when compared to Src - dependent activation. These data not only affirm the role of arrestin as an escort for signaling molecules such as Src family kinase , but also demonstrates the impact of this modulation on both the temporal and spatial properties of ERK activation.

P360018

Phenylarsine oxide inhibited the isoproterenol - induced interleukin - 6 production by attenuation of cAMP accumulation and CREB phosphorylation

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To explore the possible substrates of the Gs/ cAMP, we investigated whether tyrosine phosphatase was implicated in the ISO- induced IL - 6 stimulation in CFs. Surprisingly , phenylarsine oxide (PAO) , a tyrosine phosphatase inhibitor , dramatically repressed the IL - 6 production by ISO in a dose dependent manner. Since the cAMP , CREB and p38 are essential pathways for IL - 6 production , we determined whether PAO affected these signalling components and found that PAO significantly inhibited the CREB phosphorylation but not p38 MAPK. PAO also dose - dependently inhibited the increased cAMP accumulation by ISO or forscorlin. Moreover , Pretreatment with tyrosine kinase inhibitor , genistein further elevated CREB phosphorylation and IL - 6 production by ISO. In conclusion , inhibition of tyrosine phosphatase repressed the induction of IL - 6 production in response to b2 - adrenergic receptors activation by affecting GS/ cAMP/ CREB pathways but not the p38 MAPK in cardiac fibroblasts.

This work was supported by grants from the National Science Foundation of China (30470691) and the National Science Foundation of Beijing (7042033).

Key words : phenylarsine oxide , IL - 6 , cAMP, CREB

P360019

IL - 6 Mediates b2 - AR- induced STAT3 Activation and its Signaling Pathway in Mouse Heart

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This study was aimed to determine whether β- adrenoceptors (β- AR) activate STAT3 and to examine the underlying mechanism in mouse heart. We recently reported that b2 - AR stimulation leads to a delayed STAT3 activation via an IL - 6 family of cytokines - mediated pathway. Surprisingly , the effect of cAMP was independent of protein kinase A and the Epac (exchange protein directly activated by cAMP) - Rap1 pathway. p38 MAPK inhibitor SB203580 abrogated isoproterenol - induced IL - 6 release in cardiac fibroblasts. p38 MAPK could be positively regulated by Gs - AC - cAMP but negatively regulated by Gi - PI3K pathway. Multiple transcription factors (AP - 1 , C/ EBP, NF - κB and CREB) regulating the IL - 6 gene are activated in response to isoproterenol stimulation , which may provide essential linkage between upstream cAMP - p38 MAPK signaling cascade and downstream IL - 6 gene transcription. The present results suggest that β2 - AR mediates IL - 6 production through a noncanonical cAMP responsible pathway and p38 MAPK.

Key words : adrenoceptor , STAT3 , heart , mouse

This work was supported by the Foundation of China(30470691) and(7042033).

P360020

Effects of nitric oxide inhibitors on resuscitation following induction of head injury and hemorrhagic shock.

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We compared the effects of both a selective inducible nitric oxide synthase (iNOS) inhibitor & nonselective inhibitor on posttraumatic recovery and neuron survival by using a combined model of lateral fluid percussion injury (FPI) and hemorrhagic shock (HS). Male SD rats underwent FPI to the brain (3.5 atm) and hemorrhage to a mean arterial blood pressure (MABP) of 40 mm Hg for 1hr. Rats were then resuscitated during 1hr with bolus infusions of aminoguanidine (AG) or L - NAME. Neuronal apoptosis was determined by performing Nissl staining and in situ terminal deoxynucleotidyl transferase - mediated deoxyuridine triphosphate nick - end labeling technique. Rats infused with AG showed significant increase in survival time and cerebral tissue perfusion , although the MABP and nitrate/ nitrite levels did not significantly change compared with those in L - NAME treated rats even though both animal groups had been subjected to combined FPI and HS , FPI alone , or HS alone. Furthermore , infusion of AG also significantly decreased the number of apoptotic neurons when compared with the number in rats treated with L - NAME. This suggested that treatment with AG, might contribute to improved survival following FPI & HS.

P360021

The G- protein Selectivity of D2 - Like Dopamine Receptors

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The D2 and D3 dopamine receptors have high sequence homology but whereas D2 is thought to couple to Gαi1 , 2 , 3 , oA , D3 has been shown only to weakly couple to Gαi/ o. The objective of this study was to characterize the coupling of both D2 and D3 and investigate the structural basis of this selectivity. Two strategies were used to control the expression levels of receptor and G- protein : 1) Receptor/ G- protein fusion proteins. 2) Double stable cell lines constitutively expressing dopamine receptor and inducibly expressing G- protein αsubunit. To investigate the structural basis of G- protein selectivity a chimera was made where a 12 amino acid section from the C- terminal of intracellular loop 3 of D2 was exchanged with an equivalent region of D3. D2 had a higher affinity (Kd ~ 0. 02 nM) for 3H Spiperone than D3 and the D3/ 2 chimera (Kd 0. 1nM). Using [35S] GTPγS binding upon addition of dopamine , D2 showed coupling to all four αsubunits. D3 showed significant coupling only to Gαoa1. The chimeric D3/ 2 receptor gains D2 - like promiscuous coupling to Gαi1 , 2 , 3 and Gαoa1 , indicating that the 12 amino acid section of IC loop 3 is important for G - protein coupling.

P360022

Compound of Astragalus Extract shows anti - fibrotic effects by blocking TGF - β signaling in chronically injured livers and myofibroblast cells

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Aims : To study the effects of Compound of Astragalus Extract (CAE) on transforming growth factor - β(TGF - β) signal and try to elucidate the molecular mechanisms by which CAE can block liver fibrosis in cultural hepatic stellate cells. Methods : Protein phosphorylation and expression were analyses by immunopricipitation and western blot. The PAI - 1 transcription activity was analyses by transfection p3TP - Lux promoter and measure relative luciferase activity. Rat liver fibrosis was generated by CCl_4 and protein phosphorylation and expression in hepatocytes and mesenchymal cells were analyses by immunohistochemistry. Results : CAE inhibited TGF - β- mediated phosphorylation Smad2 , Smad3 and JNK, the complex formation of Smad4 with Smad2/ 3 and the PAI - 1 transcription activity in cultural myofibroblast - like cells. CAE inhibited Smad2 phosphorylation of α- SMA immunoreactive mesenchymal cells surrounding centrilobular areas in rat liver after chronic treatment with CCl_4. Conclusions : These results suggested that CAE exerts anti - fibrotic effects by inhibiting TGF - β signaling in chronically injured livers and myofibroblast - like cells.

Key words : Traditional Chinese medicine ; TGF - β; Smad ; Liver fibrosis

P360023

The Signaling Transduction of Integrin alpha2beta1 Agonist , Aggretin in Vascular Smooth Musde Cell and Its Crosstalk to PDGF Beta Receptor

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Aggretin , a heterodimeric platelet aggregation inducer purified from Calloselasma rhodostoma venom, was identified as a collagen - like integrin alpha2beta1 agonist. In these studies we explore the receptor and signal transduction involved in aggretin - stimulated migration and proliferation of VSMCs. Aggretin significantly increased VSMCs proliferation as determined by tetrazolium assay. Moreover , VSMCs migration toward immobilized aggretin was increased in a modified Boy-

den chamber. Incubation of VSMCs with aggretin stimulated the phosphorylation of phosphatidylinositol 3-kinase (PI3K), Akt and extracellular-regulated kinase (ERK) in a time-dependent manner. In a similar fashion, aggretin also induced phosphorylation of eNOS and PDGF beta receptor. Focal adhesion kinase (FAK) was phosphorylated within the first 5 min. The Enos activating related signaling is also elucidated. In conclusion, aggretin activates FAK, PI3K/Akt, ERK, eNOS and PDGF pathways leading to promoting of migration and proliferation of VSMCs.

P360024

The contributory role of adrenaline in colon cancer growth

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Recent evidences suggested that stimulation of beta-adrenoceptors is related to the growth of different kinds of cancers, including colon cancer. It has been demonstrated that both beta-1 and beta-2 adrenoceptors are constitutively expressed in HT-29 colon cancer cells. In the present study, it was found that HT-29 colon cancer cells produced adrenaline. The expressions of the catecholamine-synthesizing enzymes were revealed by reverse transcription-polymerase chain reaction. The inhibition of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, reduced adrenaline release along with the concomitant inhibition of cell proliferation in HT-29 cells. Moreover, nicotine, a component of tobacco smoke, stimulated cell proliferation and adrenaline production in HT-29 cells via the upregulation of the catecholamine-synthesizing enzymes expressions. These data provide strong evidences that adrenaline plays a contributory role in colon cancer cell growth and partly elucidate the carcinogenic action of cigarette smoke.

P360025

Cathelicidin: a molecule for antimicrobial or for ulcer healing in the stomach

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Objective: We investigated whether cathelicidin could contribute to gastric ulcer healing. Methods: Gastric ulcers were induced in rats and the expression of cathelicidin was determined by RT-PCR and Western blot. Overexpression of cathelicidicidin was achieved by plasmid transfection. Proliferative cells and microvessels in gastric tissue were measured. The direct action of cathelicidin on cell proliferation and its signaling pathway in cultured gastric epithelial cells (RGM-1) were determined. Results: Ulcer induction increased cathelicidin expression in the gastric mucosa. Overexpressing this peptide promoted ulcer healing by increasing cell proliferation and angiogenesis. Cathelicidin directly stimulated RGM-1 cell proliferation through a MMP-, EGFR-, and MEK-dependent pathway. TGF alpha knockdown nullified the mitogenic signals evoked by cathelicidin. Conclusion: Cathelicidin exhibits ulcer healing activity through TGF alpha-dependent transactivation of EGFR to induce proliferation of gastric epithelial cells.

Key words: Cathelicidin, gastric ulcer, proliferation, EGFR

Grant Support: CRCG grant from the University of Hong Kong and the CERG grant from Hong Kong Research Grants Council

P360027

The human cathelicidin LL-37 suppresses gastric cancer growth through transforming growth factor beta mediated pathway

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Objective: We aim to determine whether the human antimicrobial peptide LL-37 functions as a tumor suppressor by inhibiting gastric cancer growth through a defined signaling pathway. Methods: Cell proliferation and cell cycle distribution were determined by [3H]-thymidine incorporation and flow cytometry, respectively. Gene expression of transforming growth factor beta1 (TGF beta1), Smad7, p15 and p21 were measured by quantitative real-time PCR. Results: LL-37, at concentrations that can be found during inflammation or infection, suppressed the proliferation of three gastric cancer cell lines, namely, AGS, MKN-45, and TMK-1. LL-37 also induced G0/G1-phase cell cycle arrest in TMK-1 cells, accompanied by upregulation of TGF beta1, Smad7, and p21 but not p15 mRNAs. Neutralizing antibodies to TGF beta partially abrogated the antimitogenic action of LL-37. Conclusion: The human cathelicidin LL-37 suppresses gastric cancer growth through the activation of TGF beta-mediated pathway.

Keywords: gastric cancer; cathelicidin; transforming growth factor beta; proliferation

Acknowledgement: This work is partially supported by The Hong Kong Research Grants Council.

P360029

A novel antiarrhythmic target: M3R/IKM3

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This study was designed to explore the possible role of M3 subtype of acetylcholine muscarinic receptors (M3-mAChR) in cytoprotection of myocardial infarction. Studies were performed in a rat model of myocardial infarction and in isolated myocytes. We found that choline diminished ventricular arrhythmias during ischemia, which was achieved by correcting hemodynamic impairment, and protecting cardiomyocytes from apoptotic death. The beneficial effects of choline were reversed by the M3-selective antagonists but not by the M2-selective antagonist. Choline/M3-mAChR activated several survival signaling molecules (antiapoptotic proteins Bcl-2 and ERKs), increased endogenous antioxidant reserve (SOD), and reduced apoptotic mediators (proapoptotic proteins Fas and p38 MAPK) and intracellular Ca^{2+} overload. In addition, we also found that administration of choline attenuated the ischemia-induced suppression of the association between connexin 43 and M3-mAChR. We concluded that choline reduced ischemic arrhythmias via stimulating the cardiac M3-mAChRs which in turn result in alterations of multiple signaling pathways.

Key words: acetylcholine muscarinic receptors; arrhythmia; choline; signaling pathways.

P360030

Involvement of DDAH/ADMA/NOS pathway in nicotine-induced endothelial dysfunction

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Objective: To determine the involvement of dimethylarginine dimethylaminohydrolase (DDAH)/asymmetric dimethylarginine (ADMA)/nitric oxide synthase (NOS) pathway in nicotine-induced endothelial dysfunction. Methods: Thirty-nine healthy subjects, including 18 smokers and 21 nonsmokers, were recruited. Male SD rats were oral treated with nicotine (5 mg/kg/day) for 4 weeks. Human umbilical vein endothelial cells (HUVECs) were incubated with nicotine (10 μM) for 48 h. Results: The smokers had higher plasma levels of ADMA and von Willebrand factor than the nonsmokers. The level of ADMA was markedly increased in the nicotine-treated rats associated with a decrease in endothelium-dependent vasodilatation. Nicotine caused a marked increase in the level of ADMA in HUVECs. Nicotine markedly downregulated both mRNA and protein levels of DDAH-II as well as DDAH activity in endothelial cells. The antagonists of α7 nicotinic acetylcholine receptor (α7 nAChR) blocked these effects of nicotine mentioned above. Conclusion: Nicotine modulates DDAH/ADMA/NOS pathway of endothelial cell via activation of α7 nAChR, which may be involved in endothelial dysfunction associated to smoking.

Key words: Asymmetric dimethylarginine; Nicotine; Endothelial dysfunction

P360031

Aldosterone-stimulated inflammatory and profibrotic responses mediated by p38 MAPK-NF-kappaB or ERK-Sp1 signal pathway in rat vascular smooth musde cells

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Aldostrone (Ald) plays an important role in regulation of inflammation and fibrosis in cardiovascular system but the mechanism remains unknown. Using molecular and biochemical methods, we investigated regulatory effects of Ald on the expression of Cox-2 and IL-6, two important inflammatory factors, and TGF be-

ta1, a critical pro - fibrosis factor, in rat vascular smooth muscle cells (VSMCs). We found that Ald significantly increased expression of Cox - 2 and IL - 6 by 2 - to 10 - fold, respectively. Ald increased phosphorylation of p38 MAPK (p38) and NF - kappaB by 3 - and 6 - fold; while p38 inhibitor SB203580 markedly inhibited Ald - stimulated expression of Cox - 2 and IL - 6. NF - kappaB inhibitor TLCK markedly attenuated expression of Cox - 2 but not IL - 6. Also, Ald strongly induced expression of TGFbeta1. Enhanced TGFbeta1 by Ald might relate to activation of ERK - SP1 signaling pathway since PD98059, an ERK1/2 inhibitor, significantly blocked phosphorylation of ERK1/2 and function of Sp1, leading to reduced expression of TGFbeta1. These results suggest that the Ald - induced inflammatory responses and fibrosis response may be mediated by p38 - NF - kappaB pathway and ERK - Sp1 pathway in VSMCs, respectively.

Key Words aldosterone, COX - 2, IL - 6, TGFbeta1

P360032

Cydic ADP - ribose mediates calcium signaling for chemoattractants in human neutrophils

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Cyclic ADP - ribose (cADPR) derived from NAD is identified as a novel Ca^{2+} mobilizing agent which release Ca^{2+} through an IP3 - insensitive, ryanodine receptor related mechanism in many tissues. Although an increase in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) is a key signal for neutrophil functions, the mechanisms for regulation of $[Ca^{2+}]i$ is unclear. The present study examined the regulation by cADPR of chemoattractant-induced changes of Ca^{2+} dynamics in human neutrophils. cADPR induced Ca^{2+} release from digitonin - permeabilized neutrophils and the release was blocked by 8Brc ADPR, an antagonist of cADPR and FK506 and rapamycin, immunophilin ligands. In intact neutrophils, fMLP induced a transient rise of $[Ca^{2+}]i$ in the absence of extracellular Ca^{2+} and a initial rapid rise of $[Ca^{2+}]i$ and the following sustained rise in the presence of Ca^{2+} in the medium. 8Br - cADPR, FK506 and rapamycin reduced fMLP - and platelet - activating factor induced $[Ca^{2+}]i$ rise. FK506 and rapamycin caused graduate increase in $[Ca^{2+}]i$ rise. These results suggest that cADPR mediates chemoattractants - induced mobilization of Ca^{2+} by FK506 - binding protein - dependent process in human neutrophils.

P360033

Role for CD38 in cydic ADP - ribose - mediated calcium signaling in human neutrophils

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Although it is suggested that cADPR may be a mediator of chemoattractants - induced increase in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) in human neutrophils, signaling pathway for $[Ca^{2+}]i$ rise in response to chemoattractants stimulation is unclear. CD38, the best - characterized mammalian ADP - ribosyl cyclase, is postulated to be an important source of cADPR in vivo. The present study examined whether CD38 may participates in the synthesis of cADPR in human neutrophils and extracellularly formed cADPR is transported into cells to stimulate Ca^{2+} release. When NAD a substrate of ADP - ribosyl cyclase, and cADPR were added into the medium, the former increased $[Ca^{2+}]i$ and the latter potentiated fMLP - induced $[Ca^{2+}]i$ rise. fMLP - , platelet - activating factor - and NAD induced $[Ca^{2+}]i$ rise were reduced by 8Br - cADPR, anti - CD38 antibody, FK506 and several nucleoside transporter (NT) inhibitors. mRNA of ENT1, ENT2, CNT2, CNT3 are expressed in neutrophils. These results suggest that cADPR synthesized extracellularly by CD38 transported into the cells through NTs and mobilize Ca^{2+} by FK506 - binding protein - dependent process. This process may be involved in chemoattractant-induced Ca^{2+} signaling in neutrophils.

P360034

Biphasic effect of β2 adrenergic - receptor agonist on extracellular signal - regulated kinase 1/2 phosphorylation in neonatal rat cardiomyocytes

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We investigated the effect of β2 AR agonist clenbuterol on ERK1/2 phosphorylation in neonatal rat cardiomyocytes. Addition of clenbuterol evoked a dose - dependent biphasic effect comprising an initial positive effect peaking at 2 min, followed by a sustained negative effect leading to 40 % decreases in basal phosphorylation of ERK1/2 after 30 min. β2 AR antagonist ICI 118551, PTX, Ca^{2+} chelator BAPTA - AM and ryanodine receptor (RyR) antagonist ruthenium red significantly inhibited the positive effect and nifedipine slightly inhibited it. The extracellular Ca^{2+} did not affect the positive effect but RyR agonist ryanodine enhanced it. Thapsigargin and Rp - cAMP attenuated the negative effect; protein phosphatase 2A (PP2A) inhibitor okadaic acid reversed it in Ca^{2+} dependent manner. Clenbuterol had a sustained positive effect on cAMP accumulation and phospholamban (PLB) phosphorylation. These data indicate that the positive effect of clenbuterol is via Gi signaling pathway and is involved with the release of Ca^{2+} from sarcoplasmic reticulum (SR) Ca^{2+} store. Clenbuterol negatively regulates ERK1/2 through PP2A and restore Ca^{2+} into SR via cAMP dependent PLB phosphorylation.

Key words: β2 adrenoceptor, MAPK

P360035

Bay K 8644 reveals a novel regulatory effect of Bcl2 over L - type Ca^{2+} channels in PC12

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It is believed that the effect of Bcl2 is linked to its ability to form ion pores and regulate Ca^{2+} fluxes in intracellular organelles. We investigated the regulatory effect of Bcl2 on the kinetics of Ca^{2+}, focused on mitochondria, an organelle playing a central role in apoptosis, in PC12 cells: control and stably overexpressing Bcl2. Ca^{2+} was monitored using aequorins targeted to the cytosol or mitochondria. Our experiments point to the L - type Ca^{2+} channel as a new target for Bcl2, based on the following evidences: (i) the $[Ca^{2+}]c$ and $[Ca^{2+}]m$ elevations elicited by K+ depolarizing pulses were drastically depressed in Bcl2 cells; (ii) in digitonin permeabilized cells the mitochondrial Ca^{2+} entry through the uniporter was enhanced 3 - fold in Bcl2 cells; (iii) the L - type voltage - activated Ca^{2+} channel Bay K 8644 enhanced K^+ - evoked $[Ca^{2+}]m$ peak 4 - fold in Bcl2 cells and only 2 - fold in control cells; (iv) the protonophore FCCP elevated the K^+ - evoked $[Ca^{2+}]c$ peak in control cells, but not in Bcl2 cells.

Key words: Bcl2; calcium; L - type Ca^{2+}; PC12.

Acknowledgement: (1) FIS 041665; La Caixa BIN05 - 32 - 0; Grupos Emergentes UAM and Ramon y Cajal Programme, to MFCA.

P360036

Effects of lysophosphatidic acid antagonists on mitogenic responses in human breast cancer cell lines

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Lysophosphatidic acid (LPA) refers to a family of phospholipid mediators that bind to G - protein - coupled receptors (LPA1, LPA2, LPA3). This study evaluated the role of LPA in human breast cancer cells. Specifically, the effects of LPA antagonists on pro - mitogenic actions of epidermal growth factor (EGF) were investigated. Two human breast cancer cell lines were used, MCF - 7 and MDA - MB - 231. Both cell lines express mRNA for LPA1, LPA2, and LPA3. At 10 mM, 18 : 1 LPA enhances both directed (chemotactic) and random (chemokinetic) migration of MDA - MB - 231 cells. Both MCF - 7 and MDA - MB - 231 cells generate LPA; LPA levels in medium are increased by exogenous 18 : 1 LPA and by EGF. MCF - 7 and MDA - MB - 231 cells proliferate in response to EGF and LPA. LPA and EGF also stimulate activation of Erk and Akt kinases in both cell lines. LPA - induced activations of Erk and Akt kinases, as well as proliferation, are inhibited by Ki16425 and VPC32183, antagonists for LPA1/LPA3. Ki16425 and VPC32183 also inhibit EGF - induced activation of Akt (MDA - MB - 231 and MCF - 7) and Erk (MCF - 7). These studies suggest a potential role for LPA as an autocrine mediator of mitogenic signaling in breast cancer cells.

(Supported by DAMD17 - 01 - 1 - 0730)

P360037

The Involvement of Signaling Pathways in Vasopressin - induced Contraction in Mouse Penis

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Arginine vasopressin (AVP) is a peptide hormone implicated in the pathogenesis of diseases. It is a potent vasoconstrictor in the penis. The aim of the study is to investigate the involvement of different signaling pathways in AVP - induced contraction of mouse penis. AVP (10^{-11} - 10^{-7} M) induced contraction was reduced by two non - selective AVP receptor inhibitors. Western blot analysis results showed that V1 but no V2 receptor was expressed in the penis. A Rho - kinase inhibitor Y - 27632 (10^{-5} M) significantly reduced the maximum AVP-induced contractions from 59 ±10 % of KCl - induced maximum contraction to 29 ±9 % ($p < 0.01$). L - type Ca^{2+} channel blocker nifedipine (10^{-6} M) decreased AVP - induced maximum contraction by 50 %. Tyrosine kinase inhibitor genistein ($3x10^{-5}$ M) increased E50 more than 3 fold. Protein kinase C and phosphatidylinositol - 3 - kinase inhibitors had no effects on AVP - induced contraction. In conclusion, our study is the first to characterize the signaling pathways involved in AVP - induced contraction in penis. Given the powerful vasoconstrictive effect of AVP, therapies targeting on the abnormal AVP signaling may provide a new treatment for erectile dysfunction.

P360038

Heat Shock Protein - 90 Increases the Functions of Oxidative Stress - induced ERK1/2 in Rat Vascular Smooth Muscle Cells

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Aim To investigate the roles of Hsp90 in activation and nuclear translocation of ERK1/2 stimulated by oxidative stress in rat vascular smooth muscle cells (VSMC). Methods Cultured VSMCs were challenged to LY83583, a generator of reactive oxygen species, for 120 min. Western blot and immunoprecipitaion were used to analyze expression and interaction of protein. Immunofluorescence analysis was used to evaluate protein localization. Results VSMC exposure to LY83583 (1μM) for 120 min resulted in a significant increase of total, soluble and nuclear phosphor - ERK1/2, which was accompanied by a increase in Hsp90 expression. Immunoprecipitation experiment of anti - Hsp90 antibody followed by an immunoblot with anti - phosphor - ERK1/2 antibody showed that Hsp90 bound with phosphor - ERK1/2. Pretreatment of Geldanamycin (5μM), a specific inhibitor of Hsp90, attenuated LY83583 - induced phosphorylation, solubility and nuclear translocation of ERK1/2. Conclusion Hsp90 increases ERK1/2 function via facilitating solubility and nuclear translocation phosphor - ERK1/2 in responses to oxidative stress.

Key words: Heat shock protein 90; ERK1/2; oxidative stress; VSMC.

P360039

A crucial role for MIF in regulation of vulnerable - plaque function by activating MEK - ERK MAP kinase pathway

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OBJECTIVE: Our previous studies show that macrophage migration inhibitory factor (MIF) is highly expressed in atherosclerotic lesions. The aim of this study is to investigate the signaling mechanism by which MIF induces matrix metalloproteinases(MMPs) expression. METHODS: A mouse macrophage cell line (RAW264.7) was used. The adenoviral dominant - negative (DN) or wild - type (WT) vectors were constructed. RNA and protein were detected by real - time PCR and Western - blotting. The secreted MMP - 9 in the medium was analysed by zymographic analysis technology. RESULTS: The results showed that MIF was able to increase MMPs activity in a dose - dependent manner, and to activate ERK1/2, but not p38 and JNK MAP kinase in macrophages. MIF - induced MMPs expression and activation can be blocked by addition of the ERK MAP kinase inhibitor (PD98059), but not by a p38 inhibitor (SB203589) or the JNK inhibitor(SP600125). This was further confirmed by the ability of overexpressing DN - MEK and DN - ERK MAP kinases to abolish MIF - induced MMP - 9 expression. CONCLUSION: Activation of the MEK - ERK MAP kinase pathway may be a key mechanism by which MIF contributes to the instability of the atheromatous plaques by stimulating MMPs expression.

KEY WORDS: Atherosclerosis, ERK MAP kinase, MIF, MMPs.
ACKNOWLEDGMENTS: This work was supported by NSFC (30271287, 30571850) and GDNSF (015015, 04102307).

P360040

Signaling Mechanisms Involved in the Synergistic Interaction of Arachidonic Acid (AA) plus Platelet Activating Factor and AA plus Epinephrine

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The signaling mechanisms involved in the synergistic interaction of arachidonic acid (AA) with plateletactivating factor (PAF) and AA with epinephrine in platelet aggregation was investigated. Our results show that synergisms mediated by AA plus PAF and AA plus epinephrine were inhibited by cyclooxygenase (COX) inhibitors, aspirin (IC_{50} = 110uM and 105uM respectively) and as well as by COX - 2 inhibitors, nimesulide (IC_{50} = 16 and 20uM) and NS - 398 (IC_{50} = 10 and 12uM). In addition, phospholipase C (PLC) inhibitor U73122 also inhibited AA plus PAF and AA plus epinephrine induced synergism. This signaling pathways was also blocked by calcium (Ca^{++}) channel blockers, verapamil (IC_{50} = 20 and 18uM respectively) and diltiazem (IC_{50} = 15 and 5.2uM respectively). These results show a common pathway mediated through COX, PLC and Ca^{++} signaling is involved in the synergistic interactions of AA plus PAF and AA plus epinephrine.

Key words: Synergism, signaling, cyclooxygenase, phospholipase C, calcium channel blockers.
Acknowledgment: We thank Higher Education Commission, ICCS, and Aga Khan University, Karachi, Pakistan for research support.

P360041

EP4 Prostanoid Receptor Coupling to a Pertussis Toxin - Sensitive Inhibitory G Protein

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The EP2 and EP4 prostanoid receptor subtypes are G - protein - coupled receptors for prostaglandin E2 (PGE_2). Both receptor subtypes are known to couple to the stimulatory guanine nucleotide binding protein (Gs) and, after stimulation with PGE_2, can increase the formation of intracellular cAMP. In addition, PGE_2 stimulation of the EP4 receptor can activate phosphatidylinositol 3 - kinase (PI3K) leading to phosphorylation of the extracellular signal - regulated kinases (ERKs) and induction of early growth response factor - 1 (EGR - 1) (J Biol Chem 278: 12151 - 12156, 2003). We now report that the PGE_2 - mediated phosphorylation of the ERKs and induction of EGR - 1 can be blocked by pretreatment of EP4 - expressing cells with pertussis toxin (PTX). Furthermore, pretreatment with PTX increased the amount of PGE_2 - stimulated intracellular cAMP formation in EP4 - expressing cells but not in EP2 - expressing cells. These data indicate that the EP4 prostanoid receptor subtype, but not the EP2, couples to a PTX - sensitive inhibitory G - protein (Gi) that can inhibit cAMP-dependent signaling and activate PI3K/ERK - dependent signaling.

P360042

Agonist - dependent Activation of Dopamine D3 Receptors and many GPCRs is Temperature - dependent

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The effects of temperature on agonist - induced intracellular Ca^{2+} release by dopamine D3 receptors and seven other GPCRs are presented. In HEK cells expressing D3 and G15, agonist - dependent response observed at 37 °C was greatly diminished at 25 °C. Temperature had no effect on the binding Kd and Bmax of [3H] - 7 - OH - DPAT, or the functional Ki of GR - 218231. In LTK cells expressing D2, the efficacy of dopamine - induced response was reduced by 60 % at lower temperature. Similar temperature dependence was observed in dopamine - induced phosphorylation of MAP kinase in these D3 and D2 cells. In the HEK - D3 cells, ATP - induced intracellular Ca^{2+} release via the endogenous purinergic receptors had similar temperature dependence as D3. However in the LTK - D2 cells, temperature had no effect on the ATP - dependent response, suggesting that temperature effect in agonist - dependent is receptor - and cell - specific. Lower efficacy of agonist - induced intracellular Ca^{2+} release at lower temperature was also observed for cells that expressed H1, 5HT1A, 5HT2A, and alpha2A, but not M1. These results suggest that temperature has a profound effect on the efficacy of agonist - mediated intracellular Ca^{2+} release for many GPCRs.

P360043

The Involvement of Caveolae/caveolin-1 on Activation of ERK1/2 Induced by Angiotensin II in Vascular Smooth Muscle Cells

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AIM: To investigate the effects of caveolae/caveolin-1 on late-phage activation of ERK1/2 stimulated by angiotensin II (Ang II) in cultured vascular smooth muscle cells (VSMCs). METHODS: Cultured rat aortic VSMCs were challenged to Ang II 100 nmol/L for 2, 5, 10, 30, 60, 120, 240, 360, and 480 min. Western blot was used to analyze the expression of caveolin-1 and p-ERK1/2. RESULTS: Western blot showed that Ang stimulated ERK1/2 activation with two peaks at 5 min (early-phage) and 4 hr (late-phage) respectively. The late-phage activation of ERK1/2 was accompanied by a significant decrease of caveolin-1 expression. Transfection of Antisense caveolin-1 oligonucleotides enhanced Ang II-induced late-phage activation of ERK1/2. Furthermore, when caveolae structure was disrupted by Nystatin, Ang II-stimulated ERK1/2 activity was obviously attenuated. PD98059, an inhibitor of MEK-1, decreased ERK1/2 activity without effect on caveolin-1 expression. CONCLUSION: Caveolae/caveolin-1 was involved in regulation of late-phage ERK1/2 activation induced by Ang II in VSMC.

Key words: Angiotensin II, ERK1/2, Caveolin-1, VSMC.

This work was supported by the 973 Program of China (G2000056905).

P360044

Bax inhibitor-1 can regulate the ER stresses-induced accumulation of ROS

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Bax inhibitor-1 (BI-1) is an antiapoptotic protein that localizes to ER membranes, and has a specifically protective effect on ER stress-induced apoptosis. Since ER metabolism was related with the generation of reactive oxygen species (ROS) through oxidative protein folding, we focused the role of BI-1 in the regulation of ER stress-induced ROS accumulation and the association of antioxidant proteins especially Heme oxygenase-1 (HO-1). BI-1 overexpression protected against ER stresses-induced cell death where the transfection of BI-1 and HO-1 siRNA can completely abrogate the protection. The treatment of ZnPP, HO-1 inhibitor, showed the similar effect of HO-1 siRNA in BI-1 protection model. This study also showed the binding of Bcl-2 and Bcl-XL has no important role on BI-1-induced protective effects. We demonstrated here that BI-1 can inhibit the accumulation of ROS and the resultant cell death. In this study, Heme oxygenase-1 can have a critical role on the BI-1-associated protection.

Key words: Bax inhibitor, Heme oxygenase-1, Reactive oxygen species-It is supported by KRF-foundation-2005(pure basic group).

P360045

Allosterically-linked residues in heterotrimeric G protein a-subunits: Combination of evolutionary, statistical ensemble & molecular dynamic approaches

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Allosteric effects constitute a common regulatory mechanism for all protein functions. However, it is very difficult to localize allosteric effects in structural elements. Here, we used three different approaches that utilize fundamentally different and independent information to identify allosteric linkages in the a-subunits of heterotrimeric G proteins. We evaluated: 1) correlated mutations between different sites of amino acids in a multiple sequence alignment of a family of G protein a-subunits, which constitutes an evolutionary sample, 2) simulation of statistical ensemble representing the native folded state of the Gai1 or transducine, which enables one to calculate residue-specific folding free energies in GDP or GTPgS-bound forms or to determine correlations in local foldings in the protein, and 3) molecular dynamic simulations. Combination of these approaches recovered already-known details, such as switch regions that change conformation upon nucleotide exchange, or pointed to those regions that are involved in receptor, effector or Gbg interactions, but also provided additional information, which will be discussed here. This study is supported by the research grant AU. BAP. 2002-08-09-088

P360047

Coupling of b2-adrenoceptor to Gs and adenylyl cyclase in caveolin-rich low density membrane fractions. Comparison of different preparation techniques

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Caveolin-rich membrane rafts, known as caveolae, can be isolated as detergent-resistant low density fractions (DRLDF) using different solubilization and fractionation methods. As all the detergents used for solubilization potentially change properties of the proteins, each of these methods can cause artifacts. Here, we compared the localization of, and functional coupling between, b2-adrenoceptor (b2AR), Gs and adenylyl cyclase in caveolin-rich DRLDF of b2AR overexpressing HEK-293 cells obtained by: 1) Triton X-100 or 2) Octyl Glycoside solubilization, or 3) extensive homogenization without solubilization, followed by sucrose gradient fractionation. Both the membrane/DRLDF ratios and the functional properties of the proteins were found to vary considerably between the methods used, octyl glycoside solubilization being the superior one as the ligand binding properties of the b2AR and functional coupling between b2AR, Gs and adenylyl cyclase seemed to remain intact in the DRLDF obtained by this method. In any case, functional interactions between the proteins differed from what observed in bulk membrane.

This study is supported partly by the research grants AU. BAP. 2002-08-09-088 and TUBITAK 104s472

P360048

F282L mutation in transmembrane helix-6 (6.44) of b2-adrenoceptor (bAR) results in an inverse agonistresistant constitutive activity

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Constitutively active mutant (CAM) receptors have been considered as useful tools to understand molecular mechanism of receptor activation. Here we characterize a relatively unknown, but very efficacious CAM (F282L mutation) in transmembrane helix 6, which is known to be involved in the activation of rhodopsin-like receptors. F282L bAR exhibited increased affinity for agonists (Kmut/Kwt of isoproterenol = 200) and high basal adenylyl cyclase activity in HEK293 cells. However, unlike the most extensively studied bAR CAM (bAR-CAM; L272A, H269K, K267R, L266S), the membrane expression of F282L bAR was partially recovered by incubation with receptor ligands, or the inverse agonist ICI118551 was unable to abolish basal receptor activity and its affinity for the receptor was not affected by the mutation. Likewise, cellular distribution of F282L bAR, as assessed by confocal imaging of green fluorescent protein-fused receptors, was different than that of bAR CAM. All together, the results suggest that the active state adopted by F282L bAR is different than that of bAR-CAM in many respects, including their intracellular trafficking properties.

This study is supported by the research grant AU. BAP. 2002-08-09-088

P360049

Modulation of maternal care by editing of the serotonin 2C receptor (5-HT2CR)

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The 5-HT2CR has been implicated in a number of human psychiatric and behavioral disorders that can affect the quality of maternal care, including depression, anxiety and schizophrenia. RNA transcripts encoding the 5-HT2CR are modified by RNA editing events to generate up to 24 receptors with altered constitutive activity and G-protein coupling efficiency. To determine the physiologic relevance of 5-HT2CR editing, we have generated a mutant mouse strain solely expressing the non-edited (INI) isoform of the receptor. Mutant mice demonstrate several behaviors characteristic of altered maternal care including poor nest formation, pup scattering and diminished pup size. Both wild-type and mutant pups raised by mutant mice demonstrate anxiety-related behavior and a decreased growth rate compared to offspring raised by wild-type dams, indicating that the genotype of the dams is responsible for phenotypic alterations. However, mutant male

mice , but not their wildtype littermates , are hyperactive , indicating that the INI mutation produces this effect independent of maternal care. These mutant animals will aid our understanding of the role(s) that the 5 - HT2CR plays in behavioral and neuropsychiatric disorders.

P360050

ET - 1 causes p38 MAPK- dependent expression of COX - 2 through interaction with ETB receptors in Cultured Feline Esophageal Smooth Muscle Cells.

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We investigated a possible role for p38 MAPK in mediating the action of ET - 1 on induction of cyclooxygenase - 2 (COX - 2) and production of prostaglandin E2 (PGE_2) in cultured feline esophageal smooth muscle cells (ESMC). Confluent layers of ESMC were stimulated by 10nM ET - 1 ; expression of COX - 1 and COX - 2 and activation of p38 MAPK were examined by western blot analysis. Levels of PGE_2 produced by ET - 1 were measured by Elisa system. By using ETA and ETB antagonists (BQ - 123 and BQ - 788 , respectively) , the contribution of the ET receptors to COX - 1 and COX - 2 expression induced by ET - 1 was determined. Western blot analysis revealed that treatment of ESMC with ET - 1 resulted in transient expression of COX - 2 and activation of p38 MAPK in a time - dependent manner. The activation of p38 MAPK by ET - 1 reached the maximal levels at 1 hour. SB202190 , a p38 MAPK inhibitor , reduced the expression of COX - 2 , but not COX - 1. ET - 1 - induced release of PGE_2 was also blocked by SB202190. COX - 2 expression was upregulated only by ETB receptor ; COX - 1 expression was not affected by either antagonist. The data imply that ET - 1 causes p38 MAPK dependent expression of COX - 2 through interaction with ETB receptors in ESMC.

P360051

Static Pressure Up - Regulates Nuclear Factor κB - mediated Endothelial Lipase Expression through IκBα/IKK Signaling Pathways

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AIM To investigate the effect and possible mechanisms of static pressure on endothelial lipase(EL) expression. METHODS Cultured human umbilical vein endothelial cell(HUVEC) were treated with 0 , 120 , 150 , 180 and 240 mmHg in a self - manufactured pressure incubator for 24h or were treated with 180 mmHg of static pressure for 0 , 3 , 6 , 12 and 24h. RT - PCR , western - blotting , flow cytometry and immunofluorescence were used to detect the expression of EL , nuclear factor - κB(NF - κB) and IκBα(inhibitor of NF - κB) , respectively. RESULTS Static pressure significantly up - regulated level of EL protein and mRNA in a time - and dose - dependent manner with a 3 - fold increase of EL under treatment of 180 mmHg static pressure for 24h. Furthermore , static pressure induced degradation of IκBα and the nuclear accumulation of NF - κB p65 by activating the IκB kinase(IKK). CONCLUSION Static pressure induces HUVEC to secrete EL by activating the IκBα/IKK signaling pathways.

Key Words : static pressure ; endothelial lipase ; NF - κB ; endothelial cells.

This work was supported by 973 Program(G2000056905)

P360052

Sphingosine 1 - Phosphate Receptor Antagonists and Lymphocyte Trafficking

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Sphingosine 1 - phosphate (S1P) is a lysophospholipid signaling molecule that regulates numerous cellular processes including proliferation , migration and survival. S1P signals via a set of five G protein - coupled receptors ($S1P_{1-5}$). S1P signaling was validated as a target of immunomodulatory drugs when the sphingosine analog , FTY720 , was found to be metabolized in vivo to a pan - S1P receptor agonist. FTY720 alters lymphocyte trafficking such that lymphocytes accumulate in secondary lymphoid tissues ; the index of its action is lymphopenia. We synthesized a series of S1P analogs to use as tools to explore S1P biology. One compound, VPC44116 , is a competitive antagonist at $S1P_1$ and $S1P_3$ receptors. Although FTY720 - P is thought to be a functional antagonist , administration of the receptor antagonist VPC44116 caused neither lymphopenia nor lymphocytosis. Further , VPC44116 antagonized the lymphopenia evoked by its positional isomer , VPC44152 , an $S1P_{1,4,5}$ agonist , and the selective $S1P_1$ agonist SEW2871. VPC44116 and follow on compounds will enable further understanding of S1P signaling.

Key Words : Sphingosine 1 - Phosphate , FTY720 , Lymphopenia

P360053

Activation of multiple G- proteins by muscarinic M1 and M2 receptors.

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Muscarinic M2 receptors preferentially couple with Gi/o while M1 receptors with Gq/11 class of G - proteins. In addition to preferential inhibition of adenylyl cyclase , stimulation of M2 receptors by high concentrations of full agonists (carbachol , acetylcholine , furmethide and oxotremorine) stimulated also production of second messengers inositolphosphates and cAMP in agonists specific manner. These atypical responses increased with receptor density. Muscarinic M1 receptors also increased synthesis of cAMP and pertussis toxin treatment potentiated this response demonstrating activation of both Gs and Gi/o proteins. Repression of Gq/11 and Gs proteins using corresponding siRNAs diminished or abolished respective responses at both receptors whereas negative siRNA had no effect. Results of our experiments show that (a) muscrinic receptors can activate also other than conventional G - proteins and (b) various muscarinic agonists can induce distinct conformational states of the receptor resulting in unequel functional response.

Supported by project AV0Z50110509 , grants GACR305/05/P209 , GACR305/05/0452 , NIH NS25743 , LC554

P360054

P2X7 Receptors Utilize Different Pathways for Fluorescent Dye Uptake in Different Cell Types

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We investigated the P2X7 receptor mediated uptake of three different dyes (Lucifer yellow, ethidium bromide and YO - PRO1) in both RAW and rP2X7 expressing HEK - 293 cells (HEK - K4) using single cell imaging , PMT measurements and fluorescent microscopy techniques. Both RAW and HEK - K4 cells showed clear YO - PRO and ethidium bromide uptake upon ATP (1 mM) application. On the other hand while P2X7 stimulation induced an apparent lucifer yellow uptake in RAW cells , it did not stimulate any uptake in HEK - K4 cells. This clear lack of lucifer yellow uptake in HEK - K4 cells is not due to extensive disruption of the cell membrane by P2X7 receptor stimulation , rendering it incapable of holding the soluble dye in its cytoplasm, as these cells did not show any leakage of FURA2 during this stimulation period. Our results suggest that the pathway which is responsible for the fluorescent dye uptake in HEK cells is different than the one in RAW cells.

P360055

Nitric Oxide Reduces Endothelial Nitric Oxide Synthase Phosphorylation and Function by Depleting Akt

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NO can inhibit eNOS function and this is thought via feedback inhibition. Recent studies showed that phosphorylation of eNOS Ser 1179/1177 (bovine/human) by Akt is a central mechanism of eNOS regulation. Whether NO affects eNOS phosphorylation is unknown. Thus , we exposed bovine endothelial cells to NO and monitored eNOS phosphorylation. NO(1 - 20 uM) dose - dependently decreased Ser 1179 - phosphorylated eNOS. Conversely , neither the total eNOS nor eNOS Thr 497 phosphorylation was affected. In NO - treated cells , Ser 1179 - phosphorylated eNOS activity was diminished (140.6 ± 3.1 vs 9.0 ± 1.1 pmol/mg/min , $P < 0.01$, $n = 4$). NO dramatically reduced cytosolic Akt and phospho - Akt (Thr 308/Ser 473). Caspase inhibition (Z - VAD.fmk 20 uM) but not proteasome blockade (MG132 10 uM) reversed NO - induced Akt depletion and recovered Ser 1179 - phospohrylated eNOS. Akt overexpression also preserved eNOS Ser 1179 phosphorylation in NO - treated cells. These results demonstrated that besides the direct feedback inhibition on eNOS catalysis , NO profoundly influences eNOS function by affecting its phosphorylation. By activating caspases , NO depletes cytosolic Akt levels leading to the loss of eNOS Ser 1179 phosphorylation and activity.

P360056

Rosiglitazone ameliorates abnormal expression and activity of protein tyrosine phosphatase 1B (PTP1B) in skeletal muscle of type 2 diabetic rats

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PTP1B acts as a physiological negative regulator of insulin signaling by dephosphorylating the activated insulin receptor (IR) . Here we examine the role of PTP1B in the insulin - sensitizing action of rosiglitazone (RSG) . Ten-week - old , fat - fed , STZ - treated rats , were treated with RSG (10 μmol ·kg $^{-1}$ day $^{-1}$) for 2 weeks. After RSG treatment , the diabetic rats showed a decrease in blood glucose and improved insulin sensitivity. Diabetic rats showed increased levels and activities of PTP1B in muscle and liver. We found that 55 % , 48 % , and 39 % decreases in insulin - induced glucose uptake , tyrosine phosphorylation of IR β- subunits , and IRS - 1 , respectively , in muscles of diabetic rats were normalized after RSG treatment. These effects were associated with 34 % and 30 % decreases in increased PTP1B levels and activities , respectively , in muscles of diabetic rats. In contrast , RSG did not affect the increased PTP1B levels and activities or the reduced insulin - stimulated glycogen synthesis and tyrosine phosphorylation of IRβ- subunits and IRS - 2 in livers of diabetic rats. These data suggest that RSG enhances insulin activity in muscle of diabetic rats by ameliorating abnormal levels and activities of PTP1B.

P360057

Enhanced bioluminescence resonance energy transfer (BRET) between renilla proteins for the study of protein-protein interactions.

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BRET between a bioluminescent luciferase (Luc) - substrate complex and GFP occurs naturally in some renilla species. Because of a direct binding interaction between rLuc and rGFP , the excitation tranfer reaches 100 % efficiency and shows a marked enhancement of the apparent quantum yield of the luminescent reaction. This spontaneous interaction , however , is considered a potential drawback when BRET is used as a reporter system for the study of protein - protein interactions , thus GFP from different species is usually employed. To investigate if rLuc and rGFP can be useful as BRET reporters , we compared the luminescent properties of (a) coexpressed native proteins , (b) cleavable N - rGFP - rLuc or N - rLuc - rGFP fusion chimeras and (c) coexpressed mutants carrying tethered Leu - zipper peptides. BRET signals were undetectable in the wild - type pair , but readily measurable in the chimeras and zipper - mutants. Enhanced highly efficient BRET required a free N - terminus on rGFP and entailed a 15 - fold increase in luminescence. Using adrenoceptor –arrestin interactions as a model , we show that enhanced BRET provides greater sensitivity for real - time monitoring of protein - protein interactions.

Key words : BRET, luminescence.

P360058

Cerebral oxidative stress and angiotensin II signaling in chronic isoproterenol - infused rabbits

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Increased oxidative stress resulting from an increased cardiac generation of reactive oxygen species (ROS) is implicated in the progression of cardiac hypertrophy and heart failure. This study aimed to clarify the role of ROS in angiotensin II (Ang II) signaling in cerebral artery of isoproterenol (ISO) - infused rabbits. Rabbits were infused with ISO intravenously for 7 days (10 mg/ kg/ day) . Superoxide and hydrogen peroxide as well as superoxide dismutase (SOD) activity and NADH/ NADPH oxidase were measured in cerebral artery , revealing the increased superoxide/ hydrogen peroxide production and SOD activity in ISO - infused rabbits compared to control. NADH/ NADPH oxidase were not different between control and ISO - infused rabbits. We also measured the changes of ROS intensity by Ang II revealing the augmentation of ROS production by Ang II in ISO - infused rabbits compared to contrd. Beta - Adrenoceptor stimulation provokes cerebral oxidative stress. ROS may participate in cerebral dysfunction , especially in respect to Ang II mediated vasoactivity during cardiac hypertrophy.

Keyword ; Reactive Oxygen Species(ROS) , angiotensin II(Ang II) , Cardiac hypertrophy

P360059

Intercellular Calcium Signaling and Nitric Oxide Feedback During Constriction of Rabbit Renal Arterioles

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The increase in intracellular calcium concentration ($[Ca^{2+}]_i$) in vascular smooth muscle cells (VSMC) which is associated with vasoconstriction may increase endothelial cell (EC) $[Ca^{2+}]_i$. This may stimulate the endothelial nitric oxide synthase , release nitric oxide (NO) and counteract the vasoconstriction. We tested this hypothesis in microperfused rabbit afferent arterioles. Depolarisation with KCl ([100 mmol/ L]) evoked a transient vasoconstriction , which became sustained after treatment with N - nitro - L - arginine methyl ester (L - NAME) . $[Ca^{2+}]_i$ was measured by fluorescence imaging microscopy using Fura 2. After depolarisation VSMC $[Ca^{2+}]i$ increased from 162 + / - 15 nmol/ L to a peak of 555 + / - 70 nmol/ L ; (n = 7) . After a delay of 10s $[Ca^{2+}]i$ increased in EC adjacent to the VSMC. L - NAME did not affect peak values in VSMC $[Ca^{2+}]_i$. Acetylcholine caused a rapid increase in EC $[Ca^{2+}]_i$, which did not transfer to the VSMC. We conclude that the increase in VSMC $[Ca^{2+}]_i$ after depolarisation is transferred to the EC , where NO production increases and feeds back to the smooth muscle cell layer.

Key words : endothelium, calcium wave , nitric oxide , smooth muscle

P360060

LOCAL REGULATION OF CRAC CHANNELS IN T LYMPHOCYTES IS MEDIATED BY ATP FROM SUBPLASMALEMMAL MITOCHONDRIA

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As occurs with other Ca^{2+} channels , local Ca2 + microdomains act as negative feedback regulators of storeoperated Ca^{2+} (SOC) entry by promoting the inactivation of Ca^{2+} - release activated Ca^{2+} current (ICRAC) . Mitochondria , as Ca^{2+} storing organelle , may potentially control ICRAC not only by taken up Ca^{2+} ions but also through the release of soluble endogenous Ca^{2+} buffers in a metabolically dependent manner. Using the patch - clamp technique , which permits the control of the intracellular environment , we found that kinetic properties of exogenous Ca^{2+} chelators determine the extent of Ca^{2+} microdomains and hence the rate of inactivation. Moreover , we have observed that energized mitochondria located close to CRAC channels are able to regulate slow inactivation by increasing the Ca^{2+} buffering capacity beneath the plasma membrane , mainly through the release of ATP. This is the first description of the nature and modulatory effects of a mitochondrial diffusible factor on ICRAC

Key words : CRAC channels , inactivation , mitochondria , T cells.
This study is supported by BFI2002 - 01101 (MEC) , GR/ SAL/ 0522 - 2004 (CAM) and PR45/ 05 - 14162 (UCM-CAM) grants to JAG. GB-M is a MEC - FPI predoctoral fellow.

P360061

Receptor Selectivity in Growth Effects of Prostaglandins in Hepatocytes

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The aim of this study was to examine which prostanoid receptors mediate the growth - stimulatory effects of prostaglandins in cultured rat hepatocytes. Sulprostone , misoprostol , and fluprostenol strongly enhanced DNA synthesis induced by epidermal growth factor (EGF) , and inhibited glucagon - stimulated cAMP accumulation. Pretreatment of hepatocytes with pertussis toxin (PTX) abolished the growth stimulatory effect of sulprostone and misoprostol , and attenuated the effect of fluprostenol , indicating involvement of EP3 receptors. Fluprostenol was 100 - fold more potent in stimulating PLC (assessed by accumulation of inositol phosphates) than in inhibiting cAMP accumulation , indicating involvement of FP receptors. Inhibition of protein kinase C attenuated the growth - stimulatory effect of fluprostenol. EP1 - receptor antagonists (SC - 51089 and SC - 51322) did not inhibit the enhancement by prostaglandin E2 of EGF - stimulated DNA synthesis. In conclusion , the results suggest that the growth - stimulatory effects of prostaglandins in rat hepatocytes are mediated by EP3 - and FP - receptors , while EP1 - receptors appear to play a minor role.

P360062

MAGI-3 Retards Beta 1 Adrenergic Receptor Media Activation of MAPK

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Many GPCRs interact with PDZ scaffold proteins to control its trafficking and signaling. To gain a panoramic view of b1AR interactions with PDZ scaffolds, the b1AR carboxyl terminus was screened against an array of PDZ domains. These screens confirmed b1AR associations with several previously identified PDZ partners, such as PSD-95, MAGI-2, GIPC and CAL. Moreover, two novel b1AR interacting proteins, SAP97 and MAGI-3, were also identified. The b1AR was found to bind specifically to the first PDZ domain of MAGI-3, and this association was abolished by mutation of the receptor's terminal valine residue to alanine (V477A). MAGI-3 coexpression with b1AR profoundly impaired b1AR mediated MAPK activation but had no apparent effect on b1AR-mediated cyclic AMP generation or agonist-promoted b1AR internalization. These findings reveal that the interaction of MAGI-3 with b1AR can selectively regulate specific aspects of receptor signaling. Moreover, the screens of the PDZ domain proteomic array provide a comprehensive view of b1AR interactions with PDZ scaffolds, thereby shedding light on the molecular mechanisms by which b1AR signaling and trafficking can be regulated in a cell-specific manner.

P360063

Altered RNA Editing of the 2C-Subtype of Serotonin Receptor (5-HT2CR) Results in Paradoxical Alterations in Feeding Behavior and Growth in Mutant Mice

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Editing of 5-HT_{2C}R mRNA yields up to 24 receptor isoforms from a single gene locus, with the fully-edited (VGV) isoform exhibiting reduced constitutive activity and G-protein coupling efficiency. To understand the physiologic relevance of 5-HT_{2C}R editing, we created mutant mice solely expressing the VGV isoform of the receptor. Mutant mice demonstrate a dramatic decrease in growth rate during the first three weeks of postnatal development (31% of wild-type body mass at weaning), whereas the rates of growth are identical beyond this developmental stage. Despite their decreased size, VGV-expressing mice demonstrate a paradoxical increase in food consumption after weaning, consistent with reduced 5-HT_{2C}R signaling, yet the adult-onset obesity seen in 5-HT_{2C}R-null animals is not observed. To examine the cellular basis for alterations in feeding behavior, preliminary studies have focused upon increases in d-fenfluramine-induced α-melanotropin mRNA expression using qRT-PCR as an index of satiety. To further define the physiologic impact of 5-HT_{2C}R editing, future studies will examine alterations in suckling behavior and metabolism as potential explanations for the observed growth retardation in mutant pups.

P360064

ANGIOTENSIN II-TYPE 2 RECEPTOR SIGNALING IN THE INFERIOR OLIVE OF RAT BRAIN

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The signal transduction mechanism coupled to angiotensin II AT2 receptors is controversial. We assessed the effect of angiotensin II (ANG) and CGP42112A on cGMP formation in the inferior olive (IO) from young rats known to express only AT2 receptors. We show here that in the IO, ANG decreases basal and atrial natriuretic peptide (ANP)-stimulated cGMP formation. Addition of ANG + CGP 42112A had no additive effect on the cGMP inhibition. Agonist induced cGMP reduction was not altered by losartan, a selective AT1 receptor antagonist. In addition, ANG- or CGP42112A-induced decrease on basal or ANP-stimulated cGMP was blunted by sodium orthovanadate, a phosphotyrosine phosphatase (PTPase) inhibitor and with okadaic acid and caliculyn, two PP1/2A inhibitors. Our results suggest that in the IO, the inhibition of cGMP formation may be related to an ANG-stimulation of phosphatases, which may be implicated in the regulation of the particulate guanylyl cyclase activity via AT2 receptors.

Key words: angiotensin II, atrial natriuretic peptide, cGMP, phosphatases.

Grants: CDCH PI tipo A 2004; FONACIT: Ecos Nord 2003-2007

P360065

Effect of L-Arginine on healing of burn wounds

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GranulIntroduction: Nitric Oxide (NO) have an important role in healing of burn wounds This study investigated the effect of L-Arginine (Precursor of NO) on experimentally induced burn wounds. Materials and Methods: A total of 40 rats weighing 250±20 gr were used in this study. The shaved skin on the back of the rats was immersed in 100. water for 8 seconds to achieve a partial thickness scald burn. The rats were divided into four groups. In groups I and II (control groups) 100 mg/Kg of Normal Saline was injected for 7 and 15 days respectively. In groups III and IV (experimenal groups) 100 mg/Kg L-Arginine was injected intraperitioneally for 7 and 15 days respectively as 1st, 4th, 11th and 14th days after burn. 7 days postburn, the rats of groups I, III and on days 15 postburn, the rats of groups II, IV killed and the burn areas were investigated histopathologically. Changes such as epidermal proliferation, inflammation, collagen formation and blood vessels were evaluated. Results: Epidermal proliferation, collagen formation and blood vessels were higher in experimental groups(III, IV) than those observed in the control groups (I, II). Inflammation in control groups was higher than experimental groups. Conclusion: We concluded that heading of burn wound is accelerated by L-Arginine (precursor of Nitric oxide)

Key words: Burn, Wound healing, Nitric Oxide, L-Arginine

P360066

Activation of ERK1/ERK2 phosphorylation by ATP in bovine chromaffin cells

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ATP is synthesized in chromaffin cells and released in response to nicotinic receptor stimulation. Predictably, chromaffin cells contain purinergic receptors which either activate ion channels or are G protein coupled. Previous data from our laboratory have shown that ATP can increase inositol phosphate (IP3), intracellular Ca^{++} concentrations and protein kinase C activity in a time and concentration dependent manner via a P2Y receptor. The response of chromaffin cells to the increase in these effectors has not been described. We recently observed that ATP increases the phosphorylation of the extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2). The maximum effect is observed between 5 and 15 min and the increase in phosphorylation is concentration dependent (EC_{50} = $2.5x10^{-5}$ and $1.3x10^{-5}$ M for ERK1/ERK2, respectively) effects which are consistent with those for ATP on IP3 formation. Either the P2X/Y receptor antagonist, suramin, or the MEK inhibitor, PD98059, prevents ERK1/ERK2 phosphorylation by ATP. The effects of ATP on the nuclear targets of ERK1/ERK2 will be examined.

Key Words: ATP, ERK, chromaffin cells.

(Support for this work was provided by the American Heart Association # 0550030Z.)

P360067

Methylglyoxal stimulated proliferation of vascular smooth musde cells through p21/CDK2 pathway

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Increased proliferation of vascular smooth musde cells (VSMCs) is tightly linked to development of hypertension. In the present study, it was found that methylglyoxal (MG) (0.01-10μM) significantly increased DNA synthesis and proliferation of cultured VSMCs. MG treatment decreased p21 levels and increased cyclindependent kinase 2 (CDK2) activity in cytoplasmic fractions. The inhibitory phosphorylation of Tyr15 of CDK2, but not the stimulatory phosphorylation of Thr160 of CDK2, was reduced by MG, which may account for the increased cytoplasmic CDK2 activity. Phospho-pRb level was increased in MG-treated cells. MG effects were abolished by co-application of N-acetylcysteine or superoxide dismutase. In conclusion, MG at physiologically relevant concentrations stimulates VSMCs proliferation likely through the induced production of ROS, which subsequently activates cytoplasmic CDK2 and decreases p21 level. Increased MG levels in many cardiovascular disorders, therefore, may underscore increased proliferation of VSMC in these situations.

(Supported by CIHR and HSFC)

Key words: Methylglyoxal, smooth musde cells, proliferation

P360068

Rac 1 regulates peptidoglycan - induced nuclear factor - κB activation and cyclooxygenase - 2 expression in RAW 264.7 macrophages by activating the phosphatidylinositiol 3 - kinase/Akt pathway

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In this study, we investigated the role of Rac 1, phosphatidylinositiol 3 - kinase (PI3K), and Akt in peptidoglycan (PGN) - induced nuclear factor - κB (NF-κB) activation and cyclooxygenase - 2 (COX - 2) expression in RAW 264.7 macrophages. PGN - induced COX - 2 expression was attenuated by a Rac1 dominant negative mutant (RacN17), PI3K inhibitors (wortamannin and LY 294002), and the Akt inhibitor. Treatmentof RAW264.7 macrophages with PGN caused the activation of Rac1 and Akt. The PGN - induced Akt activation was inhibited by RacN17, LY 294002, and the Akt inhibitor. Stimulation of RAW 264.7 macrophages with PGN resulted in the increase in IκB kinases αβ (IKKαβ phosphorylation and p65 Ser536 phosphorylation; these effect were inhibited by RacN17, LY 294002, the Akt inhibitor, or an Akt dominant negative mutant (AktDN). The PGN - induced increases in κB - luciferase activity was also inhibited by RacN17, wortmannin, LY 294002, the Akt inhibitor, and AktDN. Treatment of macrophages with PGN induced the recruitment of p85 αand Rac1 to toll - like receptor 2 (TLR2) in a time - dependent manner. These results indicate that PGN may activate the Rac1/PI3K/Akt pathway, which in turn initiates IKKαβ, p65 Ser536 phosphorylation, and NF - κB activation, and ultimately induces COX - 2 expression in RAW264.7 macrophages.

Key words: Cyclooxygenase - 2, Rac1, PI3K, Akt, Nuclear factor - κB, RAW 264.7 macrophages.

P360069

Overview of the history and therapeutic potential of purinergic signalling

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ATP is an extracellular signalling molecule and was proposed as a neurotransmitter of non - adrenergic, non - cholinergic nerves supplying the gut and bladder in the early 1970's and later as a cotransmitter in most nerve types in both peripheral and central nervous systems. Subdivision into P1 and P2 receptors responsive to adenosine and ATP respectively was proposed in 1978. Four subtypes of P1 receptors were cloned and subdivision of P2 receptors into P2X ionotropic and P2Y metabotropic families followed. Currently, 7 subtypes of P2X receptors and 8 subtypes of P2Y receptors have been cloned and characterised. The P2X form heteromultimers and some P2Y receptor subtypes are responsive to pyrimidines. Short - term purinergic signalling occurs in neurotransmission and secretion. Long - term (trophic) purinergic signalling occurs in cell proliferation, differentiation and death during development and regeneration. There is strong current interest in the therapeutic potential of purinergic agents in diseases such as thrombosis, stroke, pain, cystic fibrosis, dry eye, osteoporosis, kidney failure, diabetes and cancer.

Key words: adenosine, ATP, purinergic, purinoceptors

P360070

Signalling mechanisms involved in induction of LRF - 1/ATF3 by G - protein - coupled receptor agonists in hepatocytes

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The aim of this study was to examine the signalling pathways involved in induction of liver regeneration factor 1 (LRF - 1/ATF3) by agonists acting on G protein - coupled receptors in cultured rat hepatocytes. mRNA and protein expression were determined by real - time RT - PCR, Northern and Western blotting. Vasopressin, angiotensin II, norepinephrine, and prostaglandin F2α rapidly induced LRF - 1. Inhibition of phospholipase C with U73122, protein kinase C with GF109203X, or reducing calcium by EGTA or BAPTA did not inhibit VP-induced LRF - 1 expression. Inhibition of each of the mitogen - activated protein kinase (MAPK) pathways ERK, p38, or JNK, with PD098059, SB203580, or SP600125, respectively, did not inhibit vasopressin - induced expression. However, the combined inhibition of the ERK and p38 pathways, as well as the ERK and JNK pathways or the JNK and p38 pathways, inhibited the expression partly. In conclusion, the vasopressin - induced LRF - 1 expression was dependent of activation of the ERK, p38, and JNK MAPK pathways. Due to redundancy in the regulatory mechanisms, inhibition of one single pathway was not sufficient to inhibit vasopressin - induced expression of LRF - 1.

P360071

Role of NO - cGMP - PKG in synaptic plasticity

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The NO - cGMP signal transduction pathway plays a role in a series of neurobiological functions. To study the effect of NO - cGMP - PKG on behavioral activity, we performed tests of rats or mice in rotarod, locomotor activity and active shuttle avoidance. It was found that nitric oxide synthase inhibitors, L - NAME and 7 - nitroindazole, caused motor incoordination on rotarod test without affecting locomotor activities. ICV injection of L - NAME, PKG inhibitor Rp - 8 - Br - PET - cGMPS and MEK inhibitor PD98059, also impaired the active avoidance learning, indicating that normal function of NO and these protein kinases in the amygdala is required during acquisition of active shuttle avoidance learning. ICV injection of L - NAME and Rp - 8 - Br - PET - cGMPS attenuated p - ERK expression in the amygdala following training. These results demonstrate the role of NO□cGMP - PKG and ERK pathways in memory acquisition of fear. We further investigated the neuritogenic action of NO in primary cortex neuronal culture. The neurite outgrowth and protein levels related to synaptogenesis were inhibited by L - NAME and PD98059 in primary cortex neuron, suggesting that NO signal transduction pathway plays an important role in synaptic plasticity.

P360072

Statin Prevents STAT3 Activation and Expression of VEGF and ICAM - 1 in Diabetic Retinas and High Glucose Treated Retinal Endothelial Cells

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This study evaluated the role of the transcription factor STAT3 in diabetes/high glucose - induced VEGF and ICAM - 1 expression. Western blotting studies of streptozotocin diabetic rat retinas showed increases in VEGF and ICAM - 1 expression that correlated with STAT3 activation as shown by tyrosine phosphorylation (PYSTAT3) and were blocked by simvastatin treatment. Treatment of retinal endothelial cells with high glucose (HG, 25 mM) also caused increases in PYSTAT3, VEGF and ICAM - 1 that were blocked by statin. HG - induced expression of ICAM - 1 and VEGF was blocked by infection with an adenovirus carrying transcriptionally inactive STAT3 but not by infection with a control adenovirus. Our results indicate that diabetes and high glucose-induced increases in ICAM - 1 and VEGF are associated with STAT3 activation. Moreover, STAT3 activity is required for HG - mediated induction of VEGF and ICAM - 1 expression in endothelial cells. Finally, statin treatment prevents STAT - 3 activation and expression of VEGF and ICAM - 1, suggesting that statin's action blocking diabetes/high glucose - induced VEGF and ICAM - 1 expression involves blockade of STAT3 activation.

Key words: Diabetes, Statin, STAT3

P360073

Protein kinase C - eta as a possible therapeutic target in breast cancer

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The objective of this study was to examine the hormonal regulation of protein kinase C (PKC) in breast cancer and to determine its role in the resistance to chemotherapy. Estradiol responsive and nonresponsive cells, apoptotic assays and an siRNA approach were used here. We show that estradiol affected differently PKC enzyme's expression. While the PKCeta isoform was specifically upregulated in the estrogen - responsive lines MCF - 7 and T47D, but not in the estrogen non - responsive line MDA - MB 231, PKCdelta was down - regulated, and PKCalpha and PKCzeta expression was unaltered. Progesterone, involved in differentiation of the mammary, reduced the estrogen - induced PKCeta expression in a time - dependent manner. We demonstrated a proliferative effect for PKCeta in these cells. Furthermore, the inducible expression of PKCeta in MCF - 7 cells provided partial resistance against cell death induced by DNA damage of camptothecin or UV irradiation. This was shown by increased cell survival and PARP cleavage and inhibition of JNK activity. Thus, the induced expression of PKCeta

by estradiol could have a role in breast cancer proliferation and resistance to chemotherapy, and thus a target for therapeutic intervention.

P360074

DIFFERENTIAL ACTIVATION OF MAPK PATHWAYS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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TgCRND8 mice exhibit cerebral amyloid deposits, cortical and hippocampal atrophy and memory impairment, and can help understanding the mechanisms of neuronal degeneration and memory impairment of AD. The MAPKs participate differentially in memory processes and inflammation. We evaluated ERK, p38 MAPK and SAPK/JNK activation in the brain of 7 months old TgCRND8. Amyloid plaques were present in brain parenchyma and P-p38 MAPK immunoreactivity (IR) increased in microglia-like and in astrocytes-like cells around the plaques and in neurons. P-SAPK/JNK IR increased in the cortex, hippocampus and thalamus while P-ERK decreased significantlyin in the piriform cortex of TgCRND8 mice. Activation of ERK was studied in vitro on TgCRND8 and wt brain slices incubated with 100 M carbachol which increased P-ERK in neurons of the thalamus, piriform cortex, in hippocampal CA1 and DG and in neurons of layer VI of the parietal cortex of wt mice. P-ERK was lower in hippocampus and piriform cortex of TgCRND8 than wt mice. Our data indicate that the three MAPKs may play different roles in inflammation and neurodegenerative processes caused by amyloid deposition.

Key words: Transgenic, Alzheimer
Grants: Università di Firenze

P360075

Possible non-immunological functions of MHC Class I glycoproteins: implications for cell differentiation and malignancy

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A deranged expression of MHC class I glycoproteins in tumors was found by us and others to regulate pivotal cellular nonimmune functions, which are impaired during malignant transformation. We investigated whether such derangements could affect proper receptor-mediated signal transduction. Malignant and H-2K murine MHC class I-deficient B16BL6 melanoma cells were characterized by the retention of major PTK receptors in intracellular compartments. The restoration of H-2K expression (and not other MHC Class I glycoproteins), abrogated their tumorogenic capacity, enhanced the translocation to the membrane of both the insulin receptor IR and the IGFR1. Insulin added to H-2K-expressing melanoma cells up-regulated the activity of (PKB)/AKT. A deficiency for H-2K, which is a characteristic of highly malignant clones, was associated with a constitutive high activity of PKB/AKT, rendering them resistant to apoptosis. The H-2K molecule was found to regulate the Interferon type I signal transduction pathway. These results strongly suggest that MHC Class I glycoproteins may possess a broad spectrum of non-immunologic functions which determine cell differentiation and cell to cell communication.

P360076

Protease Inhibition Confreres Increased Resistance to Hypoxia Induced Cell Death on NGF Treated PC12 Cells

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To investigate the response of the central nervous system to ischemic conditions we used PC12 cells. These cells are oxygen-sensitive and upon treatment with nerve growth factor (NGF), differentiate to a sympathetic phenotype expressing neurites and excitability. Hypoxia induced cell death was effected by exposing undifferentiated and NGF-treated PC12 cells to a mixture of $N_2:CO_2:O_2$ (93:5:2%) for up to 72 h. We investigated the recruitment of apoptosis using a general caspase inhibitor, benzyloxycarbonyl-Val-Ala-Aspfluoromethyl ketone (zVAD-fmk) or necrosis using calpain inhibitor Cbz-Val-Phe-H (MDL28170). PC12 cells overexpressing the panprotease inhibitor a2-macroglobulin were subjected to the same experimental conditions. Cell viability was estimated by using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]. To differentiate between apoptosis, or necrosis we used Propidium Iodide and Annexin V staining. Our findings suggest that hypoxia induced cell death on NGF treated PC12 cells shares common features between apoptosis and necrosis. Protease inhibition confreres increased resistance to hypoxia induced cell death.

P360077

Stereochemical effects on functional selectivity at the dopamine D2L receptor

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The novel mechanism of functional selectivity (differential activation of pathways linked to a single receptor) has been shown for dopamine hD2L receptor regulated endpoints such as GIRK, MAPK, ACase, etc. The current work explored the hypothesis that functionally selective ligands induce unusual receptor conformational states that lead to differential activation. We docked the propylnorapomorphine enantiomers [RNPA & SNPA] to the D2R receptor to identify potential residues of importance, and then made and expressed selected mutant receptors. WT D2R binding confirmed that RNPA forms p-OH H bonds with both S5.46 and T3.37 whereas the m-OH interacts with S5.42, while SNPA H-bonds to S5.42 (p & m-OH). When mutated, differential effects were seen on functional endpoints (e.g., S5.42A caused loss of D2R mediated effects on ACase, but not MAPK or AA release). These data demonstrate that single point receptor mutations can make a "normal" ligand become functionally selective, or change the character of a functionally selective compound. We hypothesize that ligand-specific residue interactions contribute to the conformational changes needed to result in activation of specific heterotrimeric complexes.

P360078

Interaction with CAL Regulates Beta1-Adrenergic Receptor Intracellular Trafficking

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GPCRs such as the b1AR must be trafficked to the plasma membrane in order to bind with their extracellular ligands and regulate cellular physiology. Using GST pull-down techniques, we found that the b1AR carboxylterminus directly interacts with CAL, with the last few amino acids (E-S-K-V) of the b1AR carboxyl terminus being the key determinants for the interaction. In cells, full-length b1AR robustly associates with CAL, and this interaction is abolished by mutation of the receptor's terminal valine to alanine (V477A), as determined by coimmunoprecipitation experiments and immunofluorescence co-localization studies. Consistent with observations that CAL is a Golgi-associated protein, over-expression of CAL reduces surface expression of b1AR. Interaction with CAL promotes retention of b1AR within the cell, whereas PSD-95, another b1AR associated PDZ domain-containing protein, competitively blocks b1AR association with CAL and promotes receptor trafficking to the cell surface. These data reveal that CAL modulates b1AR intracellular trafficking, thereby revealing a new mechanism of regulation for b1AR anterograde trafficking through the ER-Golgi complex to the plasma membrane.

P360079

Alteration of G Protein Signaling in Rat Brain by Age

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To explore the role of G protein-mediated signaling as a possible mechanism for aging, the brains were dissected by 9 different parts [frontal cortex, striatum, hypothalamus, hippocampus, cerebellum, cerebral cortex, thalamus, brainstem, and amygdala-septum-preoptic area] from weanling (21-day-old), young (90-day-old), adult (6-month-old) and aged (24-month-old) rats, and the localization, and both gene and protein expression levels of various G protein alpha and beta subunits were examined. The phosphorylation of Akt and ERK1/2 and the ability of purified G proteins from whole brain of each group to

activate PLC-beta, type II adenylyl cyclase, PI3K were investigated. The gene expression levels of various G protein subunits was not significantly changed, however the protein expression level of G protein beta4 subunit was significantly decreased by aging. The phosphorylation of ERK1/2 and the activity of PI3K were significantly increased by aging. The activities of PLCbeta and type II adenylyl cyclase in aged rats were decreased as compared with those in young rats. Therefore, aging induced a reduction of specific G protein subunits, which caused an alteration of G protein signaling.

P360080

Neuroprotection of ginkgolides against hypoxia-induced injury is mediated through activation of p42/p44 MAPK pathway in PC12 cells

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Hypoxia-inducible factor-1 (HIF-1) is a master regulator of cellular and systemic oxygen homeostasis. Under hypoxic conditions, Ginkgo biloba (Ginkgoaceae) extract EGb 761 has been reported to have neuroprotective effects. In this study, we investigated the effects of ginkgolides, the main constituent of EGb 761, on the content and activity of HIF-1α, a key factor to determine HIF-1 activity, in hypoxic PC12 cells induced by cobalt chloride. Our data demonstrated that ginkgolides have a significant protective role against hypoxia induced injury in the PC12 cells. The findings also strongly support our hypothesis that the protective role of ginkgolides is due to the up-regulation of HIF-1α protein expression and modification through the ginkgolides-induced activation of the p42/p44 MAPK pathway. In addition, it was evidenced that ginkgolides could significantly increase the HIF-1 DNA binding activity, which might also be associated with the protective effects of ginkgolides by promoting the expression of target genes of HIF-1 under hypoxic conditions.

Key Words: HIF-1, p42/p44 MAPK pathway, ginkgolides

P360081

Oxidative Stress Involved in Apoptosis of VECs induced by Arachidonic Acid

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To study the mechanism of apoptosis in vessel endothelial cells (VECs) induced by arachidonic acid (AA). The apoptosis of HUVECs was assessed by MTT assay, Giemsa stain, transmission electron microscopy and flow cytometic assay, etc. After 24h exposure to AA, typical morphological changes of apoptosis were observed by Giemsa stain and electron microscopy. The apoptotic ratio in VECs treated with 50 μmol/L, 100 μmol/L and 150 μmol/L AA were (20.7 ±0.6) %, (38.6 ±4.3) % and (52.5 ±7.5) % respectively. Contrarily, low concentration of AA (≤25 μmol/L) exerted no influence on cell viability by MTT assay. Intracellular malondialdehyde increased and glutathione reduced significantly in a dose-dependent manner. Western Blots show that apoptosis triggered by AA was associated with the down-regulation of Bcl-2 expression, but not with Bax and p53. Pretreatment with 50 μmol/L α-tocopherol reduced AA-induced oxidative stress and apoptosis, also inhibited the down-regulation of Bcl-2/Bax ratio. These results suggested that high concentration of AA could induce apoptosis in HUVECs probably via oxidative stress and down-regulation of Bcl-2.

Key words: arachidonic acid; apoptosis; oxidative stress

P360082

SKF83959 increases intracellular calcium in hippocampal neurons of rats

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Recent finding indicating there is a novel phosphatadinositol (PI)-linked D_1 dopamine receptor in brain which mediates the stimulation of dopamine on PI hydrolysis via activation of PLC. The present work was designed to characterize the Ca^{2+} signal regulated by a newly identified PI-linked D_1 dopamine receptor agonist SKF83959 in primary cultures of hippocampal neurons. The results indicated that stimulation of PI-linked D_1 dopamine receptor induced long-lasting increase of basal $[Ca^{2+}]i$ in a time- and dose-dependent manner. In absence of extracellular Ca^{2+}, SKF83959 was still able to induce increase of basal $[Ca^{2+}]i$. Depletion of intracellular Ca^{2+} abolished SKF83959-induced stimulation of Ca^{2+}. Indicating that SKF83959-mediated initial phase of Ca^{2+} increase from intracellular stores triggered the late phase of Ca^{2+} influx. We further demonstrated that activation of PLC/IP3 was responsible for the Ca^{2+} release. Application of AP-V attenuated SKF83959-induced late phase of $[Ca^{2+}]_i$, whereas application of CNQX only slightly lowered the late phase increase of $[Ca^{2+}]_i$. Indcated that both L-type calcium channel and NMDA receptor channel contributed to PI-linked D_1 receptor regulated $[Ca^{2+}]_i$.

P360083

Endothelin-1-induced translocation of RhoA is mediated by endothelin ETA receptors in rat bronchial smooth muscle

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A small GTPase RhoA is a key protein participating in the agonist-induced Ca^{2+} sensitization of smooth muscle contraction including airways. Although the activation pathway of RhoA via membrane receptors is not yet clear in airway smooth muscle, it is known that translocation of RhoA from cytosol to plasma membrane occurs when RhoA is activated. To clarify the receptor subtype(s) contributing to the RhoA activation by endothelin-1 in bronchial smooth muscle, the effects of BQ-123 [cyclo(D-Asp-Pro-D-Val-Leu-D-Trp)], an endothelin ET_A receptor antagonist, and BQ-788 [2,6-dimethylpiperidnecarbonyl-gamma-methyl-Leu-N_{in}-(methoxycarbonyl)-D-Trp-D-Nle], an endothelin ET_B receptor antagonist, on the endothelin-1-induced translocation of RhoA to plasma membrane were examined. Incubation of rat bronchial smooth muscle with endothelin-1 induced a distinct translocation of RhoA to plasma membrane, indicating an activation of RhoA by endothelin-1. The endothelin-1-induced translocation of RhoA was completely blocked by treatment with BQ-123, whereas BQ-788 had no effect. Thus, endothelin ET_A but not ET_B receptors might be involved in the endothelin-1-induced translocation of RhoA in rat bronchial smooth muscle.

Key words: bronchial smooth muscle; Ca^{2+} sensitization; RhoA; endothelin receptors

P360086

Simvastatin inhibits ADMA-induced inflammatory reaction via MAPK pathways in endothelial cells

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Objective: To investigate the effect of asymmetrical dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, on inflammatory cytokines, and the relationship between the protective effect of simvastatin on endothelial cells and ADMA. Methods: Tumor necrosis factor-α (TNF-α), intercellular adhesion molecule-1 (ICAM-1), nuclear factor-κB (NF-κB) were assayed by ELISA and EMSA. Activation of p38 MAP kinase (MAPK) and $ERK_{1/2}$ were also measured. Results: Treatment with oxidative low-density lipoprotein (ox-LDL) or ADMA increased the expression of ICAM-1 in a dose-dependent manner. Ox-LDL (100 μg/ml) or ADMA (30 μM) markedly enhanced the concentrations of TNF-α and ICAM-1, activity of NF-κB, p38 MAPK and $ERK_{1/2}$. Simvastatin (0.1, 0.5 or 2.5 μM) markedly inhibited the elevated concentrations of TNF-α and ICAM-1, the activity of NF-κB, p38 MAPK and $ERK_{1/2}$ induced by ox-LDL or ADMA. Conclusion: Simvastatin inhibits ADMA-induced inflammatory reaction by p38 MAPK and $ERK_{1/2}$ pathways in endothelial cells.

Key words: Asymmetric dimethylarginine; MAP kinase; simvastatin, endothelium

P360087

Agmatine inhibits Matrix Metalloproteinase expression via the regulation of ATF3 in cerebral endothelial cells

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Agmatine, a polycationic amine synthesized by the decarboxylation of L-arginine by arginine decarboxylase (ADC). In this study we will investigate the effect of agmatine administered exogenously and endogenously through overexpression of ADC. While eNOS was increased after ischemic injury, MMPs was decreased by agmatine administered exogenously and endogenously. We also showed L-NAME (NOS inhibitor) altered the suppression of MMP-9 by exogenously administered agmatine. It seems that MMP-9 suppression by exogenously adminis-

trated agmatine is mediated, at least in part, via eNOS. ATF_3 is rapidly induced in response to a variety of stress such as ischemia injury. We found that ATF_3 was increased significantly in ADC overexpression cells, but it was attenuated by NOS inhibitor. Furthermore, we found the suppression of MMP-2 and MMP-9 by agmatine were attenuated in cells transfected with ATF_3 siRNA. Our study indicate that the inhibition of MMPs expression by endogenous agmatine might be mediated via the regulation of ATF_3. Taken together, these results suggest that endogenously administered agmatine suppress the MMP-2 and MMP-9 expression via eNOS-ATF_3-MMPs pathway.

P360088

Multiple signalling pathways of the mouse β_3-adrenoceptor stably expressed in CHO-K1 cells.

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SR59230A was the first selective β_3-adrenoceptor (AR) antagonist described, However agonist actions have been reported at the β_3-AR in some rodent tissues. In CHO-K1 cells expressing mouse β_3-ARs, SR59230A has a full agonistic effect in extracellular acidification rate (ECAR) in the cytosensor microphysiometer at both high and low levels of receptor expression (high: 1118, low: 115 fmol mg^{-1} protein) while it is a classical competitive antagonist for cAMP accumulation in cells expressing low receptor levels. In this study, we examined the signalling pathways utilised by the β_3-AR in response to SR59230A and the selective β_3-AR agonist CL316243. In high expressing cells, inhibitors of adenylate cyclase, PKA, Src, PI3K and P38 MAPK blocked ECAR responses to CL316243 and SR59230A. In contrast, in low expressing cells, only the P38 MAPK inhibitor blocked ECAR responses to CL316243 and SR59230A. In conclusion, the level of expression of receptors plays a significant role in determining the signalling pathways utilised by the mouse β_3-AR expressed in CHO-K1 cells, and both cAMP and P38 MAPK have key roles.

Key words: SR59230A, β_3-adrenoceptor, signal transduction

Acknowledgments: The authors wish to acknowledge support from NH&MRC grant 236884

P360089

AMPA receptor activation up-regulates GABA-A receptor delta subunit mRNA expression via MEK protein kinases in cultured cerebellar granule cells

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Extrasynaptic alpha6-beta-delta subtype of gamma-aminobutyric acid type A receptors (GABA-A-R) mediate tonic inhibition in cerebellar granule cells (CGC). We have shown that AMPA receptor (AMPA-R) activation up-regulates GABA-A-R delta mRNA expression in cultured GCGs. AMPA-R stimulation activates MAPK signalling pathway via Lyn tyrosine kinase and PI3-K. Furthermore, AMPA-R receptor activation results in release of brain-derived neurotrophic factor that enhances the functional state of the TrkB receptor. TrkB signal transduction cascade involves activation of Ras, Raf, MEK, Rsk and CREB. In the present study we investigated the effects of protein kinase inhibitors on AMPA-R-mediated up-regulation of delta mRNA in cultured CGCs. U0126, a potent and selective MEK inhibitor inhibited 60 % of the AMPA-R-mediated up-regulation. Other inhibitors PD 98059, SB 202190, LY 294002 and K252a had no effect on the up-regulation. The results indicate that AMPA-R-mediated up-regulation of GABA-A-R delta subunit is mediated predominantly via MEK pathway.

Key words: GABA, AMPA

Acknowledgements: Supported by the Finnish Society of Sciences and Letters, the Turku University Foundation and the Oskar Oflund Foundation.

P360090

Co-Existence of G Protein-Dependent and-Independent Pathways in Angiotensin II Receptor AT_1-Mediated Transactivation of EGFR

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The objective of this study was to elucidate the signaling mechanisms for AT_1-mediated EGFR transactivation. Methods: Erk phosphorylation was detected using Western blot for EGFR transactivation in AT_1 expressing COS cells in the presence and absence of angiotensin II. Results: D125A/R126L, an AT_1 mutant incapable of activating any G proteins, induced Erk phosphorylation (~40 % of the wild type) in the absence but not presence of EGFR-specific inhibitor AG1478 and PD168393, suggesting a G protein-independent pathway. Consistently, inhibition of Gq signaling using Gq peptide, dominant negative Gq, PKC and PLC inhibitor GF203309X and U73122 failed to impair D125A/R126L-mediated Erk phosphorylation. C-terminal truncation of D125A/R126L at Leu^{314} and Phe^{309} identified a motif ($FKKYFL^{314}$) critical for the G protein-independent EGFR transactivation that was inhibited by Ca^{++} chelator EGTA and BAPTA-AM, but not by CRM197, a metalloprotease ADAM17 inhibitor. Conclusion: The results show that AT_1 simultaneously employs both G protein-dependent and-independent pathways to transactivate EGFR and the latter is Ca^{++}-dependent but EGF-independent.

Key words: AT1, EGFR, transactivation;
supported by NIH grant (HL065492) to YHF

P360091

ASK1 mediated Amyloid βpeptide-induced cerebral endothelial cell apoptosis

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A pathological hallmark of Alzheimer's disease (AD) is accumulation of amyloid-βpeptide (Aβ) in senile plaques. Aβhas been implicated in neuronal and vascular degeneration in AD because of its cytotoxic effects on neurons and endothelial cells. In the present study, we used murine cerebral endothelial cells (CECs) to explore the role of apoptosis signal-regulating kinase 1 (ASK1)-mediated signaling cascade in Aβ-induced CEC death. Aβdephosphorylated Ser967 on ASK1, leading to the dissociation of the ASK1-14-3-3 complex and transient increase of ASK1 kinase activity. In addition, Aβactivated p38 mitogen-activated protein kinase (p38 MAPK), leading to p53 phosphorylation at Ser15 and subsequent binding to DNA. The expression of Bax, a proapoptotic Bcl2 family protein downstream of p53, was upregulated following Aβexposure. Transfection of various dominant negative mutants (DNs) including ASK1 DN, MAPK kinase 3 (MKK3) DN, MKK6 DN and p38 MAPK DN suppressed Aβ-activated p38 MAPK, p53 phosphorylation and Bax expression respectively and reduced CEC death. Bax knockdown using a bax RNAi strategy reduced Bax expression and subsequent CEC death after Aβexposure. These results suggest that Aβactivated an apoptotic cascade involving ASK1-MKK3/6-p38 MAPK-p53 pathway followed by an increase in p53 binding activity to transactivate Bax expression, resulting in CEC death.

Key words: angiopathy, ASK1, Bax, cerebrovascular diseases, p53, p38 MAPK

P37. Biopharmaceuticals

P370001

THE EFFECT OF AZITROMYCIN ON SOME ANTIOXIDANT SYSTEMS IN ANIMALS WITH ULCERI STRESS

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Azitromycin was applied daily orally, 5 days before stress, and the animals were sacrified 1 hour after oral administrated 1 mL absolute ethanol, under the ether anesthesia. Antioxidative parameters (the value of reduced glutatione-GSH and the activity of glutatione paroxidase-GSHPx, glutatione reductase-GSHR, and peroxidase) were determined in liver homogenate. The quntity of GSH was lower in stressed animals, and olso was lower ($p < 0,001$) in animals treated by azitromycin either control and stressed animals. The activity of GSHPx was very reduced in stressed animals comparing to the control, and lower in azitromycin-treated animals($p < 0,001$) comparing to the control and higher than in stressed animals ($p < 0,001$). The activity of GSHR was not statistically diferent in each of compared group. The activity of Px was statistically higher in stressed animals comparing to the contrl, and olso higher in animals treated by azitromycin either in contrl ($p < 0,01$) and stressed animals ($p < 0,001$). Azitromicin protected gastric mucosa against ethanol damage, but reduced glutatione and increased peroxidase activity in the liver of stress-ulcer rats.

Key words: stress - ulcer, azitromycin, enzyme, liver.

P370002

On Combined Phytopharmacological Therapy

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Recent and earlier results ind. influence of combined herbal extracts (rad. rubiae, rad. taraxaci, herb. virgaureae, etc.: $0.1x10^{-9}$ - $100x10^{-6}$ g/ml) on contractions, spontaneous (SC) and to neurogenic electrostimulation (CES: 10/100 Hz, 0.3 ms, 3 s) of human (surgical tissue) and guinea pig preparations are summarized (compared with standard drugs - fenoterol, ouabain, etc.). 1. Vesical detrusor, pyeloureter: positive/negative ino - /chronotropic effects on SC, in - /decrease of CES. 2. Myometrium: positive ino -, but neg. chronotropic effects. 3. Vasa uterinae: vasodilation, SC - inhibition. 4. Vas deferens: CES - Inhibition. Further, 5. cardio - vascular prep. (CV: aorta, heart; fish, frog): Motor effects incl. of crataegus, valeriana, 6. also cactus (opuntia ficus - indica/elata, pfeiffera recta, etc.), 7. patients: CV, renal effects. New and modified (DiacardR, UrolR, etc.) herbal drugs (American, Chinese, Indian, etc.) for application in angio - cardiology (cardiomyopathy, hypertension), gynecology (tocolysis), urology (pyelonephritis, nephrolithiasis) (1. - 7.) could be developed. Lit.: Michailov, Neu, Hohlbrugger et al.: Indian J. Pharm. O - 155, 1985; Urol. int. 36, 225, 1981; Urol. Res. 8/4, 236, 1980.

P38. Gene Therapy

P380001

Knockdown of survivin expression inducing apoptosis of human oral squamous carcinoma cell lines KB and KBv200 by small interference RNA and its mechanism

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ABSTRACT: OBJECTIVE We apply siRNA plasmid directed against survivin in human oral squamous carcinoma cell lines KB and KBv200 to find the influence on their biological property. METHODS The transcription level of survivin gene was detected by semi - quantitative RT - PCR, the protein expression level and the apoptosis rate were analysised by flow cytometry, and the apoptotic morphology was observed under fluorescent microscope after Honchest33528 staining. MTT was used to evaluate the growth depression of tumor cells, and the activation of caspase - 3 was measured by colorimetric assay. RESULTS After transfection, the levels of mRNA and protein expression of survivin in KB and KBv200 were reduced. mu6/survivin plasmid induced apoptosis of tumor cells in time - dependent manner during 24 - 72h, and the apoptosis peak reached at 48h; the typical morphology of apoptosis was observed by Honchest 33528 staining. Also the activation of caspase - 3 was found to increase 2.5 times. MTT assay has shown their growth were inhibited significantly after transfection. Conclusions siRNA could inhibit the expression of survivin in KB and KBv200 and induce their apoptosis significantly.

P380002

Bcl - 2 siRNA increased sensitivity to 5 - fluorouracil and HCPT in HepG2 cells by induced apoptosis

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To investigated the influence of siRNA targeting Bcl - 2 on the human live cancer cells and the changes in drug sensitivity of Bcl - 2 siRNA transfected HepG2 cells. Bcl - 2 siRNA and negative siRNA expression vector were constructed and stably transfected into HepG2 cells. RT - PCR and Immunofluorescence were used to detect the target gene expression. Western Blotting was used to detect protein expression. Drug sensitivity of the cells to 5 - fluorouracil (5 - FU) and HCPT were analyzed with MTT and flow cytometry. The mRNA and protein expression level of Bcl - 2 in Bcl - 2 siRNA stable transfectants were reduced compared with negative siRNA transfected. Bax protein expression had no change and caspase - 3 protein expression showed significantly be upregulated. Bcl - 2 siRNA transfectants had higher cell inhibitory rates after treated with 5 - FU or HCPT. Bcl - 2 siRNA may be a potential therapy agent against human hepatoblastoma.

Key words: Bcl - 2, siRNA, 5 - FU, HCPT

Acknowledgement: Project supported by the National Natural Science Foundation of China (No 30300426) and the Youth Foundation of Hunan province education department (No03B034).

P380003

Small interfering RNA targeting the Bcl - 2 gene induce apoptosis of HL - 60 cell

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To investigate if bcl - 2 siRNA by in vitro transcripted can specific downregulation bcl - 2 gene expression in HL - 60 cells and increase the cell apoptosis, Methods: siRNA synthesized by in vitro transcriptional methods. Cy3 - siRNA uptake was verified by fluorescence microscopy and Bcl - 2 mRNA expression was measured by RT - PCR, the expression level of Bcl - 2 protein was detected by fluorescent staining and flow cytometry. The growth of HL - 60 cells was visualized by MTT and apoptosis was confirmed by Hoechst 33258 and flow cytometry. Results: Bcl - 2 siRNA specificly downregulated Bcl - 2 mRNA and protein expression, and reduced the number of viable cells and increased cellular apoptosis. Conclusions: Downregulation of Bcl - 2 gene expression by RNAi reduces the total number of viable cells by increasing sponstaneous apoptosis.

Key words: small interfering RNA; Bcl - 2; apoptosis; HL - 60

Acknowledgement: Project supported by the National Natural Science Foundation of China (No 30300426) and the Youth Foundation of Hunan province education department (No03B034).

P380004

siRNA blocked Bcl - XL enhanced sensitivity HepG2 cells to 5 - FU and HCPT

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To investigated the influence of siRNA targeting Bcl - XL on HepG2 cells. Bcl - XL siRNA expression vector were constructed and stably transfected into HepG2 cells. RT - PCR was used to detect the gene expression of mRNA. Westeron blot and immunofluorescence were used to detect the gene expression of protein. Drug sensitivity of the cells to 5 - FU and HCPT were analyzed with MTT and flow cytometry. Protein expression of Bcl - XL in Bcl - XL siRNA stable transfectants was observed lower than that of negative siRNA transfectarts, Bax expression has no chang and caspase 3 has increased activity. Bcl - XL transfectants had higher cell inhibitory after treated with 5 - FU or HCPT. Bcl - 2 siRNA cells combined with HCPT or 5 - FU showed lower value than that of negative siRNA. siRNA targeting Bcl - XL gene can specifically down - regulate Bcl - XL expression in HepG2 cells, and increase cell spontaneous apoptosis and sensitize cells to 5 - FU or HCPT.

Key words: Bcl - XL, siRNA, HepG2

Acknowledgement: Project supported by the National Natural Science Foundation of China (No 30300426) and the Youth Foundation of Hunan province education department (No 03B034).

P380005

Human TNF - alpha gene vaccination ameliorates collagen - induced arthritis in mice

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TNF - α is a key factor in the pathogenesis of rheumatoid arthritis. Here, we investigated whether heterologous TNF - α gene vaccination could induce anti - TNF - α antibodies via cross - reaction and prevent the inflammatory arthritis. Two plasmids, a secreted vector (pSecTag - hTNF - α) and a non - secreted vector (pTARGE - hTNF - α), were constructed respectively. The effects of these plasmids on mice with collagen - induced arthritis (CIA) were studied. Both plasmids reduced paw swelling and inflammatory cells infiltration into joints. The spleen cell from treated CIA mice displayed decreased IFN - γ mRNA levels and matrix metalloproteinase - 9 bioactivity in comparison with those from CIA

control. Furthermore, low proliferative, but high apoptotic capacities were observed in the lymphocytes after treatment. Serum levels of TNF-α were also decreased in treated CIA mice. The treatment induced both anti-human and anti-mouse TNF-α antibodies in sera. These results suggest that by inducing cross-reactive antibodies against TNF-α, human TNF-α gene vaccination can ameliorate CIA in mice.

Key Words: TNF-α, CIA, gene cross-reactive therapy
Acknowledgement: Funded by NNSF (No. 30230390).

P380006

Circadian Gene mPeriod2 Overexpression Induces Cancer Cells Apoptosis

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Period2 gene, an indispensable component of the circadian clock, not only modulates circadian oscillations, but also regulates organic function. We examined whether the overexpression of mouse Period2 (mPer2) gene in tumor cells may influence cell growth and induce apoptosis. Overexpression of the mouse PERIOD2 (mPER2) by transfecting the plasmid with mPer2 gene in the mouse Lewis lung carcinoma cell line (LLC) and mouse mammary carcinoma cell (EMT6) results in reducing cellular proliferation and increasing apoptosis, but not in NIH 3T3 cells. Overexpressed mPER2 also altered the expression of apoptosis-related genes. The mRNA and protein levels of c-Myc, Bcl-XL and Bcl-2 were down-regulated, whereas the expression of p53 and bax were upregulated in mPER2-overexpressing LLC cells as compared with control cells which were transferred with empty plasmid. Our results suggest that the circadian gene mPer2 may play an important role in tumor suppression by inducing apoptotic cell death, which is attributable to enhance pro-apoptosis signaling and attenuate antiapoptosis process.

Key Words: Chronobiology, Cancer, Circadian gene

P380007

Hypothalamic Leptin Overexpression Evokes Differential Mechanisms to Facilitate Peripheral Fat Loss

Zhang Yi*.

The Study Objective: To explore mechanisms underlying fat loss due to central leptin overexpression. Methods: Third ventricle injection of adeno-associated virus (rAAV) encoding either GFP or rat leptin. Three experimental groups include: rats given rAAV-GFP and fed ad lib (Control), rats given rAAV-Leptin and rats given rAAV-GFP and pair fed to amount of food consumed by leptin-treated rats (Pair-fed). Results: Food intake and body weight were significantly decreased in the rAAV-Leptin and Pair-fed rats. Leptin reduced fat mass by 46% relative to 12% by pair feeding. Phosphorylation of AMPK and ACC were elevated to 150% and 131% respectively, in soleus muscle in rAAV-leptin animals, but remained unchanged in Pair-fed rats. In contrast, phosphorylated-ACC was reduced with leptin and increased with pair feeding in liver and epididymal white fat (EWAT). Conclusions: Central leptin overexpression activates the AMPK-ACC pathway in skeletal muscle to stimulate fat oxidation. Liver and EWAT appear to use separate mechanism(s) to either mobilize or metabolize fat.

Key Words: Leptin, AMPK, ACC.
Acknowledgement: Supported by VA Medical Research Service and NIH.

P380008

2'-Fluoroarabino- and Arabinonucleic Acid Show Different Conformations, Resulting in Deviating RNA Affinities and Processing of Their Heteroduplexes with RNA by RNase H

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Antisense oligonucleotides (AONs), both artificial and naturally occurring ones, have been explored as potential therapeutics in last two decades. 2'-Deoxy-2'-fluoro-arabinonucleic acid (FANA) and (arabinonucleic acid) ANA paired to RNA are substrates of RNase H, which is believed to play a key role in antisense mechanism. The conformation of the natural DNA:RNA hybrid substrates appears to be neither A-form nor B-form. Consistent with this the conformations of FANA and ANA were found to be intermediate between the A- and B-forms. However, FANA opposite RNA is preferred by RNase H over ANA, and the RNA affinity of FANA considerably exceeds that of ANA. By investigating the conformational boundaries of FANA and ANA residues in crystal structures of A- and B-form DNA duplexes at atomic resolution, we demonstrate that FANA and ANA display subtle conformational differences. The structural data provide insight into the structural requirements at the catalytic site of RNase H. They also allow conclusions with regard to the relative importance of stereoelectronic effects and hydration as modulators of RNA affinity.

P380009

Lentivirus-Mediated Gene Therapy by Suppressing Survivin in BALB/c Nude Mice bearing Oral Squamous Cell Carcinoma

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Gene therapy for oral squamous cell carcinoma (OSCC) is currently under investigation. Survivin is overexpressed in OSCC, making it a promising target for gene therapy. This study was conducted to determine whether lentivirus-mediated gene therapy by suppressing survivin can be exploited in the treatment of OSCC. A lentivirus vector encoding short hairpin RNA (shRNA) targeting survivin was constructed and transfected into KB cells. The results showed that survivin was persistently and markedly reduced; the growth of KB cells was decreased by 34.2% on day 5; the apoptosis rate induced by vincristine (VCR) was increased by 29.8% and caspase-3 activity was also significantly increased; the IC_{50} value of adriamycin (ADM) were 0.09 μg/ml, which indicated that survivin-knockout KB cells were 2.1 times more susceptible to ADM than control; the clonogenic survival rate at 6 Gy of X-ray was 3.7%, less than 15.3% of the control. In the xenograft model, the development of tumors as well as the growth of established tumors was inhibited by transfection of lentivirus. Our study indicates that lentivirus-mediated gene therapy is an attractive strategy in the treatment of OSCC.

P380010

Regulation and Quality Research on Gene Therapy Products in China

Sang Guowei

Gene therapy is one of the most important bio-tech advances in the last 2 decades, yet in China it is still a new field in terms of new drug discovery and development, which requests more strict regulatory governance and comprehensive technical guidelines. In this presentation, the general China NDA application process and timeline are briefly introduced first, followed with the regulation and guidelines for gene therapy specifically, on both clinical trial and quality control research. Those key consideration points on manufacturing process and quality control for gene therapy product in the latest guideline are elaborated. The majority part of the presentation is about the quality standard research results and discussions which have been done in the national quality authority NICPBP, with the examples of Adv-p53, Adv-hIL-2, rAVV-2/hFIX etc. on assay of physicochemical characters, specification, bio-assay, impurities and safety test. The presentation ends with the current gene therapy product status in China, including 18 applications and related information, in which the top hot therapeutic area is oncology. It is aimed to provide the overall understanding of regulatory request and considerations on clinical and quality control for gene therapy applications in China.

Key Words: Gene Therapy, regulation, quality research

P380011

Identifying and charactering novel p53 regulated genes for potential anti-cancer therapy

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The p53 tumour suppressor gene is a transcription factor that can triggers cell cycle arrest or apoptosis in response to different stress stimuli, e.g. DNA damage, activated oncogenes and hypoxia. We have studied p53-dependent gene and protein expression in response to hypoxia using wild type p53-carrying and p53 null HCT116 colon carcinoma cells and a cDNA microarray containing 20,000 transcripts. Hypoxia induced p53 protein levels and p53-dependent apoptosis in the HCT116 wtp53+/+ cells. We found that only a limited number of genes are regulated by p53 in response hypoxia. Most classical p53 target genes are not up-

regulated. However , Fas/ CD95 and MDM2 were induced in response to hypoxia in a p53 - dependent manner , along with several novel p53 target genes that have been implicated in control of cell growth and survival. The functional roles of the identified novel p53 target genes in hypoxia - induced apoptosis are now being investigated. We conclude that hypoxia triggers a p53 - dependent gene expression pattern distinct from that induced by other stress agents and , novel p53 regulated genes identified here can be potentially targeted for anticancer therapy.

Key words : p53 , hypoxia , apoptosis , anti - cancer therapy

P39. Renal Pharmacology

P390001

Reflex Regulation by Intrahepatic Adenosine via A1 Receptors on Renal Water and Sodium Excretion in Healthy and Acute Liver - Injured Rats

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We showed that a decrease in hepatic blood flow, through an associated increase in hepatic adenosine , triggers a reflex that inhibits urine production in healthy and liver - injured rats. The objective was to determine which subtype of adenosine receptor is implicated in the activation of this hepatorenal reflex. Anesthetized rats were instrumented to monitor hepatic , renal circulation and urine flow. In healthy rats , blockade of hepatic adenosine A_1 receptors (8 - cyclopentyl - 1 , 3 - dipropylxanthine , DPCPX) increased urine flow dose - dependently and this response was abolished by liver denervation. Intrahepatic infusion of adenosine decreased urine flow and this response was also blunted by DPCPX. In contrast , blockade of hepatic A_2 receptors (3 , 7 - dimethyl - 1 - propargylxanthine , DMPX) had no significant influence on urine flow. Rats with acute liver injury induced by thioacetamide developed renal dysfunction ; DPCPX , but not DMPX, induced a hepatic nerve - dependent improvement in urine production. In conclusion , the activation of hepatic adenosine A_1 receptors is responsible for triggering the hepatorenal reflex that regulates urine production.

Key Words : hepatorenal reflex , Adenosine receptors , liver , urine.

P390003

Effects of Furosemide , Hydrochlorothiazide , and Benzamil on Sodium and Potassium Transport in ROMK Knockout Mice : The Type II Bartter s Mouse Model

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We have previously demonstrated that the ROMK (Kir1. 1 ; Kcnj1) null mouse has a similar phenotype to Bartter 's syndrome in patients , which manifests as salt wasting , polyuria and metabolic alkalosis. Since ROMK channel mediates K recycling to support Na/ 2Cl/ K - cotransporter (NKCC2) in the thick ascending limb (TAL) , and K secretion in the cortical collecting duct (CCD) , we compared the effects of furosemide (F) , hydrochlorothiazide (HCTZ) and Benzamil on renal functions in wild - type (WT) and ROMK null mice by metabolic and renal clearance methods. F produced diuretic , natriuretic and kaliuretic effects in WT but not in null mice. In contrast , HCTZ produced larger natriuretic effects in ROMK null than WT mice. Benzamil has similar natriuretic effects in ROMK null and WT mice. It reduced FEK by 68 % in WT consistent with the expected reduction of K secretion , due to blocking of ENaC in principal cells. The reduction of FEK by Benzamil was 50 % less in ROMK null mice. In conclusion , NKCC2 activity is diminished , but thiazide - sensitive NaCl cotransporter activity is upregulated ; ENaC activity did not change significantly , and K secretion in the CCD is compromised in ROMK null mice.

P390004

Experimental Studies of Traditional Chinese Medicine to Treat Renal Diseases

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AIM To investigate the effects of TCM, Benefit Kidney Granule (BKG) on animal models of renal diseases. METHODS (1) Nephritic syndrome : Puromycin aminonucleoside was injected into right internal jugular veins and the rats were observed for 4 weeks. (2) Renal interstitial fibrosis : PAN was injected in the same way as above and the rats were observed from 5th week through 27th week. (3) Water - loaded rats : all the rats were burdened with 1 % NaCl solution. RESULTS Compared with pathologic group : (1) the amount of 24h urine protein excretions (Uprot) and the level of cholesterol , triglyceride , blood urea nitrogen and creatinine in treated group was significantly lower , and the level of serum total protein and albumin were higher ; (2) light microscopy of treated group showed that the tubular degeneration , atrophy were alleviated ; the immunohistochemical staining assay showed the positive staining areas of TGF - β1 and α-SMA protein in renal interstitium in treated group were reduced ; (3) the value of total urine quantity in treated group was obviously increased. CONCLUSION BKG acts on multiple targets in the complicated pathogenesis of renal diseases and may become the promising drugs.

P390005

Activation of ERK by angiotensin type 2 receptor stimulation in renal tubular cells

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To clarify the role of angiotensin (Ang) type 2 receptor (AT_2R) in the renal tubular cells , we examined AT_2R mediated phosphorylation of extracellular signal - regulated kinases (ERK) in MDCK cells and rat renal primary culture cells. Rat AT_2R was stably expressed in MDCK cells. Stimulation of AT_2R - expressed MDCK cells with Ang II in the presence of angiotensin type 1 receptor (AT_1R) blocker , candesartan did not change the turnover of inositol phosphates and the cyclic AMP accumulation in the cells. The AT_2R stimulation reduced the forskolin - induced cyclic AMP accumulation and this inhibition was abolished by the pretreatment of pertussis toxin. Ang II increased the phospho - ERK in AT_2R - expressed MDCK cells. This increase in phospho - ERK was inhibited by AT_2R antagonist , PD123319 or pertussis toxin , but not by candesartan. The expression of both AT_1R and AT_2R mRNA was observed in the primary culture cells from rat renal medulla. Both candesartan and PD123319 inhibited Ang II - induced increase in phospho - ERK in the primary culture cells. These results suggest that AT_2R induces activation of ERK through Gi protein - coupled mechanisms in renal tubular cells.

Key words : angiotenisn II , kidney

P390006

AT1 receptor activation contributes to the renovascular specific PTH/ PTHrP receptor (PTH1R) downregulation in spontaneously hypertensive rats (SHR) .

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Parathyroid hormone - related peptide (PTHrP) induces renal vasodilatation which is impaired in SHR through the downregulation of PTH1R expression (mRNA and protein) in intrarenal arterioles , thus contributing to high renal vascular resistance. The objective of this study was to analyze the mechanism of this defect. We found that the PTH1R deregulation was not present in intrarenal arteries from prehypertensive SHR. In SHR with established hypertension , the defect was specific for renal arterioles. Treatment by losartan reversed the downregulation of PTH1R expression and restored PTHrP - induced vasodilatation in ex vivo perfused kidneys. In an AngII - independent model of hypertension (DOCA - salt rats) , renovascular PTH1R expression and related vasodilatation were not altered. In renovascular SMC from Wistar rats , AngII destabilized the PTH1R mRNA, a feature spontaneously observed and reversed by losartan in cells derived from SHR. Together , these data demonstrate that AngII acting via the AT1R destabilizes the PTH1R transcript in intrarenal arterioles from SHR. This process is kidney - specific and independent from the blood pressure increase.

Key words : PTH1 receptor , renal circulation , hypertension , angiotensin II.
Acknowledgement : Research supports from INSERM and Region Alsace.

P390007

Brief small intestinal ischemia lessens the renal ischemia - reperfusion injury in rats

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objective : To investigated the effect of small intestinal IPC on renal I/ R injury in rats. Methods : Renal I/ R injury was induced by a 45 - min renal artery occlusion and 2 - h or 24 - h reperfusion in rats with a previous contralateral nephrectomy , and ischemic precondtioning was induced by three cycles of 8 - min ischemia and 5 - min reperfusion of the small intestine. The concentrations of plasma creatinine

(Cr) and blood urine nitrogen (BUN), and the level of malondialdehyde (MDA) and the activities of superoxide dismutases (SOD) and catalase (CAT) in renal cortex were measured. Renal histopathology examination was also performed. Resuts: Pretreatment with intestinal ischemic preconditioning significantly alleviated renal I/R injury, as shown by a decrease in the level of Cr, BUN and MDA, an improvement of morphological changes and the better preservation of activities of SOD and CAT. Conclusion: Remote ischemic preconditioning of the small intestine protect against renal I/R injury by the inhibition of lipid peroxidation and preservation of antioxidant enzyme activities.

P390008

Role of Angiotensin II AT_2 receptors in sodium metabolism in obese Zucker rats.

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Angiotensin II AT_1 receptor antagonist treatment reduces blood pressure and promotes natriuresis to greater extent in obese than in lean Zucker rats, a model of insulin resistance/mild hypertension. We reported that the enhanced AT_1 antagonist-induced natriuresis was due to increased AT_2 receptor function in obese Zucker rats (OZR). Here we investigated the mechanism of AT_2 receptor-mediated natriuresis. We found that AT_2 receptors are up regulated in cortical membranes of obese compared to lean rats. Infusion of AT_2 receptor agonist induced natriuresis in obese, not in lean rats. In isolated proximal tubules, AT_2 agonist (dose dependently) inhibited the Na^+, K^+-ATPase (NKA) activity in obese not in lean rats. The NKA inhibition was associated with the agonist dose dependent increase in NO and cGMP and abolished by inhibiting guanylate cyclase and NO synthase suggesting the invdolvement of NO/cGMP pathway. The NKA inhibition was mediated via cGMP-, not cAMP-dependent protein kinase pathway. The data suggest that AT_2 receptors via directly inhibiting tubular Na-transporter increase renal Na-excretion serving as a compensatory mechanism to oppose the enhanced Na-retention effect of AT1 receptors in OZR.

P390009

Molecular mechanism of gender differences in progression of chronic renal failure in 5/6 nephrectomized (Nx) rats

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Women and female rats with chronic renal failure (CRF) progress to end stage renal disease much slower than males. This study was aimed at delineating key molecular pathways contributing to gender-different CRF pathogenesis. Renal transcripts of genes in essential molecular pathways in Nx rats were examined using branched DNA signal amplification assay. Male Nx rats had marked kidney injury, anemia and malnutrition; Nx females had only mild kidney injury. Compared to control male kidneys, females had higher transcripts of androgen receptor (AR), aryl hydrocarbon receptor (AhR), p53, and Cyp4a1, but lower transcripts of estrogen receptor alpha (ERa). Compared to Nx-male kidneys, females had: 1) less decrease in ERa and peroxisome proliferator-activated receptor alpha; 2) no decrease in cyclooxygenase-2 or increase in AR, cytokines, early growth response-1, c-Myc, and Fas ligand; but 3) increase in Cyp4a1 and decrease in AhR, p53, and angiotensin converting enzyme (ACE). Renal activities of ACE and caspase-3 increased in Nx-males, but not Nx-females. In conclusion, gender-divergences in ERa/AR, AhR, p53, and Cyp4a1 may explain gender differences in CRF progression and outcome of renal transplantation.

P390010

Chaihuang-Yishen Granule Improves Puromycin Amino Nucleoside Induced Renal Injury

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Objective: The current study is to investigate molecular and cellular mechanisms of CYG in puromycin aminonucleoside (PAN) induced nephrotic syndrome. Methods: Wistar rats were divided into six groups of sham operation, PAN-model, PAN-model with high-dosage CYG (CYG-H), PAN-model with median-dosage CYG (CYG-M), PAN-model with low-dosage CYG (CYG-L), and PAN-model with Fosinopri (FP). All rats were sacrificed at day 31th for blood biochemistry; kidneys histology and RT-PCR analysis. Results: PAN induced nephrotic syndrome was successfully produced in rats. CYG and FP treatments also improved protein content in blood and reduced total cholesterol and triglyceride in blood. Moreover, CYG and FP improved the damage of interstitial induced by PAN. Conclusion: Chaihuang-Yishen Granule attenuates PAN-induced kidney injury possibly through bone morphogenetic protein signal transduction pathway.

P390011

Therapeutic mechanism of Saikosaponin-d in anti-Thy1 mAb 1-22-3 induced rat model of glomerulonephritis

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Aims: The study examines the effects of Ssd on progression of mesangioproliferative glomerulonephritis induced by anti-Thy1 monoclonal antibody 1-22-3 (mAb 1-22-3) in uninephrectomized rats. Methods: Eighteen female Wistar rats were first received uninephrectomized and mAb 1-22-3 injection, and then were divided into three groups: treated daily with phosphate-buffered saline (PBS), 0.6 mg/kg and 1.8 mg/kg of Ssd. Urinary protein concentration and systolic blood pressure were evaluated and the kidneys were collected and subject to histological and immunohistological evaluation. Results: Ssd reduced the amount of urinary protein and systolic blood pressure. Ssd administration also decreased extracellular matrix expansion, crescentic formation as well as infiltration of macrophage and CD8+ T lymphocyte. Moreover, Ssd significantly reduced expression of transforming growth factor beta1 (TGF-b1) and type I collagen in the kidneys. Conclusion: Ssd inhibits the progression of mesangioproliferative glomerulonephritis through reduction of the expression of TGF-b1 and the infiltration of macrophage and CD8+ T lymphocyte.

P390012

Gender Difference in the Development of Renal Damage in Double Transgenic Rats

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Double transgenic rats (dTGRs) harboring both human renin and human angiotensinogen genes, are used to characterize renin inhibitors. In this study, we investigated whether male dTGRs are more susceptible to develop hypertension, albuminuria, impaired renal function and renal damage than females. Urine samples were collected in males (n=15) and females (n=15) from week 4 to 7 of age. In addition, blood pressure (BP) and heart rate (HR) were measured. At week 8, renal clearance was measured and kidneys were examined for structural changes. Progressive albuminuria developed in males between week 4 and 7 and was higher than in females. At week 8, renal plasma flow and glomerular filtration rate were lower in males than in females. Whereas BP and HR were not significantly different, males developed more severe vascular and tubulo-interstitial lesions in the kidney than females. All 15 female dTGRs reached week 8, whereas 7 out of 15 males died before week 8. In conclusion, both male and female dTGRs develop severe hypertension. However, males are more susceptible to develop albuminuria, impaired renal function and renal damage and show a higher mortality rate than female dTGRs.

P390013

Serve the histomorphology changes in experimental rats with pelvic inflammation treated with FuKeQianJin Soft Capsule

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Objective: To observe the influence of FuKeQianJin Soft Capsule on the histomorphology in rats with membrane inflammation in the uterus. Methods: The pathological model of membrane inflammation in the uterus was established by deep injection of phenol mucilage into vagina. 60 SD female rats were randomly divided into 6 groups, the normal group、the model control group、the HuaHongPian treated group and other three groups treated with different doses of FuKeQianJin Soft Capsule. All drugs were given orally for 12 days. After treatment, blood rheology was measured and uterus histomorphology was checked. Results: The membrane inflammation in the uterus were improved significantly in the group given FuKeQianJin Soft Capsule as compared with model control group. Conclu-

sion: FuKeQianJin Soft Capsule was effective in treating pelvic infection in experimental rats

Key words: FuKeQianJin Soft Capsule; Membrane inflammation in uterus; histomorphology; Blood rheology

P390014

Immunohistochemical and kinetic characterisation of UDP-glucuronosyltransferase (UGT) 1A and UGT2B proteins in human renal cortex and medulla.

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Immunohistochemical and kinetic approaches were used to characterise localisation and activity of UGT1A and UGT2B proteins in human renal cortex and medulla. Commercial UGT1A and UGT2B polyclonal antibodies were used for the immunolocalisation studies, and naproxen glucuronidation as a measure of the activity of cortical (HKCM, n = 7) and medullary microsomes (HKMM, n = 6). Within the cortex, UGT1A and UGT2B proteins were localised in epithelial cells of the proximal and distal convoluted tubules and were absent in the glomerulus and associated vasculature. In the medulla, UGT1A and UGT2B proteins were localised in the Loop of Henle and the collecting ducts. Naproxen glucuronidation exhibited biphasic kinetics; the apparent Km and CLint values for the high affinity component were 30.6 ± 15.8 μM; 4.6 μl/min/mg and 60.9 ± 42 μM; 1.1 μL/min/mg for HKCM and HKMM, respectively. Inhibition by fluconazole identified UGT2B7 as the predominant enzyme in HKCM and HKMM catalysing naproxen glucuronidation. These data further indicated that the intrinsic clearance of naproxen via glucuronidation is four fold greater in human kidney cortex than in the medulla.

Key words: immunolocalisation, human kidney, glucuronidation.

P390015

Chiral selective effects of doxazosin and its enantiomers on blood pressure and bladder vesical pressure in anesthetized rats

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Objective: To study chiral selective effects of doxazosin enantiomers on blood pressure and bladder vesical pressure in anesthetized rats. Method: In anesthetized rats, carotid blood pressure, left ventricular pressure of the heart and the vesical pressure of the bladder were recorded. Results: Administration of S-doxazosin at 0.25, 2.5, 25 and 250 nmol/kg iv produced a dose-dependent decrease in blood pressure, but its depressor effect was significantly weaker than that induced by R-doxazosin and racemin-doxazosin (rac-doxazosin), and the ED_{50} values of R-doxazosin, S-doxazosin and rac-doxazosin were 15.64, 45.93 and 128.81, respectively. rac-Doxazosin and its enantiomers administered accumulatively in anesthetized rats induced a dose-dependent decrease in the left ventricular systolic pressure (LVSP) and ±dp/dtmax, and a potency order of the three agents was R-doxazosin > rac-doxazosin > S-doxazosin. rac-Doxazosin and its enantiomers decreased the vesical micturation pressure dose-dependently at 2.5, 25 and 250 nmol/kg, and the inhibitory potency among the three agents was same. Conclusion: S-doxazosin has chiral selectivity between cardiovascular system and urinary system in anesthetized rats.

P390017

Cyclosporine induced nephrotoxicity: possible oxidative stress mechanism

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Recent studies indicate that Reactive Oxygen Species induced oxidative stress and lipid peroxidations are the important mechanisms implicated in the pathophysiology of nephrotoxicity with cyclosporine A (CsA). In the present study we examined the effects of CsA on oxidative stress markers in renal tunisian transplants patients. We studied oxidative stress in 33 renal transplant patients receiving two different immunosuppressive regimens (18 on CsA, 15 on azathioprine/prednisolone) and 20 normal controls. Change in lipid peroxidation (assessed as thiobarbituric acid reacting substances, TBARS), antioxidant enzyme activities (superoxyde dismutase SOD and glutathione peroxidase GSHPx) were studied. TBARS was raised in CsA group compared with controls ($p < 0.001$) and azathioprine group ($p < 0.01$). Chronic CsA treatment caused significant decrease in SOD levels as compared to azathioprine group and controls ($p < 0.05$). GSH-Px activity was reduced in the CsA group compared to azathioprine group ($p < 0.05$) and controls ($p < 0.001$). The major findings of the present study suggest that oxidative stress might play a significant role in CsA-induced nephrotoxicity.

P390018

FK506 Treatment Alters the Vascular Reactivity of Renal and Mesenteric Vascular Beds

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The contribution of endothelin-1 (ET-1) to FK506-induced hypertension, vascular dysfunction and kidney malondialdehyde (MDA) levels were investigated in rats treated with FK506 for 8 or 30 days. Kidney/mesentery of rats was perfused and perfusion pressure was recorded. The response to noradrenaline (NA) only in renal vascular beds was increased by FK506 after 8 days and this increase was prevented by Bosentan. Sodium nitroprusside (SNP)-induced decreases in perfusion pressure were attenuated by FK506, in kidney and mesentery, which was not prevented by Bosentan. After 30 days, there was an increase in blood pressure, which was prevented by bosentan, but no change in the response to NA in either kidneys or mesentery. FK506 decreased the response to SNP in kidneys, but not in mesentery. FK506 increased MDA levels in the kidneys after 30 days. Bosentan did not change this increase. Results indicated that ET-1 plays a role in the FK506-induced change in vascular reactivity to NA in kidneys and drug-induced hypertension in the rats, but not in the impaired vasodilation caused by FK506. There was no relationship between oxidative stress and FK506-induced hypertension.

Key Words: FK506, ET-1, kidney, mesentery

P390019

Protective Effects of Quercetin Preparations by Experimental Acute Renal Failure.

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Strengthening of free-radical oxidation reactions in the tissues of kidneys occupies one of the leading places in pathogenesis of acute renal failure (ARF). Antioxidant quercetin demonstrates renoprotective potential. The object of our investigation was to determine the effects of original Ukrainian quercetin preparations (water-soluble Corvitin and liposomal Lipoflavon) on experimental ARF caused in laboratory rats by intramuscular injection of 50% glycerol solution. Corvitin and Lipoflavon were administered in the dose of 8 mg/kg once intraperitoneally 40 minutes after the injection of glycerol. Administration of quercetin preparations already at the 24th hour after ARF modulation increased diuresis in 1.6 (Corvitin injection) or 2.6 (Lipoflavon) times, decreased of protein excretion in 1.6 (Corvitin) or 1.2 (Lipoflavon) times coming to the norm the creatinin blood concentration, decreasing the intensity of lipid and protein peroxidation, and increasing SH-groups' content in kidney tissues. Besides, water-soluble quercetin (Corvitin) showed renoprotective effect faster but for a shorter period, while liposomal quercetin (Lipoflavon) acted longer and mitigated the signs of experimental ARF better.

Key words: acute renal failure, water-soluble quercetin, liposomal quercetin, renoprotection.

P390020

11beta-HSD2 regulation by selective COX-2 inhibition after release of bilateral ureteral obstruction (BUO) in rats

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Previously we demonstrated that BUO for 24 h followed by 3 days release is associated with a decrease in the renal cortical expression of 11-beta hydroxysteroid dehydrogenase type 2 (11beta-HSD2), which protects the mineralocorticoid receptor from illicit activation. This could contribute to altered sodium handling after release of BUO. We tested the hypothesis that COX-2 activity regulates 11beta-HSD2 expression after release of BUO. Rats were subjected to 24h BUO followed by release for 3 days. Kidneys were removed and prepared for immunoblotting. In a subset of animals, kidneys were perfusion fixed for immunocytochemistry. Release of BUO was associated with marked polyuria and significantly increased COX-2 expression in cortex of BUO-3DR. Urinary PGE2 ex-

cretion was stimulated after release of BUO. Administration of the COX-2 antagonist parecoxib (PCOX) abolished this stimulation. PCOX treatment prevented downregulation of 11beta-HSD2 in BUO-3DR rats. Immunohistochemistry showed co-localization of EP1 receptor and 11beta-HSD2 in cortical collecting ducts. These data indicate that COX-2 activity and PGE2 through EP1 receptors may contribute to altered expression of 11beta-HSD2 in response to BUO.

P390021

Postnatal adrenalectomy stimulates kidney COX-2, impairs urinary concentrating ability and reveals a need of aldosterone for normal kidney development

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We hypothesized that inhibition of renin-angiotensin system components in the postnatal period affects kidney development through aldosterone and involves enhanced COX-2 activity. After adrenalectomy (ADX) at postnatal day 10 (P10), rats displayed normal plasma [Na^+] and osmolality and markedly elevated renin parameters at P20. ADX rats exhibited smaller outer medulla and papilla, a decreased medullary interstitial osmolality and urinary concentrating abiliy. COX-2 mRNA and protein was significantly enhanced by ADX. Combined substitution with DOCA and corticosterone corrected changes in COX expression and kidney morphology after ADX, while corticosterone alone had minor effects. Inhibition of COX-2 with parecoxib (5 mg/kg/day, P17-P20) increased body weight gain, papillary osmolality and urinary concentrating ability and lowered plasma renin in ADX rats. Weight loss and plasma osmolality increase after dehydration were attenuated significantly by COX-2 inhibtion. Thus, lack of aldosterone leads to kidney medullary maldevelopment and renal COX-2 activity contributes to the salt-loosing phenotype in mineralocorticoid-defient states.

P390022

Expression and localization of S-adenosylhomocysteine (SAH)-hydrolase in the rat kidney following of CO intoxication in vivo

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Hypoxia increases the expression levels of various proteins. These cellular changes require an enhanced gene expression associated with high transmethylation activity (mRNA cap methylation) in the nucleus. Since SAH-hydrolase regulates most SAM-dependent transmethylation reactions by hydrolyzing the potent feedback inhibitor SAH to adenosine and homocysteine we analyzed the effect of hypoxia by carbon monoxide (CO) inhalation (1200 ppm) on SAH-hydrolase gene expression and its localization in rat kidneys. CO lowered renal SAH-hydrolase mRNA expression by 64% whereas SAH-hydrolase activity was not changed during 4 hours of hypoxia 0.7 ±0.04 vs. 0.75 ±0.06 mU/mg. Using two-channel immuno-fluorescence confocal laser scan microscope SAH-hydrolase was visualized in different cells of the hypoxic rat kidney. A very bright immunofluorescence of SAH-hydrolase was observed in the nuclei of interstitial cells of renal cortex and medulla indicating translocation of SAH-hydrolase from the cytosol into the nucleus. These data suggest that SAH-hydrolase accumulation in the nucleus is involved in maintaining efficient transmethylation reactions in transcriptionally active cells by removing the product inhibitor SAH.

P390023

Role of intracellular amino acids on activation of L-arginine-nitric oxide pathway in platelets from chronic renal failure patients

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L-arginine uptake is rate-limiting for intraplatelet NO synthesis, which is essential for platelet function, and this pathway seems to be disturbed in chronic renal failure (CRF). Malnutrition is a co-morbid factor of CRF and affects the outcome of uraemia. We have demonstrated activation of L-arginine-NO pathway in well-nourished patients. This study investigates platelet aggregation, cGMP level and L-arginine transport under zero-trans conditions in platelets from CRF patients, correlating with nutritional status. 36 CRF patients were included in this study. Platelet aggregation induced by collagen was significantly impaired in eutrophic CRF patierts and basal cGMP levels in platelets were enhanced in well-nourished CRF patients compared to the other groups. Zero-trans condition did not affect L-arginine transport. In conclusion, L-arginine influx via y+L seems to be influenced by the presence of amino acids at the trans-side of the platelet membrane. In addition, we showed enhanced cGMP and decreased platelet aggregability limited to well-nourished CRF. The absence of an adaptive increase in the L-arginine-NO pathway in platelets from malnourished CRF patients may account for the thrombotic events.

P390024

Effect of renal failure on metabolic disposition of lidocaine in patients undergoing and not undergoing hemodialysis

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Objectives: The aim of this study was to investigate the effect of chronic renal failure (CRF) on the pharmacokinetics of lidocaine and its 2 main metabolites, MEGX and GX, in patients undergoing and not undergoing hemodialysis. Methods: Patients were divided in 4 groups, each including 10 subjects, on the basis of creatinine clearance (CL_{CR}): control subjects (CL_{CR} > 80 ml/min), patients with moderate and severe CRF (CL_{CR} between 30 and 60, and < 30 ml/min, respectively); anuric patients undergoing hemodialysis. Results: Lidocaine clearance decreased on average by 19% and 49% in patients with moderate and severe CRF, respectively, whereas it remained virtually unchanged in patients on hemodialysis. MEGX levels remained unchanged, whereas the levels of GX, which is mainly eliminated in urine, increase markedly in all groups of nephropatic patients. Conclusions: CRF may have a clinical relevant impact on the pharmacokinetics of drugs eliminated by liver metabolism. The observation that lidocaine clearance is restored towards normal in patients receiving hemodialysis suggests that a dialyzable uremic toxin is responsible for the inhibition of its hepatic metabolism.

Keywords: lidocaine, renal failure, hemodialysis, pharmacokinetics
This work was supported by a grant from the University of Padova.

P390025

EFFECTS OF THE STOBADINE AND TAURINE ON RENAL ISCHEMIA/REPERFUSION INJURY

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Reactive oxygen species play a role in the pathogenesis of ischemia/reperfusion (I/R) injury in kidney. Study was designed to investigate the effects of antioxidant compounds stobadine and taurine in I/R-induced renal failure. Wistar rats were allocated into six groups: Sham, I/R, stobadine-treated, I/R+stobadine-treated, taurin-treated, and I/R+taurin-treated. At the beginning of reperfusion, taurine (7.5 mg/kg) or stobadine (2.0 mg/kg) was given to the rats. I/R was achieved by occluding the renal arteries bilaterally for 40 min. Following 6 h of reperfusion, blood and tissue samples were harvested. I/R resulted a significant decrease in kidney MDA and GSH levels that were restored by stobadine or taurine treatment. Decreased activity of glucose-6-phosphate dehydrogenase observed after I/R was not changed by taurin, but significantly ameliorated by stobadine treatment. Neither stobadine nor taurine altered 6-phosphogluconate dehydrogenase activity after IR. I/R did not induce a significant difference in kidney glutathione peroxidase activity.

Supported by TUBITAK/SBAG-SLOVAK-2 (103S180) and Gazi University-BAP(01/2004-83).

P390026

The Effects of Paecilomyces cicadae (Miquel) Samson on chronic renal failure in rats

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Objective To investigate Paecilomyces cicadae (Miquel) Samson on chronic renal failure (CRF) in rats. Methods Male SD rats were induced CRF by right kidney removal and left kidney partly excision(2/3rd) or whole cauterization. Results Blood Urea Nitrogen (BUN), plasma creatinine (CRE), K^+, Na^+, Ca^{2+} were analyzed before surgery, 6 weeks after surgery and oral gavage treatment for 12 weeks. CRF rats were developed 6 weeks after surgery as evidenced by a marked

increase in BUN and CRE, the symptom of renal failure was improved by Paecilomyces cicadae (Miquel) Samson (0.6g/kg - 2.4g/kg) and Cordyceps sinenisis (Berk.) Sacc. (2g/kg) oral gavage treatment for 12 weeks by a marked decrease in BUN and CRE compare to the CRF rats without any treatment. Conclusion Paecilomyces cicadae (Miquel) Samson has benificial effect in CRF and nephriprotective for renal failure. Key Words chronic renal failure (CRF), Paecilomyces cicadae (Miquel) Samson, Blood Urea Nitrogen (BUN), plasma creatinine (CRE)

P390027

Hematide™ Erythropoiesis Activity Dependent on Renal Function

Qing Fan, Susan Winslow, Min-ja Chen, Kei-lai Fong and Kathryn Woodburn Affymax Inc and Pennsylvania Biolab, Inc Hematide™ is a PEGylated, synthetic peptide being developed for the treatment of anemia associated with chronic kidney disease and cancer.

Objective: To evaluate the pharmacokinetics and erythropoietic activity of Hematide in rats with chronic renal insufficiently which is akin to end stage renal disease in humans. These results will then be compared with normocythemic animals and will aid in defining the dose for human clinical trials in one of the target patient populations which is patients with chronic kidney disease. Methods: The plasma pharmacokinetics and erythropoietic activity were assessed in normocythemic and chronic renal insufficiency (CRI) rats following IV and SC administration of Hematide. Results: Plasma clearance was 2-fold lower in CRI rats than clearance in rats with normal renal functions, resulting in higher exposure. CRI rats were more responsive to Hematide, as measured by reticulocytes and Hgb production. Conclusion: Hematide clearance is dependent on kidney function then doses, both nonclinically and clinically, need to be adjusted dependent on indication/kidney function.

Key words: erythropoiesis, kidney impairment, dosing

P40. Drug Abuse, Tolerance and Dependence

P400001

Discovery and functional expression of brain cannabinoid CB2 receptors involved in depression and drug abuse.

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Two well-characterized cannabinoid receptors (CBrs), CB1 and CB2 mediate the effects of cannabinoids and marijuana. In mice the effects of CB2 antisense oligonucleotide injection into the brain and i.p treatment with JWH015 in motor function and plus-maze tests were evaluated. We used RT-PCR, immunoblotting, immunohistochemistry, and hippocampal cultures to determine the expression of CB2 CBrs in rat brain and in mice brain exposed to chronic mild stress (CMS) or those treated with cocaine or heroin. JWH015 reduced locomotor activities while CB2 antisense oligonucleotide microinjection induced anxiolysis. In CMS animal's expression of CB2 CBrs was enhanced and modified in brains of cocaine and heroin treated rats. Abundant iCB2 in neuronal and glial processes were detected in brain. Contrary to the prevailing view that CB2 CBrs is restricted to peripheral tissues, we demonstrate that CB2 CBrs and their gene transcripts are present in brain. The presence and functional expression of CB2 CBrs in brain may be exploited as new target in the treatment of depression and substance abuse.

Key words: Cannabinoid CB2 receptors, brain, depression, drug abuse.
Supported by WPUNJ and NIDA.

P400002

Tonic Modulation of Ethanol-Induced Ataxia by Mouse Cerebellar α- and β-Adrenergic receptors

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To further our study of neurochemical modulation of ethanol ataxia (EA), we investigated possible role of cerebellar α/β-adrenergic receptors in EA. Male CD-1 mice received intracerebellar infusion of adrenergic drugs to evaluate their effect on EA (2 g/kg ip) by Rotorod. Isoproterenol (ISP), phenylephrine (4,8,16ng each), and atenolol (AT; 2,4,8ng), propranolol (PROP;4,8,16ng), markedly attenuated and accentuated, respectively, EA indicating adrenergic modulation. Norepinephrine attenuated EA that was partly blocked by AT, suggesting a role of α_1, α_2 and β_2 receptors. The attenuation of EA by ISP was via β_2 as AT virtually blocked it. Strong tonic β_2 modulation was supported by marked accentuation of EA by AT and PROP and perhaps by prazosin-induced attenuation due to unopposed β_2 receptors. Yohimbine caused attenuation of EA that indcated α_2 involvement. It is well known that agonists of β receptor increase and of α_2 receptor inhibit cAMP production. Therefore, overall the attenuation of EA by α_1, β_2 agonists; α_1, α_2, antagonists and accentuation by β- antagonists may reflect a functional correlation to an increase and decrease, respectively, in cAMP production in agreement with our previous reports.

P400003

Diversity of functional nicotinic acetylcholine receptor subtypes in rat VTA dopaminergic neurons and nicotine dependence

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Dopaminergic (DA) neurons located in the midbrain ventral tegmental area (VTA) play important roles in nicotine reward and dependence. Nicotinic acetylcholine receptor (nAChR) a2-a7 and b2-b4 subunits are expressed as message in the VTA, but the functions of nAChR subtypes are unclear. Using patch-clamp recording from single DA neurons acutely dissociated from the VTA, we have discriminated three types of nAChR-mediated responses based on pharmacological and kinetic properties. Type I neurons (58%) responded strongly to RJR-2403, which was blocked by dihydro-b-erythroidine, suggesting a a4b2-nAChR. Type II neurons (26%) reacted strongly to choline, which was blocked by 10 nM methyllycaconitine, suggesting a a7-nAChR. However, type III neurons (16%) exhibited large, slowly-decaying current responses to both ACh and cytisine, suggesting a possibly complex mixture of nAChR subtypes (a3a4b2b4). During 10-min exposure to 500 nM nicotine, only type I neuron firing was persistently increased. Conclusion: VTA DA neurons express three subtypes of nAChR which play different roles in nicotine reward and dependence.

Key words: nAChR, VTA, DA neuron, patch-clamp.
Supported by IMHR pilot grant and ABRC grant.

P400004

Association of cannabinoid receptor CB2 gene with alcoholism and development of alcohol preference.

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We tested the hypothesis that genetic variants of CB2 gene might be associated with alcoholism in human population and this was probed using the non-synonymous polymorphisms, R63Q and H316Y in the CB2 gene in Japanese alcoholic subjects. In mice CB2 gene expression was determined in brain regions after acute administration of ethanol, and development of alcohol preference. Ethanol consumption in mice subjected to chronic mild stress and the effect of chronic daily administration with CB2 agonist JWH015 on ethanol consumption in stressed and control animals were measured. High incidence of the Q63R but not the H316Y polymorphism was found in Japanese alcoholics. Mice that developed alcohol preference had reduced CB2 gene expression and chronic treatment with JWH015 enhanced alcohol consumption in stressed but not in control mice. CB2 cannabinoid receptors are involved with the effects of alcohol along with epigenetic factors, such as stressors, and may be targeted with CB2 ligands in alcoholism.

Supported by University of Tsukuba and WPUNJ center for research.

P400005

Evaluation of the role of 5-HT_2 receptors in Dorsal and Median raphe nuclei on the morphine withdrawal syndrome in rat

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OBJECTIVES: The present study was performed to investigate the role of 5-HT_2 receptors in dorsal and median raphe nuclei on the withdrawal syndrome of morphine and acceleration the restraint of opioides. METHODES: Experiments were performed on adult male wistar rats weighing between 225 and 275 g. The control group (n=8) had 9 days s.c. injections of morphine (5, 10, 10, 15, 15, 20, 20, 25, 25 mg/kg/12h) and the last day 5 mg/kg naloxone was injected

(i. p.) and signs of withdrawal syndrome was recorded until 60 minutes. In the sham group 1 µl/ 2 min of α- methyl - serotonin vehicle was injected into the nuclei but in the test groups, 2. 5, 5. 0 and 10. 0 µg/ µl/ 2 min of α- methyl - 5 - HT (agonist of 5 - HT_2 receptors) was injected into the nuclei, and then naloxone was injected. RESULTS: Data signs were analyzed by One way ANOVA and Tukey post test. The results of this study showed a significant decrease in some of the recorded morphine withdrawal signs in test groups in comparisons with the control and sham groups. CONCLUSION: The results confirmed the important role of 5 - HT_2 receptors in raphe nucleuses on some of the morphine withdrawal signs.

Key words: 5 - HT_2 receptors, raphe nucleus, Morphine, Withdrawal syndrome.

P400006

Diadenosine tetraphosphate reduces methamphetamine - mediated neurotoxicity in dopaminergic neurons

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Previous studies have shown that diadenosine tetraphosphate (AP4A) reduced neurodegeneration caused by dopaminergic neurotoxin 6 - hydroxydopamine in rat striatum and substantia nigra. The purpose of this study was to determine whether AP4A is protective against methamphetamine (MA) - mediated toxicity in dopaminergic neurons. Primary ventral mesencephalic cultures were treated with MA. Application of MA increased LDH levels, decreased TH immunoreactivity, and increased TUNEL labeling. All these changes were reduced by pretreatment with AP4A. The protective effect of AP4A was further examined in vivo. Adult Sprague Dawley rats were injected with AP4A or vehicle intracerebroventricularly followed by 4 doses of MA (5 mg/ kg). AP4A antagonized MA - mediated bradykinesia from day 1 to day 30 after injection. Administration of MA increased caspase - 3 immunoreactivity and decreased TH immunoreactivity in striatum 3 days and one month after injection, respectively. Both effects were antagonized by AP4A. Taken together, these data show that AP4A has protective effects against MA - mediated injury in dopaminergic neurons both in vitro and in vivo. The mechanism of action may involve suppression of MA - induced apoptosis.

P400007

Effect of aqueous extract prepared from red nutshell of Pistachio (Pistacia vera) on naloxone - induced withdrawal syndrome in morphine - dependent rat

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In this study we try to examine the effect of aqueous extract obtained from red nutshell of pistachio (Pistacia vera) on withdrawal signs after administration of naloxone in morphine - dependent male rats. 42 male NMRI rats were made dependent by chronic administration of morphine during 14 days in their drinking water. Naloxone (2 mg/ kg; i. p) was injected to rats in order to produce behavioral parameters of withdrawal syndrome. Morphine - treated rats have been received an aqueous extract of nutshell of pistachio (25, 50, 100 and 200 mg/ kg; i. p; n = 8), 30 min before naloxone injection except control rats (n = 10). Findings indicated that the number of writhing in extract pretreated rats (25, 50 and 100 mg/ kg groups) significantly decreased in compare to control group. Diarrhea in all extract pretreated groups and weight loss in 50 and 100 mg/ kg extract pretreated groups decreased significantly, as well. The most decrement in withdrawal signs has been seen in 100 mg/ kg extract pretreated rats ($P < 0.01$). The results indicated that an aqueous extract of the red nutshell of pistachio could be affected dose - dependently on morphine withdrawal syndrome. However, high dose of this extract has a toxic activity.

P400008

Anabolic Effects of Enantiomers of β2 - Agonist Tulobuterol

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Tulobuterol is known as a bronchodilator by stimulation of β2 - adrenoceptor, and it was recently reported to be a potential anabolic agent. However, the β2 - agonists are usually available as a racemic mixture of two enantiomers: (-) - R form and (+) - S form, which may exert the different pharmacological activities. This study aims to compare the anabolic effects of tolubuterol enantiomers (10 mg/ kg/ day subcutaneous for 4 weeks) in Sprague Dawley rats 7 week - old. The rats were dissected and collected extensor digitorum longus (EDL) and soleus (SOL) muscle and femur and tibia bones after completing the administration period. It was found that the (-) - R - enantiomer increased body weight and wet weight of EDL significantly, comparing with untreated control. Moreover, the R - enantiomer increased the fast twitch fiber of SOL by induction via LDH isozymes. The (+) - S enantiomer hardly affected the quality and quantity of muscle. Both enantiomers had not significant effects on the bone. In conclusion, the (-) - R - enantiomer exerts anabolic potential, especially on the induction of fast twitch muscle fiber.

Key words: Tolubuterol, Anabolic Effects, Enantiomers

P400009

Cross - talk between nicotine and opioid systems evaluated by hypothalamo - pituitary adrenal function in mice

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We tried to elucidate a cross talk of nicotine and opioid systems evaluated by serum corticosterone (CS) in ICR mice. In acute experiment, we examined the ability of mecamylamine (MEC) and naloxone (NLX) to antagonize the CS increases produced by a single injection of nicotine and morphine. The CS levels were elevated by nicotine and morphine in a dose - dependent manner. The nicotine - induced CS increase was antagonized by MEC (1 mg/ kg), but not by NLX (1 mg/ kg), while the morphine - induced CS increase was antagonized by NLX, but not by MEC. In chronic experiment, we examined the effects of NLX on CS levels after chronic nicotine (3 mg/ kg, twice a day for 7 days) and of MEC on NLX - precipitated CS elevations after chronic morphine (20 mg/ kg, twice a day for 4 days). NLX (1 mg/ kg) elicited the CS increase in chronic nicotine - treated mice, and NLX - precipitated CS increase was inhibited by the pretreatment with MEC (0. 3 - 1 mg/ kg) in chronic morphine - treated mice. These results suggest that nicotine and opioid systems may show the cross talk under the condition of chronic treatments with nicotine or opioid, but not of acute treatments.

Key words; morphine, nicotine, corticosterone, dependence

P400010

Comparison of Pharmacokinetics and Pharmacodynamics of R - , S - and Racemic - Methadone in Healthy Subjects

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Methadone is a racemic mixture, with R - methadone the opioid agonist and S - methadone as ballast. We investigated pharmacodynamics and pharmacokinetics of R - (5 mg), S - (5 mg), rac - methadone (10 mg) and placebo in 6 healthy subjects after IV dosing. Blood samples and pharmacodynamic measures (pupils, respiration, POMS, MBG, MSC, immune suppression, plasma cortisol) were measured over 24 hours. There were no differences in the clearance of the enantiomers given alone or as racemate but R - methadone was cleared faster than S - methadone (10. 2 ±1. 6 versus 5. 8 ±1. 1 L/ hr). R - and rac - methadone constricted pupils and decreased respiration rate but only rac - methadone altered mood (confusion). Direct opioid effects were similar for R - and rac - methadone but nausea was greater with rac - methadone than with R - methadone and, S methadone was inactive. S - and rac - methadone caused immunosuppression and only rac - methadone increased plasma cortisol. S - methadone may contribute to some of the indirect opioid effects and further studies in a chronic dosing situation are needed.

Key words: methadone, enantioselectivity, pharmacokinetics, pharmacodynamics

Acknowledgements: NHMRC

P400011

Enhanced D1 dopamine receptor/ Gq protein coupling in female cocaine treated rats: implication for cocaine sensitization

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This study was designed to characterize the role of Gq protein activation in cocaine –induced behavioral sensitization in female rats (F). IP_3 level and G protein/ D_1 dopamine receptor (D_1DAR) coupling were assessed using brain homogenates or membrane preparations of frontal cortex (FC) in rats treated with cocaine. Acute cocaine (15 mg/ kg) produced greater behavioral responses in intact F rats than in OVX or male rats. Cocaine induced significant increases in IP3 content in F rats but not male or OVX rats. This was attenuated by SCH23390, suggesting that cocaine - stimulated IP_3 is mediated by the PI - linked D1DAR.

Daily cocaine injections (9 days) elicited greater behavioral sensitization in F rats than in males. Basal D_1 DAR/Gq coupling was increased in chronic cocaine-treated intact F rats. Furthermore, stimulation of FC membranes with a PI-linked D_1 DAR agonist, SKF83959, induced significant elevated receptor/Gq coupling in chronic cocaine-treated F rat as compared to OVX or male rats. Results indicate that activation of Gq/D_1 DAR coupling may play an important role in cocaine-induced behavioral sensitization.

Key words: cocaine, behavioral sensitization, D_1 dopamine receptor/Gq protein coupling.

P400012

An endocannabinoid hypothesis of drug reward

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Substance abuse treatment has largely been disappointing and new therapeutic targets and hypotheses are needed. Thus, an endocannabinoid hypothesis of drug reward is postulated. C57Bl/6 mice were evaluated in the plus-maze following abrupt cessation from chronic treatment with cocaine, diazepam, ethanol, methanandamide. In a separate group the ability of rimonabant, to block withdrawal aversions from alcohol and abused drugs was determined. The interaction between vanilloid and cannabinoid system was performed using selected agonists and antagonists. CB1 receptor antagonism reduced behavioral aversions following withdrawal from alcohol, cocaine, and diazepam. Treatment with capsaicin or WIN55212-2 induced aversions to the open arms plus-maze. The aversons induced with capsaicin, was dependent on gender and strain, and enhanced by pretreatment with WIN55212-2. Capsazepine reduced aversions, while rimonabant, produced dose dependent variable effects. Both capsazepine and rimonabant blocked the aversions induced by WIN55212-2 and capsaicin, indicating a cross-talk between cannabinoid and vanilloid systems. These results suggest that the EPCS may be an important natural regulatory mechanism for reward.

P400013

Inhibitory effects of (-)-epigallocatechin-3-O-gallate on morphine-induced reverse tolerance and conditioned place preference in mice

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The repeated administration of morphine produces reverse tolerance, a progressive enhancement of locomotion, which is used as a model for studying the drug-induced psychosis, and CPP, which is used as a model for studying drug reinforcement, respectively. (-)-Epigallocatechin-3-O-gallate (EGTG) inhibited reverse tolerance and CPP. In addition, EGTG inhibited the development of postsynaptic dopamine receptor supersensitivity, which may be an underlying common mechanism that mediates the morphine-induced dopaminergic behaviors such as reverse tolerance and CPP. Apomorphine (2 mg/kg, a dopamine agonist)-induced climbing behaviors also were inhibited by a single direct administration of EGTG. These results provide evidence that EGTG has anti-dopaminergic activity, as inhibiting the development of dopamine receptor supersensitivity and apomorphine-induced climbing behaviors. It is suggested that EGTG may be useful for the prevention and therapy of these adverse actions of morphine

(Supported by the Regional Research Centers Program of the Korean Ministry of Education & Human Resources Development through the Center for Healthcare Technology).

P400014

Effects of repeatedly administered morphine on locomotor activity, conditioned place preference and extracellular dopamine in GDNF+/- knockout mice

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Glial cell line-derived neurotrophic factor (GDNF) plays an important role in the plasticity of striatal dopaminergic neurons, which in turn are involved in the effects of morphine. To study the effects of reduced GDNF on behaviour related to addiction we compared effects of morphine in GDNF+/- mice and their wildtype littermates. When morphine 30 mg/kg was administered daily for four days, tolerance developed towards its locomotor stimulatory action only in the GDNF+/- mice. After withdrawal of 96 h the challenge dose of morphine 5 mg/kg stimulated locomotor activity only in the GDNF+/- mice, whereas the locomotor response seen after 10 mg/kg was similar in both GDNF+/- and wild-type mice. Morphine-induced elevation of extracellular dopamine lasted longer in the GDNF+/- than in the wild-type mice. Morphine-induced conditioned place preference developed similarly in both genotypes but it lasted longer in the wild-type mice. Our results emphasize the involvement of GDNF in the neuroplastic changes related to long-term effects of abused drugs.

P400015

Methamphetamine-induced regulation of dopamine transporter activity and subcellular localization

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In vivo high-dose administration of amphetamines (AMPH), including methamphetamine (METH), interferes with the function of the dopamine transporter (DAT). Evidence from in vitro systems indicates that AMPH may alter DAT function through internalization and trafficking to endosomes. With little in vivo data available, the objective of these studies was to determine if METH-induced alterations in DAT function is associated with DAT internalization and accumulation in endosomes. In vivo multiple high-dose administrations of METH decrease striatal synaptosomal DAT activity and, to a lesser extent, WIN35428 binding 1 h after the final METH injection, possibly indicating DAT internalization. Subcellular fractionation yielded a preparation highly enriched in the early endosome antigen 1 (EEA1) protein and devoid of the Na^+/K^+-ATPase, a marker of plasmalemmal membranes. Multiple high dose injections of METH did not alter DAT immunoreactivity among fractions containing the EEA1 and Na^+/K^+-ATPase markers at 1 h after final administration suggesting that METH does not promote the accumulation of DAT in endosomes at this early time point.

(Supported by: DA00868, DA04222, DA11389, DA00378, DA019447)

P400016

Health Canada Regulatory Guidance: Clinical Assessment of Abuse Liability for Drugs with Central Nervous System Activity

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The Therapeutic Products Directorate of Health Canada has developed a draft guidance document for the pharmaceutical industry on the clinical assessment of abuse liability for drugs with central nervous system activity. This guidance document is intended to promote a strategic approach to the assessment of abuse liability during clinical drug development. Human abuse liability studies are generally performed in experienced recreational non-therapeutic drug users. The preferred study design is usually a double-blind, multiple arm, complete crossover. Both placebo and positive control treatment arms should be included. A pre-testing qualification phase can be used to enrich the subject pool. Subjective measures of abuse liability include the Addiction Research Center Inventory and the Profile of Mood States. The results of these abuse liability studies will be used to guide risk-benefit assessments and decisions relating to drug approval, scheduling, and prescribing information.

Key Words: abuse liability, drug regulation

P400017

Effect of repeated methamphetamine exposure on prefrontal dopamine efflux under aripiprazole administration and conditioned drug reward in rats

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This study examined the profile of drug reward and conditioned dopamine (DA) efflux in the medial prefrontal cortex (mPFC) in the rats that had prior repeated administration of methamphetamine (MA). And the prefrontal DA efflux after acute systemic injection of an atypical (aripiprazole, APZ) or typical (haloperidol, HAL) antipsychotics in another group of rats with repeated MA exposure was assessed. We used conditioned place preference to monitor the drug reward and in vivo microdialysis in conscious rats to determine the DA efflux. The results showed that the mPFC DA levels significantly increased in the non MA-sensitized rats, but not in the MA-sensitized group, when exposed to the context previously conditioned with MA application. Meanwhile, in the MA-sensitized rats, the drug reward increased robustly. Further, the DA efflux is enhanced after an APZ, but not after HAL, systemic injection in the MA-sensitized rats. Together, the results indicate the neuroadaptations in the mPFC contribute to drug

reward and the modulation of prefrontal DA transmission may have therapeutic implication in drug addiction.

P400018

Effects of progesterone on morphine-induced rewarding effect and its relation to monoamine transmitters level in rat brain

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In order to investigate the effect of progenolone on morphine rewarding effect and its relation to monoamine transmitters level in rat brain. We used the conditioned place preference (CPP) test to study morphine rewarding effect, and established the high-performance liquid chromatography with electrochemical detection method to determine the levels of norepinephrine (NE), dopamine (DA), and 5-Hydroxytryptamine (5-HT) in rat hypothalamus (Ht) and striatum (Str). In result, we found that 5 mg kg^{-1} morphine could successfully induce the formation of CPP. Progenolone (5 mg kg^{-1} and 20 mg kg^{-1}) could not induce CPP effect itself, but was able to abolish the morphine CPP effect. Compared with control group, the significant increases of NE in Ht, NE, DA, and HT in Str following morphine administration were demonstrated. Compared with morphine group, this increase of DA level in Str could be attenuated by co-administered 20 mg kg^{-1} progesterone. It is speculated that progenolone may effectively attenuate morphine-induced CPP through its action on the level of DA in rat Str.

Key Words: conditioned place preference; morphine; progesterone; rat

P400019

INCIDENCE OF DEPRESSOR SUBSTANCE CONSUMPTION IN A POPULATION TREATED IN A PRIVATE ADDICTION TREATMENT FACILITY IN MEXICO CITY.

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Addiction is a chronic disorder that comprises compulsive seeking and consumption behaviour that persist despite negative consequences in the overall health of the user. Addiction to psycho-active substances is a growing phenomenon in developing countries of Latin America. The aim of this retrospective study is to evaluate the incidence of depressor substance abuse in patients seeking treatment in a private addiction treatment institution in Mexico City between 2001 and 2002. From a total of 318 patients (160 in 2001 and 158 in 2002), 79.25% were male and 20.75% were female, the ages varied between 15 years of age and older than 70, and they were categorised in 5 year increments. 96.23% of those admitted were alcohol drinkers, while 3.77% did not consume alcohol (OH). Only 19.18% were OH drinkers exclusively, 53.46% smoked marijuana (CAN), 38.36% consumed sedatives (SED) such as benzodiazepines, and 8.18% used opiods (OP). The importance of identifying the population at risk and determining the incidence of the different drug abuse populations resides in the development of prevention programs.

P400020

INCIDENCE OF STIMULANT DRUG ABUSE IN A POPULATION TREATED IN A PRIVATE ADDICTION TREATMENT FACILITY IN MEXICO CITY.

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Stimulant abuse is a growing problem in Latin America, and involves compulsive substance seeking and consumption behaviour that persist despite negative effects in the overall state of health of the user. This study evaluates retrospectively the incidence of stimulant substance abuse in patients seeking treatment in a private addiction treatment institution in Mexico City between 2001 and 2002. From a total of 318 patients (160 in 2001 and 158 in 2002), 79.25% were male and 20.75% were female, the ages varied between 15 years of age and older than 70, and they were categorised in 5 year increments. Leading the stimulant substance consumption were cocaine (CK) users with 63.84%, 18.55% consumed designer drugs (DD), 17.61% hallucinogenics, 7.86% amphetamines, and 7.23% inhaled solvent vapors. Identifying the population segments at risk and the incidence of the different drug abuse populations, is of pivotal importance in the development of drug abuse prevention programs.

P400021

Post-training infusions of dopamine receptor antagonist into the basolateral amygdala prevent morphine-induce conditioned place preference

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The present study investigated the influence of dopamine receptor antagonist SCH23390 in the basolateral amygdala on the consolidation of memory for morphine-induced conditioned place preference (CPP). Adult male Sprague-Dawley rats were confined to treatment- or nontreatment-paired compartments for 45 min on 3 alternating days. After training, rats received intrabasolateral amygdala infusions of SCH23390 (0.2 μg or 2.0 μg) or saline. The rats were then given a 15-min test session, and the time spent in each of the compartments was recorded. The results showed that immediate posttraining (but not delayed 2 hr) SCH23390 (2.0 μg) blocked acquisition of morphine-induced CPP. The finding suggests that the BLA is involved in the consolidation of memory for morphine-induced CPP and dopamine in this process plays an important role.

P400022

Lateral Hypothalamic Neuropeptide Melanin Concentrating Hormone Acts in the Nucleus Accumbens to Exert Opposite Control on Morphine and Food Seeking Behaviors in Rats

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The lateral hypothalamus (LH) is a brain region implicated in reward and motivation, but the related neurotransmitters are not clearly identified. A lateral hypothalamic-nucleus accumbens circuit mediated by Melanin-concentrating hormone (MCH) is a strong candidate. The present study designed to investigate the effects of infusing MCH into the nucleus of accumbens shell (AcSh) and LH on the seeking behaviors for food and morphine with conditioned place preference (CPP) version of the reinstatement model. The results indicate that MCH blocked morphine CPP expression, but enhanced food CPP expression; prevented morphine CPP reinstatement but had no effect on food CPP reinstatement; and blocked morphine behavioral sensitization expression in AcSh. The results demonstrated that MCH has a different and even opposite effect on the seeking behavior for morphine and food. In conclusion, motivation for natural rewards and addictive drug can be oppositely regulated. Key Words: Craving; Food; morphine; Melanin-concentrating hormone.

Acknowledgment: This work was supported by National Natural Science Foundation Grants (30230130) and National Basic Research Program Grants (2003CB515404) to Professor Nan Sui

P400023

Effects of progesterone on morphine-induced conditioned place preference and levels of amino acid transmitters in rat brain

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The present study aimed to investigate the effect of progesterone on the development of morphine conditioned place preference (CPP) and levels of amino acid transmitters in SD rat nucleus accumbens (NAc). Saline, morphine and progesterone were injected once per day for 10 days. CPP test was used to investigate the rewarding effect of morphine, progesterone and co-administration with both of them. High-performance liquid chromatography with electrochemical detection was used to determine the levels of glutamate (GLU) and gamma-aminobutyric acid (GABA) in NAc. As a result, morphine successfully induced the development of CPP. Progesterone (5 mg·kg^{-1} or 20 mg·kg^{-1}) could not induce CPP itself, but was able to abolish the morphine CPP. Compared with control, the significant decrease of GLU and increase of GABA levels following progesterone (5 mg·kg^{-1} or 20 mg·kg^{-1}) administration were demonstrated in NAc ($P < 0.01$). In conclusion, it is speculated that progesterone effectively attenuates morphine-induced CPP. The effect of progesterone on morphine CPP could be through its action on amino acid transmitters in rat NAc.

Key Words: morphine; progesterone; amino acid transmitters; nucleus accumbens

P400024

Effects of catecholamine neurotransmitters on the syntheses and release of neurosteroids by primary cultured rat brain cotical astrocytes

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The present study aimed to investigate the effects of catecholamine neurotransmitters on the levels of neurosteroids synthesized and released by primary cultured rat brain cortical astrocytes. Primary cultured rat brain cortical astrocytes were treated with dopamine (DA), norepinephrine (NE) and 5 - hydroxytryptamine (5 - HT) for 48h respectively. Unconjugated (DHEA, PREG and AP) and conjugated neurosteroids (PS, DS) in culture media were extracted, isolated by SPE and analyzed by HPLC - MS (APCI) using selected ion monitoring. Compared with NS control group, DA was shown to concentration - dependently decrease PREG level and increase AP and DS levels respectively; NE was found to significantly increase AP and DS levels but decrease PREG and DHEA levels; 5 - HT treatment elevated DHEA, PREG, AP and DS levels differently. In conclusion, DA, NE and 5 - HT could increase AP and DS levels accompanied by different effects on DHEA and PREG levels in primary cultured cortical astrocytes.

Key words: monoamine transmitters; astrocyte; neurosteroid; HPLC - MS

P400025

Effect of Pueraria lobata on behavioral functions in chronically ethanol drinking outbred rats

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It is known that the extract from radix of Pueraria lobata (Willd.) Ohwi, (kudzu), can alleviate symptoms produced by neurotoxic activity of ethanol in the hippocampus (Jang et al. 2003). Therefore, further investigation of the interaction between root of kudzu and ethanol in central nervous system seems to be of scientific importance. In our model of alcohol disease, ethanol preferring (PR) and non - preferring (NP) rats were treated with kudzu (500 mg/kg, p.o.) for 21 consecutive days and their motor activity, motor coordination, anxiety - related reactions, and long term memory were assessed. It was found out that kudzu treatment lowered alcohol intake in PR rats (86%). The kudzu administration produced an impairment of long - term memory both in PR and NP rats. The effect seemed to be specific since kudzu did not affect motor activity and led to improvement of anxiety - related reactions and motor coordination in PR rats. In conclusion, as prolonged use of kudzu and ethanol has been shown to impair long - term memory in rats, further behavioral and molecular studies may need to be carried out to confirm this hypothesis.

P400026

The regulation of agmatine on NMDA receptors expression in morphine dependent rats

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Aim: To investigate whether the regulation of agmatine on morphine dependence is associated with NMDA receptors expression. Methods: A model of chronic morphine dependence was established by repeated administration of morphine with progressive doses. Western blotting was used to examine the changes of NMDA receptors (NR1 and NR2B subunits) expression and the influence of agmatine on morphine's effect in hippocampus and nucleus accumbens of morphine - dependent rats. Results: The NR2B subunit was down - regulated significantly in hippocampus of morphine - dependent rats, while the NR1 subunit was not changed. This suggests the subtype constituent of NMDA receptors was altered. And the NR2B subunit had no change but NR1 subunit was up - regulated at nucleus accumbens, suggesting the level of NMDA receptors was changed. Agmatine co - treated with morphine could reverse morphine's regulation on NMDA receptors expression at these two regions. Conclusion: The mechanism for the regulatory effect of agmatine on opioid dependence is related with the reverse effect on the NMDA receptors' level and constituent.

Key word: agmatine, morphine dependence, NMDA receptor

P400027

Effects of morphine challenge following perinatal morphine exposure in rats.

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Objectives: Effect of morphine (MO) challenge was investigated in offspring of dams treated with MO (10 mg/kg/day) during the gestation and lactation periods. Methods: Spontaneous locomotor activity (SLA) (CONDUCTA, Experimetria Ltd), behaviour in elevated plus maze test (EPM), analgesic effect of MO (tail - flick) were measured on the postnatal day (PD) 23. The reinforcing capacity of MO was checked by condtioned place preference (CPP) test in the 1., 2. and 3. generations, too. Results: 1. There was no difference in the SLA or EPM. 2. Analgesic effect of MO was weaker in the perinatal MO exposed animals. 3. Reinforcing effect of MO was more marked in the animals exposed to perinatal MO and this enhanced sensitivity to MO was observed even in the 2. and 3. generations. Conclusion: Perinatal MO exposure changed the MO sensitivity. While the analgesic effect of MO decreased, the enhanced effect of MO in the CPP test, even in the 2. and 3. generation indcates the developing of higher risk of abuse liability, which might be the consequences of an altered maternal activity.

This study was supported by Hungarian grant OTKA K - 60999

P400028

Effects of psychostimulant challenge following perinatal psychostimulant exposure in rats

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Objectives: Effect of psychomotorstimulants [(+) methylenedioxy - methamphetamine - ecstasy, MDMA and (+) methamphetamine - MA] challenge was investigated in offspring of dams treated with either MDMA or MA during the gestation and lactation periods. Methods: Pregnant females were treated daily with either 3 mg/kg sc. MDMA or MA until the 21st postpartum day, when the offspring was separated. Spontaneous locomotor activity (SLA), drug - induced locomotor activity (CONDUCTA, Experimetria Itd), and behaviour in elevated plus maze test (EPM) were measured two or three days after separation. Results: 1. There was no difference in the EPM behaviour of offspring. 2. SLA was higher in the animals exposed to perinatal MDMA or MA. 3. The locomotor enhancing effect of MDMA or MA was reduced in animals exposed to perinatal drug treatment. Conclusion: Perinatal exposure to psychomotor stimulants alters the locomotor behaviour and decreases the sensitivity to the succeeding drug challenge.

psychostimularts, perinatal, locomotor activity

This study was supported by Hungarian grant OTKA K - 60999

P400029

Effect of memantine on morphine physical dependence in acute naloxone - precipitated withdrawal.

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Effect of memantine (N - methyl - D - aspartic acid - NMDA - receptor antagonist) on the acute opiate withdrawal induced by morphine was investigated in vitro. Male inbred guinea pigs weighing 300 - 400 g fasting for 24 h were used. Guinea pigs fasting for 24 h were decapitated after cervical dislocation and terminal portions of their ilea were taken out. After they had been placed in Tyrode solution in a container, they were nicely and throughly washed by flushing Tyrode solution through the lumen. Following a 4 hours in vitro exposure to morphine, the guinea - pig isolated ileum exhibited a strong contracture after the addition of naloxone. Memantine by itshelf had no effect on morphine dependent ilea but was able to reduce dose - dependently (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M) naloxone - precipitaded withdrawal. NMDA antagonists are able to influence opiate with-

drawal in vitro , suggesting an important functional interaction between the NMDA receptors and opioid withdrawal.

Key Words : Memantine , morphine dependence , NMDA

P400030

Effect of venlafaxine on morphine dependence in isolated guinea - pig ileum

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To investigate the effects of venlafaxine on morphine withdrawal response and acetylchline (Ach) - induced contracture in isolated guinea pig ileum. The withdrawal contracture was elicited by subjecting isolated ileum incubated with morphine (10^{-6} M) at 37.5 degrees Celsius for 4 h to naloxone (10^{-6} M) treatment. Venlafaxine (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M) was administered 1 min before and after naloxone in morphine - dependent ilea bathed in Tyrode solution containing morphine , to observe the changes in the withdrawal contracture of the ileum. The effect of venlafaxine (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M) on the contracture of untreated ileum in Tyrode solution elicited by acetylcholine was also observed. Naloxone - induced withdrawal contracture or acetylcholine - induced contracture of the ileum was significantly decreased in a dose - dependent manner , indicating that venlafaxine can inhibit morphine withdrawal symptoms in guinea pigs.

Key Words : Venlafaxine , morphine dependence

P400031

Effects of morphine on levdl of neurosteroids produced by primary cultured rat cerebral cortical neurons

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The study aimed to observe the effect of chronic morphine treatment on the level of neurosteroids produced by rat cerebral cortical neurons(CCNs). The effect of morphine(1 μmol/ L) on the level of neurosteroids was detected by using solid - phase extraction and LC- MS , with methyltestosterone or estrogen sulfate as internal standards. The dependence - like changes of CCNs were assayed by testing p - CREB levels using western blot. As a result , morphine reduced the level of pregnendone (PREG) , and dehydroepiandrosterone sulfate (DS) vs saline control group($P < 0.01$) . miu - antagonist CTAP concomitant with morphine increased the level of PREG vs morphine group($P < 0.01$). Miu - agonist DAMGO reduced the level of PREG, DS and pregnenolone sulfate(PS) , while increased the level of allopregnanolone (AP) vs control group($P < 0.01$). Simultaneously , morphine and DAMGO treatment increased the level of p - CREB vs control group respectively ($P < 0.01$) , while CTAP reduced the level of p - CREB vs morphine group($P < 0.01$). As a conclusion , miu - opioid receptor mediated , at least partly , the effect of morphine on the the level of neurosteroids.

Key words : morphine ; opioid receptor ; neuron ; neurosteroid

P400032

Effects of l - Stepholidine on contents of GFAP in VTA of morphine dependent rats

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Object : To explore the effects of l - stepholidine(L - SPD) on levels of glial fibrillary acidic protein (GFAP) in some brain regions of morphine dependent rats. Method : 30 rats were assigned randomly into five groups. Control rats were injected with saline all the time. The four treatment groups were injected with morphine subcutaneously by increasing dose for 10 days to establish morphine dependence model. After abstinence , two groups were injected with saline for 12 days and 30 days respectively , other two groups treated with L - SPD for 12 days and 30 days respectively. Brain structures needed of all rats were removed and cryo - sections were prepared. The contents of GFAP were determined on their intensities by immune - histochemistry methods. Result : Only in VTA region , levels of GFAP of morphine dependent rats were all higher than that of control group ($P < 0.05$) , while L - SPD can remarkably inhibit the increase induced by morphine. ($P < 0.05$). Conclusion : Opiate addict may specifically impair the DA neuron in VTA region , and which may be one of the most important mechanisms for addiction. L - SPD may be benefit for this kind of damage.

Key words : Opiate addicted rat , l - stepholidine , glial fibrillary acidic protein , ventral tegmental area

P400033

Correlation of tissue and plasma cocaine levels with responsiveness of β1 - and β2 - receptors to adrenergic agonists of chronic cocaine - treated animals.

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Correlation of degree of supersensitivity of cardiac β1 - and tracheal β2 - adrenoceptors with levels of cocaine in tissues and plasma of chronic cocaine administration , in which pre - or postsynaptic mechanism may be elucidated , was studied using cocaine - treated guinea - pig as model. Animals were treated with cocaine HCl 1.25 mg/ kg , or 0.9 % NaCl 1 ml/ kg , i. p. , twice daily for 14 days. After 24 hours cocaine cessation , blood sample , heart and trachea were taken from the animals. Cocaine levels were analyzed by HPLC. The responses to epinephrine and salbutamol were recorded as increase in force and rate of atria and relaxation of carbachol - induced contraction of trachea. Results showed that both tissues exhibited supersensitivity as a leftward shift of the concentration - response curves to both drugs by 7 - 10 folds for atria and 8.5 - 13 folds for trachea. Cocaine levels in plasma and trachea were 5.1 (0.6 and 7.0 (0.8 ng/ ml , respectively , but it could not be detected in atria and ventricle. According to others , these cocaine levels were unable to cause presynaptic reuptake blockade. Thus , the supersensitivity should involve postsynaptic mechanism and β2-receptors were more sensitive than β1 - receptors.

P400034

EVIDENCE FOR THE INVOLVEMENT OF NOP RECEPTOR FOR NOCICEPTIN/ORPHANIN FQ (N/OFQ) IN THE EFFECT OF BUPRENORPHINE ON ALCOHOL INTAKE IN RATS

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Buprenorphine , a mixed opioid receptor agonist - antagonist , has been shown to bind at NOP receptors for N/ OFQ. Since N/ OFQ reduces alcohol drinking in Marchigian Sardinian alcohol - preferring (msP) rats , the object of the present study was to evaluate whether buprenorphine may inhibit alcohol intake. msP rats were offered 10 % v/ v ethanol 2 hr/ day ; water was freely available. On the test day rats were IP injected with buprenorphine (0.03 , 0.3 , 3.0 or 6.0 mg/ kg) 90 min before access to ethand. The doses of 0.03 and 0.3 mg/ kg significantly increased ethanol intake , whereas 3.0 and 6.0 mg/ kg reduced it. Pretreatment with naltrexone (0.25 mg/ kg , IP) prevented the increase of ethanol intake induced by low doses of buprenorphine , but did not block ethanol drinking inhibition by 3.0 or 6.0 mg/ kg. The effect of the higher buprenorphine doses was blocked by the selective NOP receptor antagonist UFP - 101 (0 , 10 or 20 microg/ rat , ICV). These findings suggest that the interaction with NOP receptors may have an important role in the pharmacological effects of buprenorphine.

Keywords : Buprenorphine , NOP receptor , Alcohol intake
Acknowledgements : Supported EU Grant (TARGALC QLRT - 2001 - 01048)

P400035

Acute cocaine and ethanol co - administration differentially affects brain prodynorphin and k - opioid receptor mRNA expression.

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To clarify the activity of brain prodynorphin/ k - opioid receptor (KOR) system following combined treatment with ethanol and cocaine we studied their acute effects with in situ hybridization histochemistry. Adult male rats were administered

i. p. ethanol and binge cocaine 30 min later. mRNA expression of prodynorphin (prodyn) and KOR was analyzed in the dorsal striatum (DS), nucleus accumbens (NAcc); substantia nigra reticulate and compacta (SNR and SNC) and ventral tegmental area (VTA). It was found that the co-administered ethanol and cocaine significantly increased prodyn mRNA expression level in NAcc, VMS, DMS, DLS. Co-administered ethanol and cocaine significantly lowered KOR mRNA expression in NAcc, VMS, VTA and SNC. The observed effects of co-administered ethanol and cocaine might reflect their joint effects on DA release in the mesolimbic and nigrostriatal systems. This finding might contribute to clarify the close link between the dyn/KOR complex and DAergic systems in the co-abuse of ethand and cocaine.

This study was supported by the Swedish Research Council.

P400036

Effect of Carbamazepine on the Conditioned Place Preference of Morphine Dependent Rats

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Objective: To observe the effect of carbamazepine(Carb) on morphine(Mor) induced conditioned place preference(CPP). Methods: Rats were trained for 4 days by sc Mor once a day before being closed into a special box to induce Mor CPP. Carb were given during the training or after the CPP formation to observe its effect on the formation or the maintenance of Mor CPP. Results: In the process of formation, Carb 100 mg/kg could reduce the strength of Mor CPP significantly(Carb 676 ±s173.1 vs control 785 ±s60.6, $p < 0.05$); for the formed Mor CPP, Carb 50 mg/kg could greatly promote the disappearance of that(742 ±s 81.0 vs 515 ± s317.4, $p < 0.05$). Carb itself could not induce CPP of rats. Conclusion: Carb showed inhibitory effects on the Mor CPP of rats. That might mean Carb have some therapeutic usage for the craving of opiate addicts, and opiate addiction seems like a special kind of epilepsy.

Key words: carbamazepine; morphine; craving; conditioned place preference

P400037

Involvement of glutathione peroxidase in opioid dependence and amelioration of dependence by antioxidant effective natural products

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The study was undertaken to determine the commonly modulated gene by morphine, butorphanol, and nalbuphine by using microarray. We can obtain the interesting gene, glutathione peroxidase which was downregulated in the opioid-treated mouse cortex. Also this study was processed to the suitable antidote for the drug abuse by applying natural product which show the anti-oxident effect. Furtunately extract of Scutellaradix, Polygalaradix, Gardeniae fructus, and Ginseng radix show the anti-narcotic effect on the morphine dependence. The physical dependence on morphine was ameliorated by the Polygalaradix extract but the psychological dependence was not modulated. Interestingly, morphine withdrawal syndrome was aggravated in the glutathione peroxidase/catalase (GPx/Cat-/-) knock out mice. These results suggest that the oxidative stress might be involved in the opioid dependence and antioxidatant effective natural products could be used to ameliorate the opicid withdrawal symptoms.

P400039

The role of neurotransmitter systems in interaction between testosterone and morphine

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The Interaction between testosterone (T) and effects of morphine (M) has been reported (Banerjee, 1983; Bahaaldini, 1988). We investigated roles of adrenergic, dopaminergic, serotonergic and cholinergic systems in this interaction. Male mice were received T [or vehicle(V)] + agonist, or antagonist (or V) + M (or V), three times a day for three days. On the test day, the withdrawal syndrome was induced by naloxone and jump number was recorded. Apomorphine (a dopamine agonist) and Fenfluramine (a 5-HT agonist) did not induced withdrawal syndrome and did not change the effects of M or T treatment alone; but increased naloxone-induced jumping in T + M group, significantly ($p < 0.05$). Other drugs including neostigmine, atropine, clonidine and yohimbine did not cause significant change in naloxone induced signs in T + M group. In conclusion, dopaminergic and serotonergic systems are involved in this interaction.

Key words: Morphine, testosterone, Neurotransmitter systems, Drug interaction

P400040

Inhibition of agmatine on psychological dependence induced by morphine and the possible mechanism

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Aim: In the present study, the effect of agmatine on the psychological dependence on morphine and the possible mechanism was evaluated. Methods: In rat behavioral sensitization model, microdialysis and RT-PCR were used to determine the release of DA and dynorphin expression, respectively. Results: In rat behavioral sensitization model, after 4 d of morphine treatment and 3 d of withdrawal, the release of DA was not different from saline group, while the quantity of DOPAC/HVA was higher than that of control. Co-administered of agmatine with morphine inhibited the increase of DOPAC and HVA. After primed by morphine on d 8, the release of DA in morphine treated rats was increased significantly and agmatine inhibited this increase. This result inferred that agmatine modulated the adaptation after chronic morphine treatment. The expression of dynorphin mRNA in the nuclear accumbens was not changed after 4 d of morphine treatment, while decreased after 3 d of withdrawal; agmatine inhibited the decrease of dynorphin expression. Conclusion: Agmatine had inhibitory effect on morphine-induced psychological dependence through activation of imidazoline receptors. The mechanism is related to its modulation on the release of dopamine and expression of dynorphin induced by morphine.

Key words: behavioral sensitization; morphine; agmatine; microdialysis

P400041

Effects of Guiyuan tablets on Morphine-induced long-term potential

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Objection: The study was to explore the effect of Guiyuan tablets on the conditioned place preference (CPP) induced by morphine and to investigate the effect of chronic morphine on Long-term potentiation (LTP) at Dentate Gyrus(DG) granular cell synapses of rats in vivo. Method: Morphine was injected (5 mg/kg, one time/day, sc) to rats for 7 days and strong CPP was observed in rats. The rats were pretreated 15 min before each injection of morphine to during 7d training phase with Guiyuan tablets(12.5, 25, 37.5 and 50 mg/kg, sc) and treated with Guiyuan tablets(50 mg·kg^{-1}, one time/day, ig) for 12 days after formation of CPP induced by morphine. LTP at DG were examined. Results: (1) Morphine can potentiate the induction of hippocampus LTP while both doses of Guiyuan tablets itself has no effect on DG-LTP. 25 and 37.5 mg/kg Guiyuan tablets antagonize the enhancement effect of morphine on DG-LTP, while 50 mg/kg Guiyuan tablets enhance extinction of morphine-induced CPP. Conclusion: Guiyuan tablet inhibit the enhancement facilitation of LTP, which was induced in morphine dependence rats. These changes indicate that Guiyuan tablets mediate in the reinforced effect induced by morphine, and might useful in treatment of opiate dependence by acting on synaptic plasticity.

Key words: Guiyuan tablets, CPP, Synaptic plasticity, LTP

P400042

Effects of l-stepholidine on Dopamine system of morphine dependent rats

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Objective To explore the effects of l-stepholidine (l-SPD) on dopamine (DA) system in some brain regions of morphine dependent rats. Methods Rats were injected with morphine by increasing dose for 10 days to establish morphine dependence model. After abstinence, l-SPD was injected for 12 days or 30 days followed by Tyrosine 3-Monooxygenase (TM), D_1R and D_2R expression detec-

tion. Results Protein level of TM in ventral tegmental area (VTA) region of morphine dependent rats was higher than control, which could be remarkably inhibited by l-SPD. D_1R mRNA in nucleus accumbens septi (nas), amygdalae, caudatum putamen (cp), prefrontal cortex and D_2R mRNA in VTA, nas and cp significantly decreased, and both of them failed to return to normal at 30th day after abstinence. With the l-SPD administration D_2R mRNA reached control level in most brain regions at different time except in VTA at 12th day. Conclusion l-SPD could remarkably inhibit the excessive expression of TM in VTA region, promote D_1R and D_2R expression in brain and accelerate DA system functional recovery after morphine abstinence, which provide an evidence for the prevention and detoxification of opiate addiction.

Key words: Morphine dependence, l-stepholidine, TM, dopamine, receptors, gene expression

P400043

L-Tetrahydropalmatine Induces a Negative BOLD Signal in the Nucleus Accumbens and Orbitofrontal Cortex in Heroin-Dependent Rats

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Introduction. The functional MRI (fMRI) method has demonstrated that cocaine-cues induce a set of mesolimbic cortical networks in the brain of cocaine users. Moreover, the cue-induced craving rating scores were significantly correlated with the positive BOLD signal changes in regions of the anterior medial orbitofrontal cortex (BA 11) and the subcollosal cortex (BA 25). If this positive BOLD signal could serve as a biomarker for drug craving, a medication that can specifically act on these regions with a negative BOLD signal could extinguish the drug craving, thereby preventing cocaine-seeking or -taking behaviors. A present fMRI study demonstrated that the Chinese herb extract, L-Tetrahydropalmatine (L-THP), would have therapeutic potential for anticraving.

Materials and Methods. Rat preparation. Thirteen naive Sprague-Dawley rats (90-110 g, male) were treated with heroin in nine days using a progressive schedule (2 mg/kg daily for the first three days; 4 mg/kg daily for the second three days; and 8 mg/kg daily for the third three days). These rats became heroin dependent as evidenced by behavioral changes induced by naloxone. FMRI Experiments: fMRI scanning was performed within 24 hours after the last daily injection of heroin. Under urethane anesthesia (1.2 g/kg), all rats received tracheotomies and were artificially ventilated with a 30% O2/air mixture at a tidal volume of 5 ml and respiration frequency of 70 Hz to maintain stable physiological levels. Body temperature was monitored during scanning and maintained at 37 ± 1°C with a water-circulated heating pad. A femoral vein and artery were cannulated (PE 50) for drug delivery and monitoring of arterial blood gas levels, respectively. After surgery, rats were paralyzed with gallamine (250 mg/kg, iv) and an additional dose of 0.2-0.3 g/kg of urethane was administered prior to fMRI scanning. FMRI experiments were performed on a Bruker Biospec 3T/60 cm scanner using a custom-built RF birdcage volume coil (1.5"diameter ×2" length), inserted into a custom-made local gradient coil. To minimize motion artifacts, each rat head was anchored to the fixture of the RF coil with a clamping device consisting of a bar inserted under the hard palate and affixed to a nose clamp. To standardize slice anatomical locations across different rats, a medial sagittal Rapid Acquisition with Relaxation Enhancement (RARE) anatomical image (TR = 1000 ms, TE = 19 ms, matrix size 256 ×256, FOV = 3.5 cm) was obtained from each animal before functional scanning. On this slice, the interface between hard and soft palates is easily recognized and was employed as a starting point for the first imaging slice (approximately 2.2 mm from Bregma). Six 2-mm thick coronal slices were acquired. A single-shot, gradient-echo echo-planar imaging sequence (FOV = 3.5 cm, image matrix = 64 ×64 giving an in-plane image resolution of 550 ×550 μm, TR = 2 s, TE = 27.2 ms, bandwidth 125 kHz) was used for functional imaging. Experimental Design: The rats were divided into three groups. The first received a 0.1 mg/kg heroin treatment 5 min into a 25-min scan. The second group received a sham treatment with the same conditions as the first. The third group received 40-mg/kg L-THP treatment 5 min into a 65-min scan. The heroin was licensed and obtained from NIDA. fMRI Data Analysis: The BOLD fMRI signal in each voxel was fitted with a nonlinear differential exponent model, according to its pharmacological and functional responses using AFNI v2.2 software. Voxels were considered significant based on a goodness-of-fit F-test ≥10, (corresponding to $P < 0.001$ after the Bonferroni correction). Significant drug effects were determined using a Student's t-test based on changes in voxel numbers and area under the curve (AUC). Significance was set at $p < 0.05$ throughout.

Results. The present report focuses on the results from the L-THP treated group. As shown in Figure 1, L-THP induced a significant BOLD signal reduction (about 12 ±5%, n = 3) in both the right and left sides of the NAC core and shell regions, as well as the obitofrontal cortex in the heroin-dependent rats. The time course of L-THP in the NAc showed a long-lasting effect. In addition, it is intriguing that L-THP has a very high spatial specificity. It is known that these regions contain rich D3-receptor distribution. It is hypothesized that the negative BOLD signal may be a result, in part, from the antagonistic binding of L-THP with D3-receptors in the region. To test this hypothesis, the L-THP was sent to NovaScreen (http://www.novascreen.com/). The latter confirmed that the L-THP was actively bound to D3-receptors when a concentration of 1.0E-5 of L-THP was employed; the Kd (M) being 0.9E-9 of [^{3}H]7-OH-DPAT, and Ki (M) being 1.42E-9 of (+/-)7-OH-DPAT HBr. [Note: the testing compound of 7-OH-DPAT is a selective D3 receptor agonist (Kd < 1 nM). Commercial profile testing reported by NovaScreen showed that L-THP also significantly binds to D1 and D2 receptors, weakly binds to adrenergic alpha 1A and 2A receptors, as well as serotonin, 5HT1A, 5HT1D, 5HT4, and 5HT7 receptors. No other significant bindings were found among 70 receptor profile testing.

Figure 1. Left, the map of Cross correlation coefficients (CC = 0.22, $P < 0.0001$) upon L-THP administration (40 mg/kg), the green arrow points to the NAC region. Right, the time course of L-THP in the region of NAc. The black arrow points to the time L-THP was i.v. administrated.

Discussion and Conclusion. L-THP significantly induced a negative BOLD signal in the region of the NAc and the OFC in heroin-dependent rats. The long lasting effect of L-THP in these regions suggested potential therapeutic efficacy. Limited binding effects of L-THP to the other receptors indicate less possible side effects or addictive potential. These results suggest that drug cue-induced positive BOLD signal can be suppressed by administering L-THP to extinguish the drug craving. Therefore, L-THP will have a high potential in treating drug craving for heroin, cocaine, nicotine, in addition to food craving in obesity. Further clinical studies will be needed.

Acknowledgement: This work was supported by USA NIH Grants DA10214 and EB01820 and by Chinese Ministry of Science and Technology grant 2003CB51540.

P400044

Detection, Purification and Specificity Analysis of Anti-Morphine Antibody from Sera of Chinese Heroin Abusers

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Twenty-three of 57 sera (40%) of Chinese heroin abusers had positive evidence of anti-morphine antibody using original ELISA method. The polyclonal anti-morphine antibody from the abusers was purified by affinity chromatography method. The affinity specificity between the antibody and opiates was investigated respectively by competitive ELISA to determine the formation of antigenic determinants that the antibody recognized. The antibody showed high cross reactivity between heroin, codeine, Morphine-3-glucucoid and morphine (maximum ratios of inhibition range: 80%-100%, values of IC_{50} range: 10^{-6}-10^{-4} mol·L^{-1}) and lower cross reactivity between Methadone, oxycodone, etorphine and morphine (maximum ratios of inhibition range: 50%-75%, values of IC_{50} range: 10^{-5}-10^{-3} mol·L^{-1}); Naloxone and naltrexone had nearly no inhibitory effect. The results suggest the antibody had a "group specificity", the active groups (N-) of agonists to opiate receptors may be the dominant domain that recognized by the antibody, while some substituent on morphine skeleton (3-, 6-) would affect recognition of the antibody.

Key words: anti-morphine antibody; opiates; specificity; morphine;

P400045

Clinical efficacy treated with L-tetrahydropalmatine on protracted withdrawal syndrome in heroin addicts

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Objective: To evaluate the clinical efficacy of L-tetrahydropalmatine (L-THP) on the protracted withdrawal syndrome (PWS) and craving in heroin addicts. Methods: A double-blind clinical trial was adopted and approved by the IRB/IEC. 119 patients met the DSM-IV criteria for heroin dependence were randomly divided into two groups: L-THP (60 mg2t/d) and placebo. Administration lasted 30 days just 7 days after the patients admitted into the clinic and scores for PWS and craving were assessed last out. Results: The scores for pain, palpitation, anxiety, sleep disorder and drug craving of the L-THP group were significantly lower than placebo ($P < 0.05$). The abstinence rate of the L-THP group at 1 month after treatment was significantly increased (46.2% vs 14.8%). Conclusion: The results showed that L-THP treatment produced a significant reduction on drug craving and partly on PWS of opiates dependence, and further mechanistic study would elucidate the functions of L-THP treatment on heroin-dependent subjects.

Key words: L-THP, heroin addiction, craving, protracted withdrawal syndrome

P400046

Buprenorphine is protective against the depressive effects of norbuprenorphine on ventilation

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High dose buprenorphine (BUP) is used as substitution treatment in heroin addiction. However, deaths have been reported in addicts using BUP. The role of norbuprenorphine (N-BUP), a metabolite of BUP, was hypothesized to explain these fatal cases. We determined the median intravenous lethal dose (LD_{50}) of N-BUP in male rats. The effects of a single intravenous dose of 3 or 9 mg/kg NBUP alone on arterial blood gases were studied. Finally, the effect of pre- and post-administrations of BUP on N-BUP-induced changes on arterial blood gases were analyzed. N-BUP's LD_{50} was 10 mg/kg. N-BUP 3 mg/kg produces the rapid onset of sustained respiratory depression. BUP not only protected against the effects of 3 mg/kg N-BUP in a dose-dependent manner but also reversed the effects when given afterward. Binding experiments suggest a role for mu- and to a lesser extent for delta-opioid receptors in BUP protective effect against N-BUP-induced respiratory depression. In conclusion, our data clearly show that N-BUP alone causes important deleterious effects on ventilation in rats and calls into question the role for N-BUP in respiratory toxicity associated with BUP use.

P400047

Control of enkephalin on ascending and descending reflex motor responses in guinea pig small intestine model

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Peristaltic activity is due to activation of ascending excitatory and descending inhibitory reflexes subserving the circular muscle. Enkephalins were identified in intestine showing role in motor activity. In this study, using triple bath method, ascending and descending motor responses of circular muscle in guinea pig small intestine were recorded as display of functional coordination between reflex pathways and effects of Met-enkephalin were evaluated. Field stimulation (0.8 ms, 5 Hz) induced ascending and descending contractions. In nonadrenergic noncholinergic (NANC) conditions ascending contraction and descending relaxation were simultaneously observed showing coactivation of NANC excitatory and inhibitory pathways. L-NNA increased the ascending contraction and reduced the descending relaxation. L-Arginin restored the motor responses. Met-enkephalin (0.001-1 micro M) inhibited reflex responses as the EC_{50} in inhibiting the ascending contraction (39.0 ±4 nM) was more than 6 times higher than that suppressing the descending relaxation suggesting a pronounced action of opioid in reducing the efficacy of NANC descending, mainly nitric oxide-mediated reflex motor activity.

P400048

The effect of lithium chloride on morphine-induced tolerance and dependence in isolated guinea pig ileum

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The aim of the present study was to investigate the effect of lithium on acute morphine-induced tolerance and dependence in an in vitro model of isolated guinea pig ileum which has been extensively used for the assessment of these effects of opioids. Morphine inhibited electrically stimulated twitch of ileum in a concentration-dependent manner ($pD_2 = 7.27 \pm 0.16$). Tolerance to this effect was induced by incubation of ileum with $2 \times IC_{50}$ of morphine for 2 h that induced a degree of tolerance of 14.7. The co-incubation of ileum with morphine along lithium chloride (1 mM) reduced the degree of tolerance significantly ($P < 0.001$) and restored the sensitivity of ileum to the morphine inhibitory effect. Lithium chloride can also reduce the expression of tolerance to morphine significantly ($p < 0.01$). Dependence was induced by incubation with $4 \times IC_{50}$ of morphine for 2 h and was assessed based on naloxone-induced contractions (10^{-5} M). Lithium chloride (1 mM) can attenuate the development but not expression of dependence to morphine as shown by the significant decrease in naloxone-induced contractions ($P < 0.05$). These results suggest that lithium chloride can reduce the development and expression of acute tolerance to and development of dependence on morphine in the myenteric plexus of guinea pig ileum.

Key words: Ileum, guinea pig; Tolerance; Dependence; Morphine; Lithium

P400049

Involvement of glutathione peroxidase in opioid dependence and amelioration of dependence by antioxidant effective natural products

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The study was undertaken to determine the commonly modulated gene by morphine, butorphanol, and nalbuphine by using microarray. We can obtain the interesting gene, glutathione peroxidase which was downregulated in the opioid-treated mouse cortex. Also this study was processed to the suitable antidote for the drug abuse by applying natural product which show the anti-oxident effect. Furtunately extract of Scutella radix, Polygala radix, Gardeniae fructus, and Ginseng radx show the anti-narcotic effect on the morphine dependence. The physical dependence on morphine was ameliorated by the Polygala radix extract but the psychological dependence was not modulated. Interestingly, morphine withdrawal syndrome was aggravated in the glutathione peroxidase/catalase (GPx/Cat-/-) knock out mice. These results suggest that the oxidative stress might be involved in the opioid dependence and antioxidatant effective natural products could be used to ameliorate the opioid withdrawal symptoms.

P41. Pharmacological Education

P410002

An investigation of perceptions of plagiarism amongst undergraduate biomedical and biological science students

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It has never been easier for students to plagiarise course work assessments, particularly from internet sources. However, the consequences of plagiarism for students can be severe. This project investigated the perceptions of students studying bio-

science and biomedical science as to what constitutes plagiarism. A questionnaire, based on scenarios which reflect 'real-life' situations, was given to 178 undergraduate students from foundation to final year level. The results showed that students were unclear about some aspects of what constitutes plagiarism, including downloading of material from the internet. They were also uncertain about the differences between permissible group work and collusion. Based on these findings, guidelines have been produced, aimed at addressing misconceptions. The questionnaire and subsequent guidelines have been useful in raising awareness of plagiarism amongst new students. Ongoing work involves converting the exercise to an on-line form, to provide instant feedback.

Key words: plagiarism undergraduates guidelines questionnaire.

Acknowledgements: This work was funded by the Learning and Teaching Subject Network for Bioscience.

P410003

Improving the performance of bioscience students in cell and molecular sciences

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Around 200 students graduate annually from Manchester Metropolitan University's degree programmes in Bioscience and Biomedical Science. Before 2003, much of the biochemistry and cell biology was taught to first year students in the module 'Biomolecules and Cells' which was assessed by coursework and examination (50:50). The relatively large amount of basic chemistry needed by students, and the rather dense biochemical content made this module unpopular and student performance was unsatisfactory. In 2003 the teaching and assessment strategies were reviewed and realigned to improve engagement in achieving the learning outcomes. The material is now taught in two modules: 'Molecules and Cells' which is compulsory for all first year students and 'Cells in Action', which is compulsory for students on biomedical science and physiology/pharmacology programmes. The former module is assessed using a range of approaches throughout the academic year. The latter has a 70:30 course work to examination division, combined with a varied and balanced approach to the course work. This approach has been successful in terms of improving student performance.

Key words: Learning assessment biochemistry performance

P410004

History of Drugs: A Teaching Proposal at Universities

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The study of the history of drugs and chemicals is essential for the proper utility of these substances by the population at large. Since the 1950s, our knowledge of medicine and pesticides increased greatly. Students in general are not familiar with the fascinating historical events and scientific stories associated with natural or synthetic substances. It is important to note that plants containing morphine, THC, hyoscyamine, physostigmine, pilocarpine, tubocurarine, digoxin, ephedrine and reserpine were used by various cultures for centuries before pure active therapeutic constituents were isolated and chemically characterized. Template molecules were synthesized. Parallel to these developments, the science of anatomy, physiology, biochemistry and pharmacology advanced. Better testing methods developed. Causes of many diseases were better understood. Drug laws were instituted. Pharmaceutical industry flourished. Class presentations should include the panoramic view of when, where, who, how and why drugs were developed. The outline based on Topics in the History of Pharmacology, P. 294, Eds. Patil, et al., Shah Prakashan, Ahemedabad, 2005, will be presented.

Key words: Pharmacology - History, Discovery

P410005

Validity of Assessments in a South African PBL Pharmacy Programme

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Validity is an important criterion of quality in the interpretation of assessment scores. The objective of this study was to investigate the content and face validity of assessments in the integrated, modular and Problem-based Learning BPharm programme of the University of Limpopo (Medunsa Campus) and Tshwane University of Technology. Content validity was investigated by matching the questions in each of 27 summative End of Module (EOM) examinations, held from 1999 to 2003, with the general learning objectives (GLOs) formulated for the respective BPharm modules and with the outcomes for entry level pharmacists required by the South African Pharmacy Council (SAPC). Face validity was investigated in 2002 by an opinion survey of 147 BPharm students. The questions in the EOM examinations covered a mean of 96% ± 5% of the GLOs and all of the outcomes required by the SAPC. The written examinations in the BPharm program were regarded as valuable for their learning by 83% ±5% of the students. Content and face validity were therefore established for these examinations.

Key words: assessment, content validity, outcomes, PBL.

P410006

Establishment of Laboratory Teaching System of Pharmacology

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In this article we discuss the role, the content and arrangement as well as effective instructional methods of laboratory teaching of Pharmacology. Because of its importance and characteristic in functional experiment course, it is designed to have three teaching phases including general introduction, experiment and case discussion, which need about 52 class hours in all. In addition to the use of apparatuses and basic animal laboratory techniques, we introduce cellular and molecular laboratory techniques to the general introduction. The experiment part is composed of basic experiments, integrative experiments and investigative experiments, the time ratio of which is about 3:7:3. Methods adopted at our department are learning through active participation by the students through problem-based leaning, computer-assisted learning, Web-based learning, virtual laboratories, seminars, audiovisual aids and compositive quiz. Our objective is to cultivate students with modern laboratory pharmacological knowledge, the spirit of "Respect Life" and the understanding of humanity. So that when students graduate, they could serve other people and society better.

Key words: Pharmacology; laboratory teaching; reform

P410007

Pharmacology in the integrated course "Basic Medical Sciences" in Zhejiang University School of Medicine

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According to traditional teaching mode, the courses in preclinical medicine including pharmacology are separately run. This mode causes a series of disadvantages including loose connection between knowledge in different disciplines and weak ability to bridge basic predinical knowledge and clinical practice. In order to overcome the disadvantages and promote the teaching efficiency, we constructed a new integrated course - Course of Basic Medical Sciences, which includes 6 traditional courses, anatomy, histology and embryology, physiology, pathology, pathophysiology and pharmacology. We integrated these courses based on the human organ systems and according to the principle - "From macro to micro, From morphological to functional, From normal to abnormal and From disease to drug therapy" and published the series of textbook in 2004. The contents of pharmacology are taught just after pathology and pathophysiology in every organ system. In comparison with the traditional teaching mode, teachers of pharmacology need not spend a lot of time to review preceding knowledge of anatomy and histology, physiology, pathophysiology and pathology. This is helpful in saving time and improving the teaching efficiency

P410008

Protective effects of curcumin on injury of HUVEC and its molecule mechanisms

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AIM: To observed the cytoprotection effect of curcumin and its molecular mechanism on the cell injury caused by TNF-α and thrombin in isolated human umbilical vascular endothelial cells (HUVEC) in vitro. METHODS: The adherence of platelets, leukocytes to HUVEC was determined by [^{3}H]-Adenine labeled and

Myeloperoxidase. The expression of P-selectin, GPIIb/IIIa and ICAM-1 mRNA and protein was detected by RT-PCR, FCM and Western blotting, respectively. The extent of cell livability was assessed by MTT viability assay. RESULTS: It's showed that the increase of adhesion between activated HUVEC and platelets and leukocytes were significantly inhibited by Curcumin in a concentration dependant manner. The expression of ICAM-1 and P-selectin can be inhibitied by Curcumin respectively. It's demonstrated that in the TNF-a group, the cells suspended in the culture medium, While group pretreated with Curcumin had showed no obvious injury character in the tests. CONCLUSION: Curcumin could act against the endothelial cell damage caused by activated platelet and TNFa, which could be attributed to a poly-pathway mechanism.

P410009

An Original Means Might Be Promising In The Teaching Of Pharmacdogical Experiment

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Objective: To evaluate an original teaching means in pharmacological experiment. Methods: One hundred students were randomly divided into two groups with fifty students each group. Group I treated with normal means: Teachers narrated the procedure before students started an experiment. Students left classroom after they finished the experiment and teachers did not instructed them any more; Group II treated with original means: students learned an experimental procedure and did the experiment all by themselves. If they got into trouble they would immediately found instruction from their teachers. Then they discussed the experiment with their teachers after experiment finish. Results: It showed that the pharmacological experiment grades of students in group II were significantly better than that of the students in group I ($P < 0.05$). Conclusion: It is therefore suggested that the original means might be promising in the teaching of pharmacological experiment.

Key Words: original means, teacling, experiment

Acknowledgement: This study was supported by School of Pharmacy, Yantai University.

P410010

The discussion of "Bilingual" teaching in Pharmacdogy

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Objective: To explore the present situation of Pharmacdogy "Bilingual" teaching in our college. Methods: To know development of "Bilingual" teaching combining with our college practical circumstances. Results: "Bilingual" teaching of Pharmacology have a lot of insufficient in our college. Conclusion: To need further know teaching content, form and test for Pharmacology "Bilingual" teaching.

Key words: "Bilingual" teaching; Pharmacology; Explore

P410011

The Comparison About Teaching Styles Between Medical Cdlege And Pharmacy College

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Objective: To compare medical college with pharmacy college about teaching styles. Methods: Students of medical cdlege and pharmacy cdlege were divided into two groups accordingly. Through observing separately the two groups of students studying condition in class include theory class and experiment class within a semester, much attention had been paid to compare medcal cdlege with pharmacy college about teaching styles. Results: Experiment class had important proportion in the two colleges. But, because of different position they based on, the emphasis they paid on training student was different. Pharmacy college emphasis paid on cultivating students ability of new drug research, whereas medical college emphasized on learning how to put their theory knowledge into use in the clinical disease research skillfully. Conclusion: Training students practical capability was paid attention by both colleges. And just theirs emphasis particular on training students was different owing to different teaching background.

Key Words: comparison teaching styles

Acknowledgement: This study was supported by School of Pharmacy, Yantai University.

P410012

Pharmacology teaching in African schools of medicine, pharmacy, dentistry and nursing

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A survey was conducted in 12 schools of medicine (SOM), 12 schools of pharmacy (SOP), 6 schools of dentistry (SOD) and 12 schools of nursing (SON) in order to find out how pharmacdogy teaching is organized in Africa. The department of pharmacology was only found in the SOM and SOP. A fair distriibution of academic staff at all levels was found in some schools. However, senior positions were more vacant in many others. The number of hours allocated to pharmacology teaching were: 106 ± 20 in the SOM, 135.6 ±... in the SOP, 113.3 ±... in the SOD and 115.1 ±.... in the SON. The time devoted to research ranges from 7 to 15 %. Involvement of students in research and seminar presentation is popular in SOM and SOP and inexistent in SOD and SON. Teaching material and evaluation methods are far from becoming getting standardized. Cooperation and exchange of teaching material between the different institutions should be encouraged. A workshop on teaching pharmacology should be organized in order to build a common vision and define action plan to be followed on the African continent.

Key words: Pharmacology, teaching, Africa.

Acknowledgement: WHO (Department of Essential Drugs and Other Medicines) for financial support of the study.

P410014

Perceptions of Student Nurses Regarding the Use of a Factual Novel (autobiography) as a Teaching Tool

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Resent studies encourage educators in nursing to use innovative and non-traditional teaching methods, such as using popular movies, posters, portfolios and surfing the internet, to stimulate students' interest, participation and interaction to enhance academic performance as well as knowledge retention. In this, descriptive cross-sectional study, we used self-administered questionnaires with statements graded on 5-points Likert scale (quantitative measures) and open-ended questions (qualitative measures), to assess the feasibility and students' perceptions of using factual novels as teaching tools. At the beginning of the lecture copies of selected chapters from Armstrong & Jenkins (2001), were given to students. Willing students were requested to read for the whole class while the lecturer interjected periodically to explain and expound on certain pharmacological concepts. Eighty percent (80%) of participants felt that the use of a factual novel stimulated their interesting in cancer drugs and 84% agreed/strongly agreed that it contributed to their knowledge of pharmacdogy. Using Lance Armstrong's novel to teach cytotoxic drugs is a worthwhile and rewarding exercise from the student's perspective.

Key words: pharmacdogy, cytotoxic drugs, student nurses, teaching methods, popular novels.

P42. Others

P420001

β2 Adrenoceptor Blockers "Carazdol" and Conception Rate In Buffalo In Scope of The Artificial Insemination

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Effect of Carazolol on the pregnancy rate in buffalo and cows were studied. I/V injection of Carazolol (2.5 mg/animal) were given for 68 buffalo and 90 cows 10 minutes before insemination (treated group). Another 114 animals (41 buffalo and 73 cows) were injected with 5 ml saline (control group). The uterine tone was measured rectally following injection of Carazdol. The results revealed that 65% (44/68) and 71% (64/90) of treated buffalo and cows proved pregnant at day 42 post insemination respectively, whereas in control animals 51% (21/41) of buffalo and 58% (42/73) of cows were diagnosed pregnant at day 42 post in-

semination. The differences between treated and control buffalo and cows were significant ($p < 0.05$). The animals that have a good uterine tone (+++) during estrus, give a high percentage of conception. Finally, injection of 2.5 mg of β_2 adrenoceptor blockers Carazolol can relief the effect of stress on the uterus during the heat period and it will improve the conception rate in both buffalo and cows.

Key words: buffalo, Carazolol, conception, insemination.

P420002

Protective effect of puerarin on cultured cerebral cells injured by anoxia-reoxygenation

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OBJECTIVE: To study the protective effect of puerarin(PUE) on cultured cerebral cells injured by anoxia-reoxygenation. METHODS: The anoxia-reoxygenation injury model were developed, anoxia for 60 min and reoxygenation for 30 min. the effect of PUE on cerebral ultrastructure was observed. $[Ca^{2+}]_i$ was estimated with Adherent Cell Analysis and Sorting 570(ACAS 570) Laser Cytometer and measured with fluorescent dye Fura-2-AM, the lipid fluidity of cellular membrane was determined by fluorescence polarization technique. RESULTS: PUE could obviously improved the ultra-structure of cerebral cells and dose-dependently decrease $[Ca^{2+}]_i$ and increase the lipid fluidity of cellular membrane, PUE also could markedly reduced the chromaticity value of pseudo-colour graphic model of Ca^{2+}. CONCLUSION: Puerarin has the obvious protective effect on cultured cerebral cells injured by anoxia-reoxygenation, this may be related to its effect of decreasing $[Ca^{2+}]_i$ and increasing the lipid fluidity of cellular membrane.

Key words: puerarin; Anoxia-reoxygenation; Calcium; Membrane fluidity

P420003

Experimental Study of the Affection of Puerarin on Glaucomatous Optic Neuroprotection

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Objective: To observe the protective effect of puerarin on optic nerve of chronic intraocular pressure elevated(IOP). Method: IOP was reduced to normal through conventional glaucomatous trabeculetomy. Injection puerarin was used everyday in the treated group for 4 weeks. The levels of the glutamic acid and NO in retina was measured. The number of retinal ganglion cells(RGCs) were observed. Results: The glutamic acid and NO levels of the treatment group was significantly lower than those in experimental group($P < 0.05$, $P < 0.01$). The result indicated that compared with experimental group the damage of retina and optic nerve axons relatively gentler. The number of RGCs of treatment group was more than the experimental group ($P < 0.05$, $P < 0.01$). Expression level of bcl-xl and BDNF in retinal was enhanced in treated group. Conclusion: Puerarin can protect the optic nerve from elevated IOP efficiently by alleviated the damages of the retinal and optic nerve axons ultrastructure induced by chronic ocular hypertension, alleviating the apoptosis of RGCs, alleviated the toxicity of NO and glutamic acid and enhanced the expression level of bcl-xl and BDNF in retinal.

Key words: glaucoma; puerarin; protection of optic nerve;

P420004

Polypeptide from Chlamys farreri inhibits HaCaT cells apoptosis and modulates UVB induced signaling pathway activation

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Polypeptide from Chlamys farreri (PCF) has been identified as a potent antioxidant and photoprotective agent. In this study, we investigated whether PCF could inhibit apoptosis of HaCaT cells induced by ultraviolet B (UVB) and explored the role of the MEK-ERK pathway and Caspase cascade on HaCaT cells cultured in vitro. We found that PCF attenuated UVB caused DNA fragmentation in HaCaT cells. Caspase inhibitors substantially blocked the UVB-induced DNA fragmentation and the inhibition of MEK-ERK pathway enhanced UVB-induced DNA fragmentation. However, PCF potently stimulated the phosphorylation of MEKs and ERKs and bated the activation of Caspase-3. The results indicate that PCF had protective effects against UVB-induced apoptosis in HaCaT cells, and part of the antiapoptotic effect of PCF might be mediated by its ability to modulate the MEK-ERK pathway and Caspase-3 cascade.

Key words: Polypeptide from Chlamys farreri; Ultraviolet B; Mitogen-activated protein kinases; Caspase-3

Acknowledgements: The authors are very grateful to technical assistance of Dr. Yao Ru Yong. This work was funded by both the National science Natural Foundation of China (No. 30471458) and the science Natural Foundation of shandong province (No. Y2003c02).

P420005

Somatostatin (SRIF) infused in the globus pallidus increases locomotor activity and cFos expression in rat brain areas implicated in motor control

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This study investigated the effect of SRIF and selective ligands on locomotor activity when infused in the rat globus pallidus (GP), and the resultant changes in neuronal activity. Male Sprague-Dawley rats were infused bilaterally in the GP with SRIF (60, 120 ng/0.5μl/side), L-797,591 (sst1 agonist, 60, 120, 240 ng/0.5μl/side), L-779,976 (sst_2 agonist, 120, 240, 480 ng/0.5μl/side), L-803,087 (sst_4 agonist 240 ng/0.5μl/side), SRA-880 (sst_1 antagonist + SRIF, 120ng/0.5μl/side) and CYN154806 (sst_2 antagonist + SRIF, 120 ng/0.5μl/side) or saline. Locomotor activity was measured for 60 min. Brains were processed for c-fos like immunoreactivity. SRIF increased the locomotor activity of the rat in a statistical significant manner, by activating sst_1, sst_2 and sst_4 receptors. C-fos expression was increased in the motor areas of the prefrontal cortex, the striatum, and the hippocampus. This study provides functional evidence for the presence of sst1,2,4 in the GP. Investigations are in progress in order to delineate the neurochemical routes via which SRIF mediates the enhancement of locomotor activity.

Key words: somatostatin receptors, basal ganglia; co-funded by the Eur. Soc. Fund Natl Res, Heraklitos

P420006

Therapeutic Update of the Traditional Medicine in Cuba

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Cuba has a prodigious flora that offers therapeutic alternatives to Public Health and Veterinary Medicine. New investigations are being carried out in order to get natural health product (NHP). The traditional medicine has played an important role in the treatment of diverse pathologies, mainly in the developing countries. The objective of this work is to describe the characteristics of the traditional medicine in Cuba and the main requeriments for the registering of herbal medicinal products in Cuba. The market and the main challenges are analysed in the investigation of the phytomedicines as well as the tendencies in the growth of this attractive sector. Another important aspect is, the importance of clinical trials in order to guarantee the safety quality and efficacy of NHP, the main mistakes in Clinical Trials of natural products are explained. The strategies for the development of herbal medicinal products in Cuba are showed as well as some of the interactions between natural and synthetic drugs in Cuba. The natural health products are considered a very important source for the health in Cuba.

Key words: Cuba, regulatory, phytomedicines

P420007

The effect of continuous darkness and light on the reactivity of vasa defrentia in rats

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Environmental and psychological stress cause an immediate and significant release of vasoactive substances such as noradrenaline (Nad). Rats subjected to environmental stress through continuous exposure to light or darkness were examined for changes in their smooth muscle reactivity. Sixteen male albino Wistar rats weighing 70-90g were divided into two groups. One group is exposed to continuous light and the other to continuous darkness for 4 weeks. A control group (n = 6) was kept at normal day light cycle. At the end of the exposure period, the vasa defrentia were isolated and isometrically tested for its reactivity to Nad and 5-HT. Vasa defrentia from rats of continuous darkness showed a significant decrease in their responses to 5-HT and Nad compared to controls. Similarly, vasa defrentia from rats subjected to continuous light showed a significant decrease in the

responses to 5-HT and Nad compared to control groups. The above results may be explained by a down regulation mechanism that could be resulted from prolonged exposure to excessive vasoactive substances release due to the environmental stress.

P420008

Loco-regional radioimmunotherapy (RIT) of high grade malignant gliomas using the humanized monodonal antibody, h-R3, labeled with 188-Re.

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RIT may improve the management of malignant gliomas. A Phase I clinical trial was performed to evaluate the toxicity and clinical effect of intratumoral administration of a single dose of the humanized h-R3 MAb directed against epidermal growth factor receptors. 3 patients with anaplastic astrocytoma (AA) and 7 with glioblastoma multiforme (GBM) were treated with 3 mg of MAb labeled with 10 or 15 mCi of 188-Re. In patients treated with 10 mCi (n=6) transitory worsening of pre-existing neurological symptoms were observed. Patients treated with 15 mCi (n=4) development severe neurological symptoms. In the group treated with 10 mCi, 1 GBM patient died in progression after 6 months of treatment, 2 patients (1 GBM and 1 AA) development stable disease during 3 months. One GBM patient has partial response for more than 1 year and 2 patients (1 GBM and 1 AA) were asymptomatic and in complete response after 3 years of treatment. Maximal tolerated dose of the radioimmunoconjugate h-R3-188-Re is 10 mCi. RIT using the h-R3 MAb labelled with 188-Re at the dose level of 10 mCi, may be relatively safe and a promising therapeutic approach for treating high grade gliomas.

Key words: RIT, gliomas, h-R3 MAb.

P420009

Effects and Mechanisms of Anisodamine to Prevent Liver Fibrosis in Rats

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Objective: To investigate the protective actions and mechanisms of anisodamine on liver fibrosis. Methods: The experimental liver fibrosis model was produced by CCL4. All therapeutic groups had been treated by anisodamine intraperitoneal injection once a day for six weeks. The pressure of portal vein, serum indices, liver slices and the contents of MDA and NO in livers were compared. Expressions of transforming growth factor beta1 (TGFβ1) and IV collagen were observed by immunohistochemistry. RT-PCR was used to detect the mRNA expressions of inos, enos, matrix metalloproteinase2 (MMP2) and its tissue inhibitor (TIMP2) in livers. MMP2 was determined by gelatin zymography. Results: Anisodamine diminished the degeneration, necrosis and extracellular matrix (ECM) deposition in fibrosis livers. The portal vein pressure, biochemical indices and TGFβ1 were significantly reduced in anisodamine treated groups. The mRNA expressions of inos, enos, MMP2, TIMP2 and the protein of MMP2 were significantly reduced in anisodamine treated groups. The expression ratio of MMP2 and TIMP2 was adjusted. Conclusion: Anisodamine can ameliorate liver fibrosis by inhibiting lipid peroxidation and ECM deposition.

Key words: Anisodamine; Liver fibrosis; MMP2

P420010

THE STUDY OF RHIZOMA PINELLIAE ON VOMITING IN MINKS

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Aim To study emetic and anti-emetic effects of rhizoma pinelliae in minks. Methods The emetic effect of raw pinellia 2 g.g^{-1} ig was investigated in minks; three preparations of rhizoma pinelliae (processed with ginger) were made by ethanol extraction, water extraction and water decoction respectively and their effects on emesis model induced by cisplatin (7.5 mg.g^{-1}, ip) or apomorphine (1.6 mg.g^{-1}, sc) were then studied; the effect of rhizoma pinelliae (processed with ginger) by decoction on rotation-induced emesis model in minks was also observed. Results Raw pinellia can induce emesis in minks ($P<0.01$), on the other hand, rhizoma pinelliae (processed with ginger), metoclopramide and ondansetron significantly inhibit the emesis model induced by cisplatin and apomorphine ($P<0.05$) in minks while showing no effect on the emesis induced by rotation in minks. Conclusion Pinellia tuber showed anti-emetic effect in minks and its mechanism is probably related to its inhibiting property on central nervous system

Key words: pinellia tuber; emesis; mink

P420011

The reasearch on the bioactivities of betaine

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Object: to study the effect of betaine on EGF receptor and the lipotropic effect of betaine in hepatic steatosis induced by ethanol in rats. Methods: using radioligand binding assay of receptor, comparing the binding of 125I-EGF to its receptor between the test group and the control group; Using the HPLC to determine the levels of Sadenosylmethionine in the rat liver cells to compare the differences between groups. Results: 26n mol L^{-1}-5.2 mmol L^{-1} betaine inhibit the binding of EGF receptor in a noncompetitive way, 0.5% betaine in the diet prevented hepatic steatosis induced by chronic dietary feeding. And promote the generation of Sadenosylmethionine compared with control group dramatically ($P<0.05$). Conclusion: betaine can inhibit the binding of EGF receptor and it has the ability to prevent the hepatic steatosis induced by ethanol.

Key words: betaine, EGF receptor, S-adenosylmethionine

Acknoledgement: Thanks for the Committee of National Natural Science Foundationof China, by which the project is supported. (NO.30400352, NO.30300284)

P420012

ATP potentiates effects of prostaglandin in human pregnant uterus

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The aim of the study was to test the functional activity of P2 receptors in human uterus. In vitro experiments were performed on myometrial samples obtained from women undergoing caesarean section at different stages of pregnancy. Concentration-response relationships for α,β-methylene-adenosine 5-triphosphate (α,β-meATP), for ATP, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and their combination were obtained using pharmacological organ bath technique. An in vivo study was carried on pregnant women with dysfunctional abnormalities of the active stage of labor where Controls received intravenously $PGF_{2\alpha}$, while the ATP group received $PGF_{2\alpha}$ concomitantly with ATP. We found that α,β-meATP evoked contractions of isolated uterus which were significantly higher in full term than in earlier pregnancy. ATP at low concentrations potentiated the responses of the isolated uterus induced by $PGF_{2\alpha}$. Patients receiving ATP as a supplement to $PGF_{2\alpha}$ treatment had a significantly shorter second stage of labor and needed lower total dose of $PGF_{2\alpha}$. In conclusion, since P2 receptor-mediated contractions are increased with progression of the pregnancy, ATP could be a useful supplement drug to increase uterine contractility at labor.

P420013

The Inhibitory Effect of Nobiletin on Human non-small Cell Lung Cancer Cell Line A549

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Objective To investigate the inhibitory effect of nobiletin (5,6,7,8,3'4'-hexamethoxyflavone) on A549 cell line and its mechanism. Methods The inhibitory effect of nobiletin on A549 cells was evaluated by MTT, growth curve, clone-forming assay, microscope, flow cytometric analysis and agarose gel electrophoresis. Results After treated with nobiletin for 24、48、72 hours, MTT assay showed IC_{50} of nobiletin to A549 in 24h、48h and 72h were 38.2 μg/ml, 25.7 μg/ml and 16.7 μg/ml respectively; IC_{50} of nobiletin to A549 cells in clone forming test was 25.9 μg/ml. The dose-effect and time-effect relationship were described in the growth curve. The characteristic morphology typical for apoptosis was observed under microscope. The cell cycle was arrested in G2/M phase, cells in G0/G1 phase decreased. The percentage of apoptosis increased. The sub-G1 peak, DNA ladder typical for apoptosis, Significant raise of bax

expression and the ratio of bax/bcl-2 was observed. Conclusions Nobiletin can inhibit the growth of A549 cells in vitro, its mechanism is probably associated with the apoptosis induction.

Key words: Nobiletin; A549 cell line; apoptosis

P420015

Pathogenicity of a gene encoding a fibrinogen-binding protein (fbe gene) from Staphylococcus epidermidis

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Objective To study the pathogenicity of fbe gene from Staphylococcus epidermids. Methods Homologous recombination method was used to acquire a S.epidermidis fbe mutant which have the same gene background as a fbe-positive strain HB except fbe. A rat central venous catheter (CVC) infection model was established to compare the in-vivo pathogenicity of S. epidermidis HB with its fbe gene mutant S. epidermidis HB-ermB. Additionally, an ELISA method was used to compare the adhesion to fibrinogen (Fg) between fbe-positive and fbe-negative strains in vitro. Results The fbe mutant S. epidermidis HB-ermB was constructed. The difference in adhesion to Fg between fbe-positive and fbe-negative strains in vitro was significant ($P < 0.01$). The infection rate of HB group (100%) was significantly higher than that of HB-ermB group (20%). The CFU (colony forming unit) recovered from catheters, blood and tissues of HB group were larger than that of HB-ermB group, and the differences were all significant ($p < 0.01$). Conclusions Defect of fbe gene could lower pathogenicity of S. epidermidis, implying that fbe gene is an important factor to induce S. epidermidis infection.

Key words: S. epidermidis; fbe gene; pathogenicity

P420016

Advancement in drug treatment of osteoarthritis in articular genu

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Osteoarthritis (OA) in articular genu is the most common one in all kinds of arthritides. Odynolysis is still a key issue in OA treatment in spite of .the increasing knowledge on its pathology at these days. Drug treatments, including local, intra-articular and oral administration, have been taking an important rcle in the alleviation of pain caused by OA. Desirable effect can be achieved by local administration of 0.025% capsaicin gel and 5% Brufen gel. As to intra-articular administration, besides corticosteroid, hyalurate is now used widely with the effectiveness on protecting arthrodial cartilage, lubricating articular cavity, improving intra-articular milieu and painkilling, etc. Among various oral preparations, COX-2 inhibitor, such as Celebrex, is the prefered one for severe or medium pain sufferer as well as elder patients because of its relatively minor side effects on gastrointestinal tract. It is also a preference for sufferers of rheumatoid arthritis and acute pain, but its side effects on cardiovascular systems huold be cautioned if used for long.

P420017

Prescription of prophylactic antibiotics for neurosurgical procedures in teaching hospitals in Iran

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Objectives: To assess the appropriateness of surgical antibiotic prophylaxis in neurosurgical procedures, using the American Society of Health-system Pharmacists guideline as reference. Methods: We recruited 110 patients by random selection from a sampling frame of 2 hospitals. Data were collected prospectively from patients' medical records in 2004. The data collection forms for each patient contained patient demographics, type of surgery and type of antimicrobial prophylaxis regimen. Results: A major discrepancy about antibiotic prescription was seen between current administration and the ASHP guideline. The direct cost of prophylactic antibiotics was 14 times greater than what it would have cost to administer prophylactic antibiotics adhering to the ASHP guideline (US $802 vs. US $59). Conclusion: This study indicates the need for interventions to improve the rational use of antibiotic prophylaxis in Iran to prevent the complications of inappropriate administration of antimicrobials and decrease unnecessary costs.

Key words: neurosurgery; antimicrobial prophylaxis; compliance
Acknowledgement: Thank Deputy for Research at the Shiraz University of Medical Science (grant no. 83-2168).

P420018

Dissolution profile as a means for quality control of botanical products - a pilot study of Gegen-Danshen capsule

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Objective: In vitro dissolution profile has been utilized internationally as a standard measure for quality control (QC) of formulation of conventional drugs. The present study assessed the dissolution profiles of multiple components of Gegen-Danshen capsule to determine the characteristics suitable for QC of such a botanical product. Methods: An HPLC assay for quantification of 10 components of a Gegen-Danshen capsule was established. The dissolution tests for two batches of capsules were performed at pH2.0 and pH7.4 (sequentially) using the standard USP method. Results: Of 10 components, 7 water soluble ones were detected for studying dissolution profiles. Their cumulative % dissolved ranged 50-100%. The time to reach 50% of the total dissolved was similar among the 7 components. Only 3 components had similar profiles between 2 different batches. Conclusions: Dissolution profiles of multiple components provided unique characteristics reflective of the formulation effect, and are thus suitable as well as needed for the QC measure of a given botanical product with multiple active components.

Key words: Dissolution; Gegen-Danshen
Acknowledgement: AoE grant (AoE/B-10/01) by UGC, H.K.

P420019

Increasing de novo neurogenesis for the therapy of motor neuron degeneration in ALS-like mice

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Using transgenic mice mimicking ALS, we showed that there was an increase of neural progenitor cell (NPC) proliferation, migration, and neurogenesis in the lumbar region of adult spinal cord in response to motor neuron degeneration. The proliferation of NPCs detected by BrdU incorporation and LacZ staining was restricted to the ependymal zone surrounding central canal (EZ). Once the NPCs moved out from the EZ, they lost the proliferative capability, but maintained migratory function vigorously. During ALS-like disease onset and progression, NPCs in the EZ migrated initially toward the dorsal horn direction, and then to the ventral horn regions, where motor neurons have degenerated. More significantly, there was an increased de novo neurogenesis from NPCs during ALS-like disease onset and progression. The enhanced proliferation, migration, and neurogenesis of (from) NPCs in the adult spinal cord of ALS-like mice may play an important role in attempting to repair the degenerated motor neurons and restore the dysfunctional circuitry which resulted from the pathogenesis of mutant SOD1 in ALS. Treatments of ALS-like mice with neurogenic Rx-087 delayed disease progression and extended lifespan.

P420020

Neurogenic and dopaminergic neurogenic responses in the substantia nigra (SN) of MPTP-induced Parkinson's disease-like mice

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Using nestin promoter controlled LacZ reporter transgenic mouse model coupled with MPTP lesion system, we demonstrated there are neural progenitor cells (NPCs), basal levels of neurogenesis, and DA neurogenesis in the normal adult mouse SN. In addition, we also showed there is not only a significant increase in the number of NPCs, but also a dramatic increase of neurogenesis from the NPCs in the SN and the midline region adjacent to the SN of the PD-like mice, compared with that of normal controls. More importantly, we demonstrated there is an increase of DA neurogenesis in the SN of the MPTP lesioned mice. The increased DA neurogenesis in the MPTP lesioned mice was derived from the NPCs and BrdU positive cells. Intracerebroventricular transplantation of embryonic NPCs (eNPCs) in the MPTP-lesioned mice, promotes neurogenesis and DA neurogenesis

in the SN. The increased NPC migration, integration and differentiation in the MPTP lesioned mice further suggest that experimental approaches to promote neurogenesis may provide an effective therapy to PD by functional replacement of degenerated DAs.

P420021

Effects of Osthol on testosterone and testis androgen receptor level in The reproduction system disturbance mice

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AIM: To investigate the effects of Osthol(Ost) on testosterone and testis androgen receptor level in The reproduction system disturbance mice. METHODS: The reproduction system disturbance model was established by injecting cyclophosphamide in mice. They were treated i.g. with Ost daily for 20 d. The level of testosterone in serum and the auxiliary sexual organ coefficient were calculated. The testis and rogen receptor (AR) was determined by immunohistochemistry. RESULTS: The Ost-treatment(150 mg/kg) significantly increased the level of serum testosterone and the coefficient of epididymis($P<0.05$); The Ost-treatment(150 mg/kg, 75 mg/kg) increased significantly increased the coefficients of seminal vesicle($P<0.05$). The specific AR immunostaining was observed in Leydig cells, peritubular myoid cells, spermatogonia. The Osttreatment(150 mg/kg) increased significantly the AR positive cells percentage of peritubular myoid cells($P<0.05$). CONCLUSION: Osthol could increase testosterone levels and the AR positive cells percentage of peritubular myoid cells in The reproduction system disturbance mice.

Key words: osthole; cyclophosphamide; testosterone; testis androgen receptor

P420022

Effect of L-Arginine on healing of burn wounds

ilkhanizadeh behrouz*.

Nitric Oxide (NO) have an important role in healing of burn wounds This study investigated the effect of LArginine on experimentally induced burn wounds. A total of 40 rats weighing 230-270 gr were used in this study. The shaved skin on the back of the rats was immersed in 100 °C; water for 8 seconds to achieve a partial thickness scald burn. The rats were divided into four groups. In groups I and II (control groups) 100 mg/Kg of Normal Saline was injected for 7 and 15 days respectively. In groups III and IV (experimenal groups) 100 mg/Kg L-Arginine was injected intraperitioneally for 7 and 15 days respectively as 1st, 4th, 11th and 14th days after burn. 7 days postburn, the rats of groups I, III and on days 15 postburn, the rats of groups II, IV killed and the burn areas were investigated histopathologically. Changes such as epidermal proliferation, inflammation, collagen formation and blood vessels were evaluated. Epidermal proliferation, collagen formation and blood vessels were higher in experimental groups(III, IV) than those observed in the control groups (I, II). Inflammation in control groups was higher than experimental groups. We concluded that heading of burn wound is accelerated by L-Arginine.

P420023

Beneficial effects of n-hexacosand on STZ-induced diabetic rat trachea

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Objectives: In order to investigate the diabetes-associated neuropathy and the effects of n-hexacosanol (FA) in trachea, we studied its effect on diabetic-induced hyper-reactivity in the rat trachea. Methods: Eight weeks old male SD rats were divided into 5 groups. One group was as age-matched control rats and others were induced diabetes by streptozotocin (50 mg/kg, i.p.). Four weeks after the induction of diabetes, rats were randomly divided into four groups: immediately sacrificed rats to perform experiments, and diabetic rats treated with FA (0, 2 or 8 mg/kg, i.p. every day) for the another 4 weeks. The serum glucose and insulin levels were determined, and the contractile responses of the trachea induced by carbachol and KCl were investigated. Results: Treatment with FA did not alter the diabetic status of rats, i.e., body weight, thickness of the trachea, serum glucose levels, and serum insulin levels, but significantly improved the diabetic-induced hyper-reactivity of the trachea in a dose-dependent manner. Conclusion: Our date indicates that this drug can improve hyper-reactivity in the diabetes-induced rat trachea.

Key word: trachea, n-hexacosanol, diabetes

P420024

INVOLVEMENT OF INCREASED ARGINASE ACTIVITY IN IMPAIRED ENDOTHELIUM DEPENDENT CAVERNOUS RELAXATION WITH AGING IN THE RABBIT

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Objective: Whether arginase is involved in impaired endothelium dependent cavernous relaxation with aging in the rabbit.

Materials and Methods: Young adult and aged rabbits were used. Cavernous tissues were processed for isometric tension experiments, measurements of cyclic guanosine monophosphate (GMP), nitric oxide synthase (NOS) and arginase activities, endogenous methylarginines and L-arginine.

Results Carbachol induced endothelium dependent relaxation was significantly impaired in aged specimens without change in sodium nitroprusside induced relaxation. Cyclic GMP production was significantly decreased in aged. NOS activities remained unchanged. The tissue contents of endogenous methylarginines and L-arginine were decreased in aged. Arginase activity was significantly higher in aged. Impaired relaxation in aged was normalized in the presence of NG-hydroxy-L-arginine as an arginase inhibitor or excess L-arginine.

Conclusions: These results suggest that impaired endothelium dependent cavernous relaxation with aging is due to decreased NO production, which would result from increased arginase activity and probably from decreased L-arginine content.

Key words: penis, rabbits, aging, arginase

P420025

Evaluation of QT interval in conscious guinea-pigs and dogs instrumented with telemetry.

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Early evaluation of the cardiovascular safety of potential development compounds, in particular, the effect on the QT interval of the electrocardiogram (ECG), is a key requirement in drug discovery today. In this study, the effects of sotalol on mean arterial pressure (MAP) and ECG intervals were assessed in conscious guinea-pigs ($n=6$) and beagle dogs ($n=8$). In guinea-pigs instrumented with telemetry, oral administration of sotalol at 10 and 30 mg/kg had no effect on MAP, whereas at 100 mg/kg MAP was decreased. Furthermore, sotalol dose-dependently increased the heart rate-corrected QT interval (QTc): at 100 mg/kg, QTc increased by 13 $\pm$2% ($p<0.001$) and the RR interval by 23 $\pm$3% ($p<0.001$). In telemetry dogs, sotalol showed little effects on MAP and RR interval after oral administration of 1, 3, and 10 mg/kg but dose-dependently increased QTc: at 3 mg/kg QTc increased by 11 $\pm$2% ($p<0.001$) and at 10 mg/kg by 18 $\pm$3% ($p<0.001$).

In conclusion, in conscious guinea-pigs and beagle dogs sotalol induced significant QTc prolongations.

Telemetric dogs and guinea-pigs could, therefore, be used to assess the cardiovascular safety of drug candidates.

Key Words: ECG; QT interval, blood pressure, telemetry

P420026

The effect of ozone on isolated guinea pig tracheal preparations and its influence on the action of drugs

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Ozone is a major pollutant formed from common atmospheric pollutants such as hydrocarbons or nitrogen oxides. On the other hand, ozone has a variety of potential uses in industry, homes and medicine. Inhalation of ozone can induce rapid damages to epithelial cell membranes in the pulmonary airways. In vitro

methods, employing isolated tracheal preparations, offer a unique possibility for studying the adverse effects induced by inhaled ozone. Although the in vitro study of ozone poses a special problem due to the short half life of ozone in Krebs solution, this study was adapted to perform in vitro studies of ozone on isolated guinea pig trachea as well as its effect on the action methacholine (Mch) and isoproterenol (Isopr).

The results indicated two direct effects on the trachea: (i) contraction of the trachea, and (ii) a hyper responsiveness to Mch. It was concluded that ozone has no adverse effect on muscarinic receptors. Ozone has a desensitizing effect on the response of Isopr, while Isopr relaxed the ozone-induced tracheal contraction.

This study emphasised that the inhalation of ozone should be avoided, and especially by those with airway diseases.

trachea ozone methacholine isoproterenol

P420027

HYDROGEN PEROXIDE MODULATES ANGIOTENSIN II-INDUCED CONTRACTION OF DIABETIC MESENTERIC ARTERIES VIA AN INDOMETHACIN-SENSITIVE PATHWAY

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Hydrogen peroxide is increased in diabetes. We explored its role in angiotensin II (Ang II)-induced contraction of mesenteric arteries from streptozotocin-induced diabetic rats (DM) using the Mulvany-Halpern myograph. Catalase (H_2O_2 scavenger, 800 U/ml) raised Ang II contraction in DM but not the normal (WKY) tissues, suggesting H_2O_2 inhibits contraction in DM. Superoxide anion scavenger, SOD (150 U/ml) reduced contraction in both groups, suggesting that superoxide mediates Ang II contraction in both tissues. L-NAME (0.1 mM) significantly raised the contraction in WKY and DM. Catalase did not alter the L-NAME effect in WKY, but synergised with L-NAME in DM, suggesting that Ang II contraction stimulates a relaxant mechanism which is NO-mediated in WKY but NO and H_2O_2 mediated in DM. The COX inhibitor, indomethacin (10 μM) had no effect on WKY or DM contraction but reversed the catalase effect on DM. This suggests that Ang II contraction in WKY or DM is independent of a COX product, but the increased H_2O_2 production in DM stimulates a relaxant PG which inhibits Ang II contraction.

Key words: Diabetic blood vessel, oxidative stress,

Supported by the Int. Med Uni. Malaysia, grant No. IMU 099/2005

P420028

Endothelial cell, shear stress and biomechanopharmacology

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Shear stress (SS) is the friction force between flowing blood and endothelial cell (EC). Generally, arterial SS within physiologic range induces endothelial quiescence and an atheroprotective gene expression profile. Low SS may stimulate atherogenic phenotype, whereas high SS may induce prothrombotic state. So, the biomechanical impact on EC should be fully considered in pharmacology. In fact, the pharmacological in vitro dose-response pattern of EC functions can be significantly modified by in vivo SS. Biomechanopharmacology is forming at the boundary between biomechanics and pharmacology. It will likely consist of both pharmacological intervention on biomechanical factors and biomechanical influence on pharmacokinetics and pharmacodynamics, as well as the joint effect of biomechanical factors and pharmacological factors. It remains to be seen if EC protector/regulator with biomechanical interactive effects of flowing blood can write a new chapter in pharmacology. Physical exercise should be emphasized for gaining joint biomechanical and pharmacological effects.

Key words: endothelial cell, shear stress, biomechanics, pharmacology

Acknowledgement: Grants from NSFC (No. 10272116 and No. 90209055)

P420029

Double Roles of Estradiol in Benign Hyperplasia Prostate of Rat

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AIM: To investigate the role of estradiol (E) in rat prostate. METHODS: 1. 30 male SD rats divided into five groups, after castration, four groups were treated with 0.05, 0.1, 0.2 and 0.4 mg.kg^{-1} E respectively for 14 d, and all animal were treated with 0.5 mg.d^{-1} testosterone propionate (TP). 2. 12 male SD rats divided into control and E groups at random, all animal in the latter were treated with 5 mg.kg^{-1} E for 14 d. After animal being killed, freed the prostate, measured the prostate weight, calculated the prostate index (PI), and analyzed the height of epithelial cell (HEC) and acinar luminal area (ALA) with MIVNT. RESULTS: 1. After being administrated by E, the mean prostate wet weight increased from 0.65 to 0.72 g; The mean PI increased from 0.29 to 0.35; The HEC and the ALA also increased ($P < 0.01$). 2. After being treated with 5 mg.kg^{-1} E for 14 d, the mean prostate wet weight reduced from 0.82 g to 0.25 g ($P < 0.01$), the mean PI reduced from 0.21 to 0.08 ($P < 0.01$), and the HEC and the ALA shrinked ($P < 0.01$). CONCLUSION: In rats, E plays double roles in hyperplasia prostate, it either promotes or stops prostate proliferating.

Key words: estradiol; prostate; proliferation; rat

P420030

Pharmacological activity of acetyl-2,5,7,8-tetramethyl-2-(4'-methylpentene-3'-yl)-6-oxychroman under chemical lesions of liver

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This study is part of investigation of E-vitamin activity of alpha-tocopherol derivative with the shorten side chain-acetyl-2,5,7,8-tetramethyl-2-(4'-methylpentene-3'-yl)-6-oxychroman (Evit). The experiments were performed in males Wistar rats intoxicated by xenobiotics with hepatotoxic action: carbon tetrachloride and acetaminophen. Pharmacological effects of Evit evaluated on its antioxidant and hepatoprotective action.

It was shown, that administration of Evit to animals (10 mg/kg, per os) intoxicated by xenobiotics inhibited processes of lipid peroxidation (LPO) in liver, as on a stage of superoxide anion formation and initial products of LPO (diene and triene conjugates and hydroperoxides) as at terminating stages of formation of interaction products with thiobarbituric acid. This antioxidant effects were accompanied with its hepatoprotective properties, like reduction of aminotransferases (1,5-2 times), alkaline phosphatase (20%) activities and total bilirubin (50%) in the serum.

Data clearly indicate the key role of chroman hydroxyl group in biological activity of tocopherols. The identification of pharmacological effects of Evit stipulates for development it as analog of vitamin E

P420031

The heart-specific miRNA expression in the human bone mesenchymal stem cells (hMSC) induced by 5-aza#

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OBJECTIVE: To investigate the heart-specific miRNA expression in the hMSCs induced by 5-aza. METHODS: The hMSCs isolated from human bone marrow were cultured for 2 weeks, then the cells were induced by 5-aza for another 2 weeks. Then the total RNA extracted from induced cells was used for the first-strand cDNA synthesis with the controls of hMSCs and human cardiomyocytes. Primers for miR-208, miR-181a, miR-143, miR-206, miR-1-1 and miR-1-2 were used for first-strand synthesis, and these primers (reverse) and the corresponding forward primers were used for PCR amplification. The PCR products were analyzed by 1.5% argarose gel eletrophoresis and DNA sequencing identification. RESULTS: The 6 miRNAs were all expressed in cardiac myocytes, only miR-181a was expressed in the hMSCs, miR-208, miR-143 and miR-206 could also be expressed after inducing by 5-aza, but miR-1-1 and miR-1-2 were failed to be expressed. CONCLUSION: Some heart-specific miRNAs could be expressed in the hMSCs induced by 5-aza.

Key Words: hMSC, miRNA, Cell differentiation

Supported by NSFC (30271287, 3030042, 30571850) and GDNSF (015015, 04102307.33189).

P420032

Potentiating effects of distigmine on the guinea - pig urinary bladder contractility evaluated in in vitro **and** in vivo **studies .**

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Effects of distigmine , a long - acting acetylcholinesterase inhibitor , on the guinea - pig urinary bladder contractility were investigated in in vitro and in vivo studies . In the isolated detrusor smooth muscle , distigmine (0 .3 - 3 micro M) strongly potentiated acetylcholine (ACh) - induced contraction without increasing basal tone whereas neostigmine profoundly increased muscle basal tone in the same concentration ranges . Potentiating effect of distigmine on ACh - induced urinary bladder contraction was also shown in in vivo studies using balloon - inserted bladder . In the studies to monitor intravesical pressure changes using cystometry method , distigmine (0 .03 - 0 .1 mg/ kg , i .v .) was shown to markedly increase the maximum intravesical pressure during the micturition reflex without affecting the minimum intravesical pressure at the initiation of urine storage and without decreasing bladder capacity and voided volume . These results suggest that distigmine improves the bladder - voiding functions by increasing the bladder contractility without decreasing the storage capability , which supports a basis for the usefulness of this drug in the treatment of voiding dysfunction associated with impaired detrusor contractility .

P420033

Exposici - solar y esclerosis mutiple . Estudio caso control en Cuba

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Esclerosis M- tiple (EM) es una enfermedad inflamatoria , desmielinizante e inmunol - ica que afecta al SNC y constituye causa de invalidez m- frecuente en el adulto , se evalu rd del sol como factor ambiental protector , se diseun estudio casos - control pareado en 70 pacientes y se recogi : diagn - tico de la enfermedad , criterios de apareamiento , lugar de residencia , pr - tica de deportes , lugar de vacaciones , horario de exposici - solar y horas luz como promedio d - / a - , se utilizaron pruebas de estad - tica no para m- rica (Wilcoxon para variables nodicoticas) , nivel del 95 % y p < 0 .05 , con este trabajo tratamos de demostrar la teor - planteada por autores de la Universidad de Limoges Francia acerca del papel protector del sol en la EM y su impacto sobre la sociedad y la comunidad cient - ica internacional . Obtuvimos : controles se exponen con mayor frecuencia y durante m- horas al sol estableci dose diferencia estad - tica para Z = - 2 .6375 y p = 0 . 0084 , existen m- controles que pasan sus vacaciones a orillas del mar por lo que deben estar m- soleados Z = - 2 .4326 y p = 0 .0150 , adem- practicaron deporte con mayor frecuencia que los casos .

Descriptores : esclerosis m- tiple , exposici - solar , casoontrol .

P420034

Are physiological loads suitable for non - pharmacological control in thorough QT/ QTc study

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Thorough QT/ QTc (TQT) studies are performed to study potential effects of drugs on ECG QT prolongation using active controls such as moxifloxacin . According to the ICH E14 guideline , non - pharmacological control may be used . In this study , effects of physiological loads on QT/ QTc were studied . Subjects and Methods : Seventy - four healthy male subjects aged 20 - were included . ECGs were recorded 5 times ; 3 times in supine position after 5 minute 's rest , 1 in standing position for 5 minutes , and 1 during Valsalva maneuver . QT was measured on papers by the method consistent with ICH - E14 guideline at Quintiles ECG Services . QT was corrected by Bazett 's (QTcB) and Fridericia 's (QTcF) method . Results : After 5 minutes 'standing , QTcB increased (7 .374 msec , [4 . 293 - 10 .455] , mean , 90 % CI) while QTcF decreased (- 9 .162 msec [- 11 . 296 —7 .028]) . Valsalve maneuva did not cause significant change in QT/ QTc . RR - QT relationship was preserved in case of standing records , but was weak in case of Valsava maneuva . Conclusion ; Changes in QT/ QTc after standing is influenced by heart rate , and an acute autonomic load like Valsalva maneuver may not be suitable as a control because of RR - QT hysteresis .

P420035

Effect of vine shoot extract - vineatrol against pentylenetetrazole induced seizures in rats

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The effect of vineatrol , a polyphenolic compound with potent antioxidant activity was investigated against pentylenetetrazole (PTZ) induced seizures in rats . Vineatrol at doses of 10 , 20 and 40 mg/ kg i .p . 20 min prior to convulsive challenge of PTZ (60 mg/ kg , i .p .) , dose dependently increased the initial latency and reduced the percent incidence of generalized tonic cloric convulsions . There was insignificant difference between the initial latencies and percent incidence of convulsions of the PTZ (60 mg/ kg) , i .p and the vehicle treated PTZ rats . Pretreatment of vineatrol at the doses 10 and 20 mg/ kg , i .p significantly ($p < 0.05$) increased the initial latency of seizures in vehicle treated PTZ rats . The values being 115 ±5 and 345 ±45 s as compared to vehicle treated PTZ rats (81 .6 ± 11 .4 s) respectively . The percent incidence of generalized tonic cloric convulsions was also significantly ($p < 0.05$) reduced in the vineatrol treated groups as compared to the vehicle treated PTZ rats (100 %) . Vineatrol at the dose of 40 mg/ kg offered 100 % protection against PTZ induced seizures in rats . The findings of the present study suggest that potential anti convulsant activity of vineatrol .

P420036

Solubility measurements at 37°C

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The primary objective of this study was to obtain a method for easy solubility measurements at a different temperature other than at room temperature . Most published solubility values have been measured at 25°C . The human body is at 37°C , but it 's difficult to make tradtional shake - flask solubility measurements at this temperature . This study presents a way to measure aqueous solubility of drugs at 37°C by Chasing Equilibrium Solubility (CheqSol) . CheqSol is a new technique of measuring equilibrium solubility during a UV - assisted pH - metric titration , which can be done automatically in a temperature - controlled glass vial maintained at 37°C . CheqSol requires accurate pKa values . pKas of poorly water - soluble samples must be measured in water - solvent , but solvent evaporates quickly at 37°C , and volume changes during the experiment will affect the result in traditional pKa measurement techniques . Therefore , a new pH - UV method , named Fast D- PAS , was developed to measure pKa values in 4 minutes at 37° C . The speed of the titration means that very little solvent evaporates in 4 minutes , thereby providing accurate pKa values at 37°C . Sulfamerazine was 50 % more soluble at 37°C than at 25°C , while diclofenac was 100 % more soluble at 37°C than at 25°C . Solubility differences like these could affect bioavailability , as drugs need to be in solution before they can permeate through membranes in the body . The results are supported by recently published papers .

P420037

Identification and Characterization of Novel Genes Highly Expressed in the Mantle of Pinctada fucata : a New Way Towards Treatment of Osteoporosis

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There are many similarities between the biomineralization processes of pearl and bone . Mantle of Pinctada fucata from the South China Sea was used . Using the mantle cDNA library and the ESTs we have colend through suppression subtractive hybridization (SSH) , ten full length novel genes have been obtained through nested PCR . Then we performed mantle in situ hybridization . The results of GST - PFMG1 on $CaCO_3$ crystallization showed significant affects on nucleation and precipitation of $CaCO_3$, which shows that it may be a potential drug for the treat-

ment of osteoporosis. The 3T3-E1 cells which were transfected with these genes can be used to screen drugs for osteoporosis. All this work can pave the way for the bulk cloning of new genes related to biomineralization and may accelerate research on the treatment of osteoporosis.

Key words: biomineralization, osteoporosis, Pinctada fucata, novel genes

Acknowledgement: This work was financially supported by the National High Technology Research and Development Program of China (2001AA621140), the National Natural Science Foundation of China (No. 30472162) and the Tsinghua-Yue-Yuen Medical Sciences Fund (THYY20040008).

P420038

Biomineralization Activation of New Genes From Pinctada fucata: Screen Potential Drugs for Osteoporosis

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It has been reported that nacre can activate bone marrow cells and bone formation. We have found that QM which isolated in pearl oyster (Pinctada fucata) from the mantle cDNA library overexpressed in MC3T3-E1 enhanced cell differentiation and mineralization. Alkaline phosphatase (ALP) activity and nodule mineralization were increased in MC3T3-E1 from QM overexpression cultures. The protein of QM may be a potential drug for the treatment of osteoporosis. So we isolated another ten genes in pearl oyster (Pinctada fucata) from mantle cDNA library using the method of suppression subtractive hybridization (SSH) and nested PCR. First we will overexpress the genes in MC3T3-E1 to test the activation of biomineralization, then purify the proteins from recombinant E.coli. We do this in order to screen potential drugs for osteoporosis and accelerate research on the treatment of osteoporosis.

Key words: QM, biomineralization, osteoporosis

Acknowledgement: This work was financially supported by the National Natural Science Foundation of China (No. 30472162) and the Tsinghua-Yue-Yuen Medical Sciences Fund (THYY20040008).

P420039

The effect of dexamethasone ointment on the clock gene expression in mouse skin

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In mammals, circadian oscillators involving a set of clock genes reside in most tissues including brain, liver and heart. In the skin, however, the physiology of the clock gene system remains to be determined. To address this issue, we investigated whether the clock gene system acts in mouse skin tissue by measuring their mRNA expression with a real-time PCR method. In addition, the effects of single- and multiple-dose dexamethasone ointment on the clock gene system were examined. In the skin tissue of HR-1 hairless mice, all transcript levels of the clock genes examined (Bmal1, Per2, Cry1, and Dbp) clearly showed 24-h rhythms. The single application of dexamethasone ointment at the onset of light phase advanced the phase of clock genes expression, whereas the treatment at the onset of dark phase delayed the phase. A 2-wk treatment of mice with dexamethasone ointment did not affect the phases of the clock genes, but the transcript level of Per2 significantly increased throughout a 24-h period. These results suggest that dexamethasone can affect both the phases and expression levels of clock genes, and that these effects may depend on the time of day of application and the duration of treatment.

P420040

Daily rhythms of P-glycoprotein expression and activity in rat small intestine

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Aim: The pharmacokinetics of many medications vary depending on the time of day of dosing. In this study, we examined whether the expression and transporting activity of P-glycoprotein exhibit daily rhythmicity. Methods: Male Wistar rats were maintained under a 12 h light/12 h dark cycle for 2 weeks, and thereafter small intestine was obtained at every 6 h during a 24-h period. P-glycoprotein gene (Abcb1a and Abcb1b) and protein expression levels were determined by the real-time PCR and western blot analysis, respectively. Transporting activity of digoxin, a P-glycoprotein substrate, was assessed using an excised intestine perfusion system. Results: The mRNA expression of Abcb1a and Abcb1b showed clear 24-h rhythmicity and peaked at the onset of the dark phase. The protein expression also exhibited a daily rhythm, with a peak occurring in the dark phase.

Consistent with the expression profile, the activity of P-glycoprotein peaked during the dark phase. Conclusion: In the rat intestine, both the expression and function of P-glycoprotein exhibit the 24-h rhythmicity. Circadian variation in this function might be involved in various chronopharmacological phenomena.

P420041

Fingerprint Analysis of Chinese Traditional Medicine of Aristolochia Plants by Capillary Electrophoresis with Electrochemical Detection

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Fingerprint analysis of Aristolochic acids (AAs) in six different traditional Chinese medcines (TCMs) herbs was achieved by capillary electrophoresis (CE) with electrochemical detection (ED).

AAs are main bioactive ingredients in the most of Aristolochia plants, which are used to make dietary supplements, slimming pills and TCMs. Excessive ingestion of AAs can lead to serious nephropathy. It is, therefore, quantitative analysis and quality control for the plants containing AAs is of great importance.

Recently, CE-ED has been widely used in analytical science, especially in the pharmaceutical industry. We utilized the CE-ED method to analyze AAs contents in plant extracts. The results indicated that the contents of AAs in each part of Aristolochia debilis Sieb. Et Zucc. plant were different. Meanwhile, the CE−ED method was applied for fingerprint analysis of medicine herbs. Six herbs (Radix Aristolochiae, Fructus Aristolochiae, Herba Aristolochiae, Caulis Aristolochiae Manshuriensis, Caulis Clematidis Armandii, Caulis Akebiae) were well distinguished by comparing their electropherograms obtained by CE−ED method.

P420042

Soluble Dispersal Mixture of Chicken Collagen Type II: A Novel Potent Drug for Osteoarthritis Treatment

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AIM: To evaluate the prophylactic and therapeutic effects of soluble dispersal mixture of domestic chicken collagen type II (CCII, patent number: ZL 02 1 45192.3) on rat osteoarthritis (OA) and analyze concomitant immunohistochemical and biomolecular changes in articular cartilage of osteoarthritic rats. METHODS: OA models were surgically induced, experiments were set up with prophylactic and therapeutic groups. In prophylactic groups, treatment began at the day of operation while in therapeutic groups, treatment began on the seventh week after operation. Morphology of articular cartilage was done by HE staining, immunohistochemistry of Matrix metalloproteinase (MMPs) and Cathepsin K was done by ABC method while special mRNA levels were evaluated by RT-PCR method. RESULTS: Oral administration of CCII prophylactly or therapeutly reduced the morphological, immunohistochemical and biomolecular changes of osteoarthritic cartilage.

CONCLUSION: Oral CCII has prophylactic and therapeutic effects on delaying articular cartilage degradation of osteoarthritic rats and may be a potent drug candidate for OA treatment in clinic.

KEY WORDS osteoarthritis; chicken collagen type II; MMPs; cathepsin K

P420043

The Impact Of Puerarin On SOD Activity And MDA Level In Exhausted Exercise Mice

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Objective: To study the impact of puerarin on superoxide dismutase (SOD) activi-

ty and malonaldehyde (MDA) level in exhausited exercise mice. Methods: Swimming training models were established, then measure the activity of SOD and the content of MDA in the blood of being given puerarin mice. Results: Puerarin can obviously enhance the swimming capacity of mice, prolong the swimming time, significantly enhance the SOD activity, significantly degrade the content of MDA. Conclusion: Puerarin has the antioxidant effect

Key words: Puerarin; superoxide dismutase; malonaldehyde; exhausted exercise

P420044

Effect of Puerarin on Experimental Prostatic Hyperplasia in Mice

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Objective: To study the inhibiting effect of Puerarin on Benign Prostatic Hyperplasia of mice.

Methods: Models of Benign Prostatic Hyperplasia were established by subcutaneous injection testosterone propionate in mice. We observed the prostate glandular wet weights, indexes of prostate glandular and morphological changes of prostate glandular to investigate the effect of Puerarin on Benign Prostatic Hyperplasia model of mice. Results: Puerarin can apparently inhibit Benign Prostatic Hyperplasia in mice induced by testosterone propionate. Conclusion: Puerarin have significant inhibiting effect on Benign Prostatic Hyperplasia induced by testosterone propionate in mice.

Key words: Puerarin; Prostatic hyperplasia; model; Mice

P420045

ANTIOXIDANT EFFECT OF QUERCETIN ON THE NITRERGIC NEUROTANSMITTER IN THE MOUSE GASTRIC FUNDUS

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The aim of this work was to investigate whether the antioxidant effect of quercetin on the nitrergic neurotransmitter in the mouse gastric fundus. Nitrergic nerve stimulation (EFS; 4 Hz, 25 V, 1 ms, 15s-train), exogenous nitric oxide (NO; 10 micromol) and isoproterenol (5 nM) induced relaxation in mouse gastric fundus preparations. The superoxide anion generators, pyrogallol (10 micromol), hydroquinone (100 micromol) and LY83583 (5 micromol) inhibited relaxation to EFS and NO, but not to isoproterenol. The inhibition observed with pyrogallol, hydroquinone and LY83583 was prevented by quercetin (0.1 micromol). Also, the antioxidants, SOD (100 U/ml), ascorbic acid (500 micromol) and glutathione (100 micromol) prevented the inhibitory effect of superoxide anion generators on relaxation to EFS and NO. The Cu/Zn SOD inhibitor, diethyldithiocarbamic acid (DETCA; 8 mM), inhibited the relaxation of gastric fundus to EFS and NO but not those to isoproterenol.

DETCA-induced inhibition on EFS and NO-induced relaxation was partially prevented by quercetin, glutathione and ascorbic acid. These results suggest that quercetin can act as an antioxidant in mouse gastric fundus.

P420046

INHIBITOR EFFECT OF COLCHICINE ON LUMINOL-ENHANCED CHEMILUMINESCENCE (CL) OF STIMULATED HUMAN LEUKOCYTES AND CELL-FREE SYSTEMS

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Colchicine exerts an antiinflammatory effect by inhibiting neutrophilic functions. Its direct quenching effect on oxygen-centered free radcals (OFRs) was not evaluated clearly. In the present study, the inhibitor effect of colchicine on OFRs generated by N-formyl-methionly-leucyl-phenylalanine (FMLP) and phorbol myristate acetate (PMA)-stimulated human leukocytes and cell-free systems has been investigated by using luminol-enhanced CL. A luminometer was used to assay free radical generation (H_2O_2), hydroxyl radical and hypochlorous acid (HOCl)-induced CL responses were initiated by H_2O_2, $FeSO_4$ and NaOCl. Colchicine inhibited the peak CL of H_2O_2 (1.6×10^{-2} M) $FeSO_4$ (5×10^{-8} M) and HOCl (5×10^{-3} M) dose dependently. In FMLP (4×10^{-6} M) and PMA (5×10^{-7} M)-stimulated human leukocytes, colchicine also produced an inhibitor effect on the peak CL. These data suggested that antiinflammatory potency of colchicine might be due to either its inhibitory activity on the polymorphonuclear leukocytes or direct scavencing activity against OFRs.

P420047

Roles of increased arginase activity and decreased nNOS protein expression for the impaired neurogenic relaxation of corpus cavernosum in aged rabbit

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We investigated whether the changes in arginase activity and nNOS protein expression are involved in impaired neurogenic cavernous relaxation in the aged rabbits. The cavernous specimens of young adult (3 to 6 months) and aged (36 to 48 months) rabbits were used for the isometric tension experiment, Western blot analysis, cyclic GMP determination and measurements of NOS and arginase activities. The neurogenic relaxation, but not sodium nitroprusside-induced one, was significantly impaired in the aged group. The impaired relaxation was accompanied by the significantly decreased cydic GMP production stimulated with electrical field stimulation, almost abolished nNOS protein expression and enhanced arginase activity without change Ca^{2+}-dependent NOS activity per se. Supplementation of excess L-arginine or S-(2-boronoethyl)-L-cysteine as an arginase inhibitor partially restored the impaired neurogenic relaxations in the aged group. In conclusion, the impaired neurogenic and NO-mediated relaxation of corpus cavernosum with aging is possibly due to not only enhanced arginase activity but also decreased nNOS protein expression.

Key words: nNOS, arginase, erectile dysfunction

P420048

LC Determination of Omeprazole in Human Plasma Using a Monolithic Column

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A rapid and sensitive HPLC method using a monolithic column has been developed for quantification of omeprazole in plasma. The method was specific and sensitive with a quantification limit of 10 ng/ml. Sample preparation involves simple, one-step extraction procedure and analytical recovery was complete. The separation was carried out in reversed-phase conditions using a Chromolith Performance (RP-18e, 100 ×4.6 mm) column with an isocratic mobile phase consisting of 0.01 M disodium hydrogen phosphate buffer-acetonitrile (73:27 v/v) adjusted to pH 7.1. The wavelength was set at 302 nm. The calibration curve was linear over the concentration range 20-1500 ng/ml. The coefficients of variation for inter-day and intra-day assay were found to be less than 7%.

P420049

The Roles of the Opioidergic System and Nitric Oxide in the Analgesic Effect of Venlafaxine

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The noradrenalin and serotonin re-uptake inhibitor venlafaxine has an analgesic effect that is independent of its antidepressant activity; however, the mechanism of this effect remains to be elucidated. This study was performed to investigate the possible roles of the opioidergic system and nitric oxide (NO) pathway in the analgesic effect of venlafaxine. Eighty Wistar rats of both sexes were allocated to 10 groups. The hot plate test was used to assess the antinociceptive effect. The temperature of the hot plate was adjusted to 52.5 ±1oC and the cut-off period was set to be 50 sec. Venlafaxine alone (25 mg/kg) showed marked analgesic activity ($p < 0.05$). N-ω-nitro-L-arginine (LNOARG) alone (20 mg/kg) and naloxone alone (2 mg/kg and 4 mg/kg) showed no analgesic activity ($p > 0.05$).

Coadministration of low-dose naloxone (2 mg/kg) and both doses of L-NOARG (20 and 40 mg/kg) with venlafaxine (25 mg/kg) did not modify the analgesic effect but high-dose naloxone (4 mg/kg) decreased it significantly ($p < 0.05$). In conclusion, these results suggest that the opioidergic system but not the NO pathway has a role in the analgesic effect of venlafaxine.

Key words: Analgesia, venlafaxine, naloxone, L-NOARG

P420050

Dexamethasone treatment inhibits IGF-I synthesis and astrogliosis after stab wound in the cerebral cortex of adult rats

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Objectives I: Quantify the IGF-I concentration in cerebral cortex at 4 days after unilateral stab wound, and the DXA effects. II: Analyze the astrocytosis and microglial reactivity at 22 days after bilateral lesion, and the DXA influence. Methods: I: two groups were assembled (8 male rats): Control: 3 days before lesion received vehicle; the other rats were likewise injected with 0.5 mg/kg of DXA and sacrificed for cerebral IGF-I quantization II: other three groups were assembled: Intact controls and cerebral cortex injured rats, previously injected either with vehicle, or DXA. Double immunolabeling for astrocytes and microglia proliferation (GFAP+PCNA) and (Isolectine-B4+PCNA) respectively was conducted. Results: DXA inhibited the IGF-I synthesis at third day postlesion. In DXA injected animals, a decreased total population of astrocytes and microglia were found; the proliferative index of microglia was also reduced but not for astrocytes; a reduced cytoplasmic complexity also resulted for both by DXA influence. Conclusions: The prophylactic DXA dosage inhibited the IGF-I synthesis and glial reactivity in adult rats that suffered a bilateral stab wound in fronto-parietal cortex

P420051

Validation of pharmacodynamic assessment method after administration of voglibose in healthy subjects

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BACKGROUND Voglibose is an alpha-glucosidase inhibitor. Due to negligible oral absorption, measuring drug concentration in the blood is impractical. So we proposed a pharmacodynamic assessment method to reflect drug effect, and this study aimed to validate this method.

METHODS A placebo-controlled, selective two-period clinical study was conducted in 20 healthy male subjects. Period I: On day 1, subjects received a placebo and a sucrose-rich fluid meal 20 min after dosing.

Blood samples were taken during 3 hours. On day 2, subjects received 0.3 mg voglibose instead of placebo.

Period II: 9 subjects in whom effects of drug were observed in Period I participated in a multiple dose study (placebo: 8, 11 pm on day -1, and 9 am on day 1 / voglibose: 2, 8, 11 pm on day 1, and 9 am on day 2).

RESULTS The average percent decreases of AUEC1h (area under the serum glucose level-time curve to 1h) and Gmax (maximum serum glucose level) were 19.6% ($P < 0.001$) and 22.2% ($P < 0.001$), respectively.

CONCLUSIONS Significant drug effects of voglibose were revealed after multiple doses. Changes of AUEC1h and Gmax compared to placebo may be alternative parameters to AUC and Cmax for an equivalence study.

P420052

$G\alpha_{i2}$ maintains CSF homeostasis in rat brain

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The heterotrimeric G protein $G\alpha_{i2}$ has restricted and conserved localization in cilia of different tissues, including the ependymal cilia. The role of $G\alpha_{i2}$ in the CNS is largely unknown. We used intracerebroventricular antisense administration to clarify the physiological role of $G\alpha_{i2}$ in the rat ventricular system. High resolution MRI studies revealed that continuous icv-infusion of $G\alpha_{i2}$-specific antisense oligonucleotide caused unilateral ventricular dilatation restricted to the antisense-receiving ventricle. Ciliary beat frequency measurements in vitro indicated that antisense administration resulted in ciliary stasis. Our results establish that $G\alpha_{i2}$ has an essential regulatory role in ciliary function and CSF homeostasis.

Key words: G protein, ependymal cilia, ciliary beat frequency

P420053

Study on Chemical Composition of the Ether Extracts of Dated Commercial Semen Plantaginis

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The paper studied the chemical composition of ester extracts of Semen Plantaginis after 2 years storage. Material powder was exhaustively extracted with regurgitant ether. The extracts were condensed and added decuple ethanol (v/v) and placed overnight under -20°C (Lin). Then the essential fraction was abtained after centrifugating and evaporating the upper liquid. The chemical composition of the essential fraction was deduced from GC-MS analyse. It is found that two main components are (Z,Z)-9,12-Octadecadienoic acid (79.22%, the highest content) and n-Hexadecanoic acid (13.63%, second). Moreover, there have small quantities of Octane (0.03%), (Z)-2-Heptenal (0.07%), 2-Cyclohexen-1-ol (0.06%), (E,E)-2,4-Decadienal (0.12%), Z,Z-10,12-Hexadecadien-1-ol acetate (0.04%), Stigmasterol (0.09%) and 22,23-dihydro-Stigmasterol (1.06%). It would be presumed that volatile components were badly losed after 2 years storage, compared with the paper reported (Kameoka H). So volatile components cannot be highly dependent to evaluate the quality of this medicinal materials.

Keywords: Dated Semen Plantaginis, Ether extracts, Chemical composition

Acknowledgement: Center of Analysis and Testing intramural for GC-MS analysis.

P420054

Treatment of *Cryptosporidium parvum* gut infection with Nitazoxanide prevents long term ileal hypersensitivity in immunocompetent rats

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Aim: The aim of this study was to determine whether nitazoxanide (NTZ) treatment of unweaned immunocompetent rats infected with *Cryptosporidium parvum* (genotype II) oocysts preverts long-term jejunal hypersensitivity to distension Methods: Five-day-old suckling Sprague-Dawley rats were orally infected with 10^5 *C. parvum* oocysts. Twenty infected rats were treated with 200 mg/kg/day NTZ from day 1 to day 14 post infection. Twenty infected rats were untreated as control. On day 20, intestinal infection was assessed by measuring oocyst shedding which was terminated by day 25. On day 120, jejunal / rectal sensitivity to distension was measured as the threshold distending volume inducing a significant drop of arterial pressure (pain threshold). And myeloperoxidase (MPO) activity (/g protein) was determined on jejunal specimens. Results: Pain threshold to distension was lower in infected rats by comparison with control animals with a threshold ≤ 0.2 ml in 87.5% of infected rats vs. 33.3% in controls ($p < 0.01$). In contrast, pain thresholds did not differ between NTZ treated rats and uninfected control rats ($p > 0.05$). Jejunal MPO activity was higher in both untreated and NTZ infected treated rats than in controls ($p < 0.05$). Conclusion: Present data suggest that in suckling rats, cryptosporidiosis induces long term jejunal hypersensitivity to distension which is prevented by an early use of NTZ treatment.

Key words: *Cryptosporidium parvum*; distension; Myeloperoxidase

P420055

Ghrelin ameliorates oxidative hepatic injury and fibrosis in rats

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The possible therapeutic effects of ghrelin, a peptide produced predominantly by the stomach, were evaluated in cirrhotic rats. Bile duct ligated (BDL) and sham-operated Sprague Dawley rats were treated with either ghrelin (1 μg/kg, sc) or saline for 28 days. Rats were then decapitated and blood samples were collected. In the saline-treated BDL group, hepatic malondialdehyde and collagen levels, myeloperoxidase activity were increased, as compared to sham-operated group, while serum aspartate aminotransferase and alanine aminotransferase levels, as indices of hepatic function were also elevated ($p < 0.001$). Serum levels of TNF-α, IL-1β and IL-6 were increased ($p < 0.001$) in saline-treated BDL group. These biochemical alterations, as well as hepatic damage assessed microscopically, were reversed by ghrelin-treatment ($p < 0.05$-0.001). Since ghrelin administration alleviated BDL-induced oxidative injury of the liver and improved the hepatic structure and function, it seems likely that ghrelin may be of potential therapeutic value as an anti-inflammatory and anti-fibrotic agent, in protecting the liver against chronic hepatic injury.

Key words: Ghrelin, myeloperoxidase, liver injury

P420056

Inhibition of human B-cell lymphoma by an anti-CD20 antibody H147 and its chimeric Fab and F(ab')2 fragments via induction of apoptosis

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Objective: To study biological activity of an anti-CD20 antibody H147 and its chimeric Fab and F(ab')2 fragments. Methods: Binding of H147 and its fragments to Raji cells were examined by FACS. The cytotoxic effect of H147 and its fragments on Raji cells were determined using both MTT and nude mice bearing Raji xenografts. Raji cells apoptosis induced by H147 and its fragments were assayed with Annexin V-FITC and PI.

The bcl-2/bax gene expression were assayed using RT-PCR and western blot. Results: Both Fab and F(ab')2 fragments competed efficiently with H147 for binding to Raji cells and inhibited proliferation of Raji cells in a dose-dependent manner. H147 and its fragments induced a significant degree of B-cell apoptosis. In this apoptosis procedure, several events were involved, including burst of ROS, change of bcl-2/bax gene, and release of cytochrome c. Further, both the F(ab')2 and Fab fragments when administered in vivo significantly inhibited the growth of human B-cell lymphoma xenografts in nude mice. Conclusion: H147 and its fragments most likely exert their antitumor activity through induction of cell apoptosis.

Key Words: B-Cell Lymphoma; anti-CD20 antibody; apoptosis;

P420057

Lysophosphatidic Acid (LPA) and Angiogenesis

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The role of Lysophosphatidic Acid (LPA) in angiogenesis is uncertain. Thus goal of our study was to determine whether LPA, acting via the LPA receptors (LPA_{1-3}), evokes an angiogenic response. We used the chicken chorio-allantoic membrane (CAM) assay to evaluate LPA and LPA analogs selective for individual LPA receptors. We found that LPA elicited a significant increase in vessel number. LPA-induced angiogenesis is blocked by VPC32183, an antagonist for LPA1 and LPA3 receptors. Further, the LPA_3 selective agonist, S-OMPT, induced angiogenesis. An invertebrate lysophospholipase D (produces high amounts of LPA) likewise evoked angiogenesis in the CAM assay and this response was blocked by VPC32183. A catalytically inactive mutant form of the enzyme did not induce vessel growth. We conclude that LPA is angiogenic in vivo and that its response proceeds via activation of the LPA_1 or LPA_3 receptors, or both. Further in vivo and in vitro angiogenesis studies using mammalian systems are in progress.

(Supported by R01 GM052722 and 1 F31 HL79881-01)

P420058

ROLE OF L-ARGININE-NO PATHWAY ON DAY-NIGHT AND GENDER VARIATION OF ANTINOCICEPTIVE EFFECT OF METOPROLOL

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The aim of our study was to investigate the role of L-arginine-NO pathway on temporal and gender variation of antinociceptive effect of metoprolol on mice parabenzoquinone (PBQ) writhing test.

Experiments were performed on male and female Swiss-albino mice synchronized to 12:12 LD (HALO=07:00).

PBQ writhing test was used at 09:00 and 21:00. Saline, metoprolol (20 mg/kg s.c), L-NAME (75 mg/kg s.c), Larginine (2 mg/kg s.c), morphine (ED50 = 0.13 mg/kg s.c) and morphine + metoprolol, metoprold + L-NAME, L-arginine + metoprolol, morphine + L-NAME, morphine + L-arginine, morphine + L-NAME + metoprolol, morphine + L-arginine + metoprolol combinations were administrated 15 minutes before PBQ (2 mg/kg i.p) administration. After intraperitoneal administration of PBQ, withes were counted for 15 minutes. Results were shown as normalized (arcsin) % antinociception values and analyzed by using parametric and nonparametric ANOVA followed by post-hoc when it is necessary. The antinociception value of L-name + metoprolol combination at 21:00 was higher in females than males. In 09:00 male group, combination with L-arginine increased the metoprolol antinociception. L-arginine-NO pathway may have a role on metoprolol antinociception.

P420059

Comparative Effects of Alpha2 Adrenoceptor Agonists on Electrical Field Stimulated Contractions of Rat, Human and Guinea-Pig Urinary Bladder

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α2 adrenoceptor agonists have been shown to inhibit neurally evoked contractions in urinary bladder smooth muscle, however the relative efficacy and potency of a range of agonists has not been explored within or across species. Potential agonist and species differences were explored on neurally evoked contractions of rat, guineapig and human bladder smooth muscle strips using standard tissue bath methodology. Phasic neurally evoked responses were stable for up to 4hr and inhibited by 1mM tetrodotoxin. The α2-adrenoceptor specific agonists UK-14304, PGE-6201204, dexmedetomidine and clonidine, the α2A/D preferring agonists guanfacine and oxymetazoline and the endogenous agonist noradrenaline caused concentration dependent inhibition of evoked contractions. The relative efficacy and potency of this effect varied not only between agonists but also species. None of the agonists inhibited carbachol or potassium chloride induced contraction. The collected data suggests that α2-adrenoceptor agonists readly demonstrate partiality in native tissues, EC50s and Emax's probably governed by receptor expression and coupling, and that this varies between species (rat > guinea-pig > human) in regard to the bladder.

P420060

Alpha1 Adrenoceptor Mediated Increases in Pudendal Nerve Evoked Intraurethral Pressure Rises Measured In Vitro

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Although a1 adrenoceptors are known to cause urethral smooth muscle contraction their possible influence on urethral striated muscle activity is unknown. We have utilised an in vitro preparation consisting of whole female rat urethra and attached pudendal nerves to study a1 adrenoceptor effects on intraurethral pressure due to smooth muscle contraction and pudendal nerve evoked striated muscle contraction. Phenylephrine caused an increase in baseline and pudendal nerve evoked intraurethral pressure by 49.0 ± 9.7% and 108.8 ± 15.4% of control values (EC50s 2.03 ±0.25 and 1.54 ± 0.23 mM) respectively (n = 8). The a1A/L agonist A-61603 caused an increase in both baseline urethral and pudendal nerve evoked pressure of similar magnitude with EC50s of 52.5 ± 2.6 and 20.0 ± 3.1 nM respectively (n = 6). 300 nM A-61603 induced a sustained increase in both baseline pressure and pudendal nerve evoked responses, application of the a1A/L selective antagonist 5-methyl-urapidl reversed both baseline and pudendal nerve evoked activity to control values with IC_{50} values of 6.3 ± 1.4 and 9.7 ± 2.1 nM respectively (n = 5). In conclusion, a1 adrenoceptor agonists potentiate

pudendal nerve evoked urethral striated muscle activity.

P420061

The 5-HT2C Receptor Agonists Ro-60-0175 and CP-809101 Increase Voided Volume in Conscious Spontaneously Hypertensive Rats

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The spontaneously hypertensive rat (SHR) is a genetic model of hypertension which is also known to exhibit abnormal bladder function, in particular reduced bladder capacity, voided volume, increased urinary frequency and occurrence of non-void contractions. Presently we have utilised the SHR to explore the influence of 5-HT2C receptors on bladder function in conscious animals. SHRs received either Ro-60-0175 (0.1, 0.3, 1 and 3 mg/kg, n=8 per dose) or CP-809101 (0.1, 0.3, 1 and 3 mg/kg, n=8 per dose) sub-cutaneously. All animals received the vehicle for the respective agonist with at least 4 days between treatments, application of agonist doses and vehicle was randomised. SHRs were subsequently placed in metabolic cages over a urine capture system consisting of a conical sponge (which deflects faecal pellets) placed within a container on a balance to record both voided volume and frequency. Both agonists caused a significantly increased voided volume (0.73 ±0.12 ml with 3 mg/kg Ro-60-0175 vs 0.26 ±0.02 ml with vehicle) and decreased voiding frequency, with no significant change in total volume voided. 5-HT2C agonists increase bladder capacity and may be useful in the treatment of bladder dysfunction.

P420062

THE EFFECTS OF HIGH-RATE FREQUENCY MODULATION TREATMENT ON MALONDIALDEHYDE IN DIABETIC POLINEUROPATY

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This study was planned to investigate the effects of TENS treatment on patients which have diabetic polyneuropathy. So that, 14 diabetic polyneuropathy patients suffering ischemic pain were examined during the cure. Malondialdehyde and glycemia levels were determined by collecting 5 cc blood samples from 14 patients 24 hours before beginning the treatment. In case 50 Hz signals as high-rate frequency modulation, were applied to patients as long as 20 days as a seance of 20 minutes a day, TENS treatment increased significantly free oxygen radicals. The levels of MDA before TENS were compared to the levels of MDA after TENS and the end of the following term of 20 days by paired sample test, and a meaningful increase was seen significantly ($p < 0.01$).

Besides, glycemia levels were decreased significantly before TENS - after TENS treatment. Moreover, it was observed that MDA levels were decreased significantly between in the final of the treatment and the end of the following term of 20 days Glycemia levels were not changed significantly after TENS and the end of the following term of 20 days

P420063

Hormone replacement therapy decreases noradrenaline release in human myometrium

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Aim: The aim of the present study was to investigate the effect of hormone replacement therapy (HRT) on noradrenaline release profile of human myometrial tissue. Samples were collected from women with different hormonal status (regular cycle, postmenopause, receiving HRT).

Method: Samples were loaded with [^{3}H]-noradrenaline and transferred into a chamber for superfusion after excision. After a wash-out period, 3-min fractions were collected. In the 5th and 15th fraction tissues were stimulated with electric field. [^{3}H]-noradrenaline content of the fractions was determined together with remaining amount in the tissue for fractional release calculation.

Results: Myometrial [^{3}H]-noradrenaline release and uptake was substantially decreased among patients in postmenopause and in patients who have received HRT compared to control group. These differences were more pronounced in HRT-treated patients than in postmenopausal patients.

Conclusion: HRT decreases the noradrenaline content of myometrial neurons and their stimulus-evoked noradrenaline release. These results support previous findings that found HRT to inhibit sympathetic activity.

Keywords: hormone replacement, myometrium, noradrenaline

P420064

EVALUATION OF RELATIONSHIP BETWEEN ARTERIAL AND VENOUS BLOOD GAS VALUES IN THE PATIENTS WITH TRICYCLIC ANTIDEPRESSANT POISONING

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Objective: To evaluate the relationship between arterial blood gas (ABG) and venous blood gas (VBG) values in tricyclic antidepressant (TCA)-poisoned patients. Methods: Samples of 50 TCA-poisoned patients for ABG and VBG analysis were obtained during initial evaluation. Laboratory data were analyzed by paired Student t-test. The degree of agreement between the arterial and venous pH measurements was evaluated by Bland and Altman method. Results: There were significant differences between mean differences of ABG and VBG parameter values. There was also relationship between arterial and venous pH on the initial evaluation. Conclusion: In TCA poisoning, the peripheral venous pH measurement is a valid and reliable substitute for arterial pH.

Key Words: Tricyclic antidepressant poisoning; Arterial blood gas; venous blood gas

Acknowledgement: The authors would like to thanks the members of Anesthesiology and Intensive Care Department for their valuable supports.

P420065

Role of Oxygen-free radicals on the motility of rat ileum: Effects of Xanthine plus Xanthine Oxidase

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To characterize the effects of oxidants generated by xanthine (X) plus xanthine oxidase (XO) on isolated rat ileum motility, the effects of three concentrations of X/XO on the basal tone of the rat ileum preparation were studied. Also the effects of 2X concentration of X/XO in the presence and absence of superoxide dismutase, catalase, mannitol, histidine, and deferoxamine were evaluated. Xanthine puls xanthine oxidase produced relaxation of ileum. Superoxide dismutase and catalase did not protect ileum from effects of X/XO suggesting that neither superoxide anion nor hydrogen peroxide involve in X/Xo-induced relaxation of ileum.

Dimethylthiourea and mannitol offered protection against X/XO-induced relaxation of ileum suggest formation of hydroxyl radical within the cells. Pretreatment with deferoxamine, a potent iron chelator reduced the relaxation of ileum. In addition the ability of exogenously administered histidine to reduce relaxation suggests that singlet oxygen is another oxygen derivative which is responsible for relaxation of ileum-induced by X/XO.

Key words: Ileum, Xanthine, Xanthine oxidase, Free radicals

P420066

The effect of vitamin E on plasma antioxidant capacity, lipid peroxidation and diabetic nephropathy in rat

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We investigated the effect of vit E on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation. Twenty four male wistar rats were chosen., then 16 rats were diabetized by STZ The rats were divided into 3 groups (n=8) of control, non-treated diabetic and Vit. E treated diabetic. After 8 weeks all rats were anaesthetized. After blood sampling, kidneys were removed and kept in 10% formalin buffer. Plasma and red blood cells were separated. Plasma antioxidant capacity by FRAP method, and MDA as lipid peroxidation indicator were mea-

sured. Also, renal samples were studied for focal cells proliferation and tubular changes. MDA levels showed a decrease in treated diabetic comparing to non treated diabetic rats($P < 0.01$) .. Plasma antioxidant capacity in treated rats showed a significant augmentation comparing to the other groups ($P < 0.05$) . In non-treated rats diffused glomerular proliferation, and inflammation were seen. Also arteries wall thickened. While these changes showed a significant reduction in treated rats. Our results indicated that Vit. E caused a decrease in lipid peroxidation, nephropathy and an increase in plasma antioxidant capacity

P420067

Protective Effect of Naoxinxueshuankang(NXK) on the Experimental Cerebral in Mice

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Objective: To observe the protective effects of NXK on cerebral ischemia reperfusion injury.

Methods: The method of ligating both common carotid arteries and vagus nerves was used to make acute cerebral ischemia reperfusion injury in mice. Results: NXK increased notably brain SOD and NO content, and at the same time decreased brain MDA content on the cerebral ischemia($p < 0.05$) . Conclusion: NXK may have protective effect on the cerebral ischemia injury in mice.

Key words: NXK; mice; acute cerebral ischemia

P420068

Effect of Salvianolic acid A on rat liver mitochondria

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The present study was conducted to observe the effect of Salvianolic acid A (Sal A) ,a compound isolated from the Traditional Chinese medicine, Salvia miltiorrhiza Bunge, on rat liver mitochondria. Male Wistar rats were decapitated and their livers were harvested. Respiratory parameters of isolated liver mitochondria were measured polarographically using a Clark-type oxygen electrode at 30 A measurement of 10 mM L-glutamate plus 5 mM L-malate were used to quantify complex Ⅰ-dependent respiration, while 10 mM succinate was used to quantify complex Ⅱ-dependent respiration. The mitochondria were incubated with Sal A for 5 min, and then the substrates, ADP was added. Results showed that whether in complex Ⅰ-dependent respiration or complex Ⅱ-dependent respiration, Sal A 10^{-4}, 10^{-6} M both decreased the rate of state 3 and state 4 very significantly. In complex Ⅰ-dependent respiration, 10^{-5} M Sal A increased the RCR from 4.98 ± 0.23 to 5.37 ± 0.14, but with no significant difference, such a change would be result in an increase in state 3 respiration. Our results suggested that Sal A could change the mitochondrial respiratory rate under normal conditions and may affect the functions of mitochondrial membrane.

Key words: Salvianolic acid A, mitochondria, respiration, oxidative phosphorylation

P420069

Prevalence of Coronary Artery Disease and Effects of Revascularization in Diabetics with Left Ventricular Systolic Dysfunction in Indian Patient Population

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Objective: To compare coronary artery disease pattern and effects of coronary artery bypass grafting (CABG) in diabetics having left ventricular (LV) systolic dysfunction (LVD) with those in non-diabetics. Methods: The study included patients with LVD (ejection fraction (EF) less than 35% on echocardgiography) and undergoing CABG. Group-I included diabetics and group-II included non-diabetics. Records of coronary angiography were compared. Pre- and post-CABG echocardiographic data were also compared. Results: Out of 267 patients included, 116 were in group-I and 151 in group-II. Relatively more patients in group-I had significant stenosis in left anterior descending, obtuse marginal and right posterior descendng artery than those in group-II.

Consistently, there was reduced LV contractility before CABG, in group-I (EF: 27.5%) as compared to group-II (29.5%). However, improvement following 2-months of CABG was greater in group-I (EF: 35.3%) than group-II (34.4%). Reduction in LV diameters was also greater in group-I. Conclusions: Indian diabetics having LVD and undergoing CABG are found with more stenosed coronary arteries. Diabetics gain greater improvement by CABG than non-diabetics.

P420070

Effects of bicydol on dimethylnitrosamine-induced liver fibrosis in mice and its active mechanism

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The aim was to investigate the suppressive effect of bicyclol on hepatic fibrosis (HF) induced by dimethylnitrosamine (DMN) and its mechanism. HF was established by intraperitoneal injection of DMN 8 mg/kd/d on three consecutive days of each week for four or five weeks. Bicyclol treatment markedly reduced the levels of alanine aminotransferase, total bilirubin, hydroxyproline, prolidase, tumor necrosis factor < alpha > ;(TNFalpha;) , transforming growth factor beta-1 (TGFbeta1) , type Ⅰ collagen in serum and the score of HF. In addition, bicyclol treatment inhibited liver TGFbeta1 and tissue inhibitor of metalloproteinase 1 (TIMP-1) mRNA expressions, liver and serum TIMP-1 levels, and increased the liver collagenase activity (CA) . The result suggested that bicyclol attenuated DMN-induced HF in mice. Its active mechanisms may be related to the hepatoprotective and anti-inflammation properties, the down-regulation of liver TGF-beta1, TIMP-1 expressions and the increase of net CA in liver.

Keywords: Bicyclol; Dimethylnitrosamine; Hepatic fibrosis;

P420071

Arsenic Trioxide Induced Synovial Tissues Apoptosis in the Rat Model of Collenge and decreased the levels of TNF-a,IL-1

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Objective: We firstly observed the effects of arsenic trioxide (As_2O_3) on the apoptosis of synovium and the levels of IL-1, TNF-αin CIA rat. Methods The experimental models of collagen inducing arthritis rats were used. 72 rats were divided into normal control group, model group, and As_2O_3 treatment groups. The knees' synovium, cartilage and bone tissue of the rat were taken out, waiting for being observed with light microscope and the electron microscope and measured apoptosis by TUNEL after the 15th day of treatment Meanwhile the level of IL-1 and TNF-αwere measured by ELISA method. Results: The pathological injury were improved and the apoptosis of synoviocytes were increased in the As_2O_3 treatment groups, compared with the model group. Compared with the model group, the levels of IL-1 and TNF-a were decreased in the arsenic trioxide treatment groups, especially in 4.0 mg/kg and 6.0 mg/kg As_2O_3 groups ($p < 0.01$) . Conclusions: These results suggested that arsenic trioxide might play a protective effect by inducing apoptosis of synoviocytes and decreasing the levels of IL-1, TNF-α.

Key words: Rat Model of Collenge; Arsenic Trioxide; apoptosis

P420072

Arsenic Trioxide Induces Apoptosis and Decreases the xpression of NF-kappaB mRNA in RA-HFLS

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Objectives: Observed the effect of arsenic trioxide on RA-HFLS with respect to apoptosis and NF-kappaB mRNA in vitro. Methods: RA-HFLS cultures were treated with control group or mediums supplemented with 0.5, 2, 8 μmol/L AS_2O_3 respectively. RA-HFLS apoptosis cultured with different concentrations of AS_2O_3 for 72h were investigated under light and electron microscope. Apoptosis exponent was measured by (TUNEL) .

MTT assay were carried out in continuous 5days. Moreover, the NF-κB mRNA level of RA-HFLS was measured by RT-PCR after treated with AS_2O_3 for 24 h. Results: AS_2O_3 induced the apoptosis of RA-HFLS in morphology. Apoptosis exponent were increased in a dose dependent manner in TUNEL experiment, especially in the cells treated with 2 and 8 μmol/L AS_2O_3($P < 0.05$) . RA-HFLS proliferation was inhibited in both dose and time dependent manner when cultured with AS_2O_3. Meanwhile, the NF-κB mRNA level was decreased in

AS_2O_3 treated groups, which was especially significant in mediums cultured over 2 μmol/L AS_2O_3 ($P < 0.05$). Conclusions: AS_2O_3 depressed the RA-HFLS proliferation and may increase the RA-HFLS apoptosis through decreasing the expression of NF-κB mRNA. Key words: RA-HFLS; apoptosis; AS_2O_3

P420073

High fat emulsion induced rat model of nonalcoholic steatohepatitis

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To establish a high fat emulsion induced rat model of NASH. Male SD rats were fed a high fat emulsion via gavage for 6 weeks. Animals were examined for serum and hepatic biochemistry, insulin sensitivity, hepatic malondialdehyde, superoxide dismutase and morphological evaluation, as well as Cytochrome P-450 2E1 and Peroxisome Proliferator-activated Receptor α expression in the liver. The results showed that rats treated with high fat emulsion became obese, demonstrated abnormal aminotransferase activity, hyperlipoidemia, hyperinsulinemia, hyperglycemia and insulin resistance. The model rats exhibited an increased concentration of serum TNF-α, total cholesterol, triglyceride, MDA and reduced SOD levels in the liver. Immunoblot analysis showed that the expression of CYP2E1 was increased, whereas PPARα was reduced in the NASH model rat liver. Morphological evaluation revealed that hepatic steatosis, inflammation and mitochondrial lesions were also reproduced in this model. In conclusion, a new rat model of steatohepatitis was established by feeding with high fat emulsion via gavage. This model reproduces many of the clinical indices of human NASH.

Key words: Animal model, Nonalcoholic steatohepatitis.

P420074

A Report On The Experimental Study of The Theory of Channel Tropism

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Research the relation between the theory of Channel Tropism and the regulation of Neuro-endocrine immunity (NEI) network. We use the experimental spleen-deficiency rats (ESDR) and observe the effects of Huangqi (HQ, Radix Astragali), Fuling (FL, Poria) and their couples (HFC, HQ:FL 1:2) on vasoactive intestinal peptide (VIP) levels in brain-gut axis, etc.. Result: (1) HQ, FL and HFC increase the rats' D-xylose content of serum. (2) HFC and HQ can recover the falling Creatine Kinase (CK) activity of muscle because of spleen-deficiency. HQ represents better than HFC, but FL has no effect on CK. (3) HQ, FL and HFC reduce VIP content of hypothalamus, and enhance VIP content of the mucous membrane of artrum pyloricum and jejunum. HQ increases VIP content of plasma. (4) The changes of VIP levels in brain-gut axis are correlated with D-xylose content of serum, CK activity of muscle only when HQ combined with FL through multiple correlation analysis. The experiments show herbs' attribution to Spleen Meridian are perhaps correlated with the regulation of brain-gut axis, and complex prescription maybe influence herbs' selective attribution to Meridian.

Key words: Channel Tropism; Huangqi; Fuling

P420075

Human bone-marrow-derived mesenchymal stem cells (hMSCs) express a unique set of microRNAs

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OBJECTIVE: To identify the expression profile of microRNA (miRNA) in hMSCs. METHODS: hMSCs were isolated from bone marrow and cultured up to the amount of 10*6 cells. Low molecular weight RNA fraction from hMSCs was extracted, and polyadenylated by poly(A) polymerase. A 5'RNA adaptor was ligated to poly(A)-tailed RNA using T4 RNA ligase. After reverse transcription, the cDNA was amplified by PCR with two adaptor primers. The PCR product approximately 110 bp was recovered and subcloned into pUCM-T vector. And the small RNA sequences cloned were identified by DNA sequencing and database searching. RESULTS: A cDNA library was generated and total 220 clones were characterized by DNA sequencing and database searching. The result showed that the cloned RNAs represent several kinds of cellular RNA fragments such as mRNA, tRNA, rRNA, snRNA and snoRNA. And 3 novel miRNAs and 18 known miRNAs were discovered in hMSCs. CONCLUSION: A large diverse population of miRNAs may function to regulate gene expression in hMSCs, and the newly identified miRNAs may also serve as molecular markers for hMSCs.

Key Words: hMSC, microRNA
Supported by NSFC (30330042, 30571850) and GDNSF (33189, 04102307).

P420076

Biocompatibility and safety evaluation of beeswax

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To investigate the biocompatibility and safety evaluation of beeswax as cosmetic surgery material to confirm whether the material has potential harmful effect on human body. Methods: ①Cytotoxicity: By the method of agar overlay test, using 3T3 cells and laying beeswax's extraction ②Muscles implant test: The histologic examination and gross observation of beeswax and silicon model after hypodermic implantation for 1, 2, 3, 4, 5 and 6 months were contrastively analysed ③Haemolysis test: Beeswax's extraction was mixed with blood. Compare with control groups, the effect of resoluvent blood of the material was evaluated. Results: ①The extraction of material groups were similar to the extraction medium contrast groups, no dissolved cells have been seen and the cellular reaction target was R=0/0 ② Beeswax had a mild inflammatory reaction in the early days of planting and after 2 months the inflammation basically disappeared ③The haemolysis degree was 0.15% and demonstrated that beeswax didn't resolve red blood cells. Conclusion: All the results indicate that beeswax is a kind of material with good biological compatibility, no cytotoxicity and no haemolysis.

Key words: beeswax biocompatibility safety

P420077

Dual Action of Nitric Oxide in Pathogenic Mechanism of Ischemia/Reperfusion-Induced Mucosal Injury in Mouse Stomach

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We examined the roles of NO/NOS isoforms in the pathogenesis of I/R-induced injury in mouse stomachs.

Under urethane anesthesia, the celiac artery was clamped for 30 min, and then reperfusion was achieved for 60 min through removal of the clamp. L-NAME and 1400 W were given 30 min, L-arginine was given 60 min before ischemia. Multiple hemorrhagic lesions were observed in the gastric mucosa with I/R treatment. Pretreatment with L-NAME significantly increased the severity of these lesions, and this effect was significantly antagonized by L-arginine. By contrast, pretreatment with 1400 W significantly prevented I/R-induced gastric lesions. The expression of eNOS mRNA in the mucosa remained unchanged under normal and I/R conditions while the iNOS expression was markedly up-regulated in following I/R with an increase in the mucosal NO content. The increased NO production during I/R was completely attenuated by L-NAME and partially mitigated by 1400 W.

These results suggest that endogenous NO plays a dual action in the pathogenesis of I/R-induced gastric lesions; NO derives from eNOS is protective while NO derived from iNOS is proulcerogenic in the stomach during I/R-induced conditions.

P420078

EFFECTS OF TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION ON MOTOR AND SENSORIAL NERVES FOR DIABETIC POLYNEUROPATHY PATIENTS BY USE OF ELECTROMYOGRAPHY

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This study was planned to investigate the effects of TENS treatment on patients which have diabetic polyneuropathy. About 20~30% of diabetic patients are affected by DP. TENS and electrical has been proposed as physical therapies. Effects of HRFM with TENS on motor and sensorial nerves in patients with DP are investigated. Patients with type 2 diabetes and DP (n=14) both upper extremities

were treated for 20 min daily for twenty consecutive days. The patients' values of glucose, amplitude and latance were measured by use of EMG at before TENS, after TENS and following term of TENS. Patients were similar in terms of baseline characteristics, such as age, duration of diabetes, neurological symptoms scores and neurological disability scores. Differences among glucose levels related to before TENS, after TENS and following term of TENS are found statistically significant ($p < 0.05$). Differences for amplitude was not statistically significant. Differences on latances belong to motor and sensorial nerves were found statistically significant ($p < 0.05$). This study indicates that TENS treatment has been positive effect on polyneuropathy.

P420079

Regulation of the expression of microsomal PGE synthase by progesterone in ovarian granulosa cells

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Ovarian expression of the microsomal PGE synthase-1 (mPGES-1) and cyclooxygenase is observed in granulosa cells (GCs) of the mature follicle. Based on sequence homology, the mouse mPGES-1 5'-flanking region contains progesterone receptor (PR) binding sites. Effect of progesterone (P4) on mPGES-1 mRNA expression was determined in cultured GCs. Addition of P4 or LPS increased mPGES-1 mRNA expression. Amount of PGE_2 released into the media was also enhanced by P4 treatment. In a newly established mouse GCs line, G-tsT, P4 or Norgestrel (P4 receptor agonist) stimulated mPGES-1 mRNA expression. When we connected genomic DNA fragments upstream of the transcription initiation site of mPGES-1 gene with a promoter-less luciferase reporter cassette and transfected the mix to G-tsT cells, P4 enhanced the reporter activity in this assay, and a 150 bp upstream region of mPGES-1 gene was responsible for that. These data suggest that P4 augments the transcription of mPGES-1 gene in ovarian GCs.

Key words; ovary, microsomal PGE synthase, progesterone, granulosa cell

P420080

COMPARATVE STUDY OF DETOXICATION ENZYMES IN CATALYSING DEFLUORINATION

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To investigate the possible mechanism of fluoroacetate tolerance, a comparative study was performed between the fluoroacetate specific defluorinase (FSD) activity, overall glutathione S-transferase (GST) activity, and GST Theta 1 (GSTT1) and GST Zeta 1C (GSTZ1) specific activities of the liver cytosolic fractions of brushtail possums (Trichosurus vulpecula) from Western Australia (WA) and Southern Australia (SA). The results show that there is no significant difference in FSD activity between tolerant and sensitive brushtail possums. However, WA brushtail possums had significantly greater liver cytosol GSTT1 and GSTZ1 activity (0.39 ± 0.05 and 1.84 ± 0.16 μmol/mg protein/min, separately, both $P < 0.05$) compared with SA brushtail possums (0.17 ± 0.07 and 1.28 ± 0.15 μmol/mg protein/min, separately). The mitochondria of WA brushtail possum liver contained significant higher percentage of total FSD activity than that of SA brushtail possum ($P < 0.05$).

The results indicated that more than one of these GST isoenzymes may contribute to fluoroacetate tolerance. Enzyme defluorination is a critical step in fluoroacetate detoxication, but may not be the main factor that induces fluoroacetate tolerance.

Key Words: Fluoroacetate tolerance, FSD, GST, mitochondria.

P420081

Effect of exposure to nicotine in utero on fetal adrenal steroidogenesis in rats[1]

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Effect of exposure to nicotine in utero on fetal adrenal steroidogenesis was elucidated in this study. The pregnant rats were treated with nicotine from gestational day (GD) 8 until sacrificed on GD21. Radioimmunity and quantitative PCR analysis were done. The cortisol level in dam's blood was enhanced by nicotine. In dam's adrenals, steroidogenic acute regulatory (StAR) and cytochrome P450 cholesterol side chain cleavage (P450scc) mRNA increased in nicotine group, but in fetal adrenals, they presented obvious decreasing tendency. Nicotine had no influence on CYP1A1/2 and aryl hydrocarbon receptor (AhR) mRNA of dam's and fetal adrenals. However, in placenta, CYP1A1 and AhR mRNA were much higher after nicotine treated. Meanwhile, placental 11β-hydroxysteroid dehydrogenase type 2 (11β HSD-2) mRNA was reduced by nicotine. These results suggest that nicotine increase the dam's corticosteroids and impaire the placental barrier to maternal glucocorticoids. Overexposure to maternal glucocorticoids appears to impairment of fetal adrenal steroidogenesis.

Key words: nicotine; fetal; adrenal; steroidogenesis.
Supported by the grant from the Youth Foundation, Family Planning Commission of Hubei, China (2003)

P420082

Lifestyle interventions on body weight gain olanzapine-induced: result from a randomized-controlled trial.

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The objective of this study is to value a psycho-educational program to diminish the weight increase in a group of patient in treatment with olanzapine.

The first group of patients (A) with psychotic disease (22 patients: 12 females and 10 males) has assumed olanzapine (10/20 mg/die), has practised about 30 minutes of slight jogging for 3 times a week with a dietetic regimen reduced of about 500 Kcal/day. The second group (B) composed by 14 patients has followed only the therapy with olanzapine (10/20 mg/die). The patients, belonging to both groups, have been weight at the beginning of the observation and every week for 12 weeks.

After three months of observation, the group A has highlight a medium weight increase of about 0.3 Kg (medium increase of BMI of 0.3) while the group B has shown a medium weight increase of about 3.5 Kg (medium increase of BMI of 1.3) with a difference of about 3.2 Kg ($p < 0.005$) between the two groups. The group A has shown a statistically significant reduction of the weight increase in comparison with the patients of the group B, demonstrating the efficacy of the program to reduce the weight increase associated at the use of the atypical antipsychotics.

P420083

The interface between clinical practice in NRDS and laboratory research in ARDS.

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Surfactant replacement is commonly used today in the clinical management of newborn babies with Respiratory Distress Syndrome (RDS). In Cuba, the natural exogenous pulmonary surfactant SURFACEN has proved to be effective in RDS. There are evidences that this treatment might be effective in other lung disease for example, Acute Respiratory Distress Syndrome (ARDS). The uses of SURFACEN in other diseases need the evaluation in the first instance of anti-inflamatory and bactericidal properties in "in vitro" and animal models. SURFACEN, administrated intratracheally in rats challenge with LPS, showed the inhibitory effect on myeloperoxidase activity, malonaldehyde levels and total cell number. Also was able to reduce the TNF level produced in LPS-stimulated monocytes and inhibit the ICAM-1 in cell assays. SURFACEN was able to reduce of colony forming units in all types of bacteria tested, showing antibacterial effect on bacteria causing lung disease. These results demonstrate that SURFACEN can be considering as adequate preparation to improve the physiological status of ARDS patients.

Key words: SURFACEN, ARDS, anti-inflammatory, bactericidal.

P420084

Carrier-Mediated Uptake of Levofloxacin, By BeWo Cells, a Human Trophoblast Cell Line.

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Objective: Placental transfer of Levofloxacin (LF), a broad spectrum fluoroquinolone antibiotic, and its inhibition was investigated in BeWo cells, a human trophoblast cell-line.

Methods: The uptake experiments of LF by BeWo cells were performed after preincubation and in the presence of: P-glycoprotein (Pgp) inhibitors - Cyclosporin A (CsA), Verapamil and Quercetin, OAT substrate - Cimetidine and MCT substrate - Lactic acid.

Results: Pgp inhibitors increased the uptake of LF in BeWo cells. The increase in accumulation by CsA, Verapamil and Quercetin was by 30, 90 and 80%, respectively. Cimetidine - the OAT substrate and Salicylic acid - the MCT substrate increased the inward transport of LF by 48 and 200%, respectively.

Conclusions: The uptake of LF by human trophobast cells is mediated by multiple transporters as well as passive diffusion.

Key words: Levofloxacin, placental transporters, BeWo cell line.

P420085

Difference of apoptosis in nifedipine responder cell, non-responder cell and normal human gingival fibroblast.

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Objective: We have previously reported that the gingival fibroblast from nifedpine (NIF) reactive patients (nifedipine responders, NIFrs) gave trends toward better cell proliferation rates, DNA synthesis, and collagen synthesis than those from non-reactive patients (nifedipine non-responders, NIFns) in the presence of 1μM of NIF. Fujimori et al. demonstrated that the inhibition by NIF of LPS-induced apoptosis in human gingival fibroblasts might be the mechanism of gingival overgrowth. In this study, we compared the effect of NIF on LPS-induced apoptosis in NIFr, NIFn, and non-treated gingival fibroblast. Methods: We monitored the occurrence of apoptosis in each cell using APOPercentage Apoptosis Assay Kit. Results and Discussion: The less number of apoptotic cells in NIFr cells was found compared to these in NIFn and non-treated cells. Therefore, difference of apoptosis in NIFr cell, NIFn cell and non-treated control cell might relate the gingival overgrowth caused by NIF.

This study was supported in part by Grant-in-aids for 2003-Multidisciplinary Research Projects from MEXT.

P420086

Promoting action and mechanism of emodin on the experimental wound healing in rabbit

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AIM To observe the effect of emodin on restoration of dermal wounds. METHODS Full-thickness excision wounds were made on the back of rabbit and the ointment of emodin was applied to the wound once daily for 7-14 d and the effect of drug-treated wounds were measured by wound area, bacteria amount and histopathological examinations. The content of hydroxyproline and protein on wound tissues were measured. Semi-quantitative RT-PCR, Western blotting and immunohistochemistry were used to detect the expression of transforming growth factor (TGF-β1) and Smad 2, 3, 4, 7 protein on wound site respectively. RESULTS Emodin (100, 150 and 200 μg·g^{-1}) improved rates of wound contraction and with increasing emodin dose and days. what's more, total protein and total collagen content of granulation tissues increased with increasing emodin dose too. Also, TGF-β1 mRNA and Smad 2, 3 protein expression were both up-regulated by emodin with concentration-dependently compared with vehicle control. Otherwise there was no significant change on Smad 4 between emodin and vehicle control group. Emodin 150, 200 μg·g^{-1} decreased Smad 7 protein expression and emodin 200 μg·g^{-1} increased Smad 2,3 protein expression. Emodin 150, 200 μg·g^{-1} decreased Smad 2,3 protein expression compared with rhEGF group. CONCLUSION Emodin has ability to accelerate healing of cutaneous wounds which is relate to TGF-β1/Smad signaling pathway.

Key words: emodin; wound healing; transforming growth factor-β1; smad

P420087

Facilitation of the functional reendothelialization in improving the accelerated intimal hyperplasia with estrogen in the rat

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Present experiments were designed to investigate the effects of ovariectomy (OVX) and estrogen replacement (ER) on the intimal hyperplasia (IH) following balloon injury of the rat carotid artery. Twelve weeks old female rats were divided into 3 groups of sham operation, OVX, and OVX plus ER. Bilateral OVX significantly accelerated the IH. The acceleration was accompanied by the enhanced impairment of NO generation, attenuated reendothelialization and enhanced accumulation of asymmetric dimethylarginine (ADMA) as an endogenous NOS inhibitor (NOSI). Meanwhile, ER effectively improved the accelerated IH following OVX through improving the impaired NO generation and accumulated ADMA, and facilitating reendothelialization. The plasma estrogen level in the ER group was maintained under the physiological level. These results suggest that ER effectively improves the accelerated IH following OVX by recovering the impaired NO generation through reducing NOSI and facilitating the functional reendothelialization.

P420088

Single technology appraisal (STA): the feasibility of early assessment of cost-effectiveness of new drugs

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For 4 years SMC has provided the health service in Scotland with rapid STA on all new medicines, and new indications/formulations of existing therapies. Our objective was to review lessons learnt now that the National Institute for Clinical Excellence (NICE) is also planning STA.

Submissions from pharma companies undergo pharmacy/health economics assessment, then review by SMC in 2 stages, including an objective review and a societal view of need. Comparative cost-effectiveness is assessed and recommendations on use made close to the launch on the UK market.

Over 200 drugs were assessed to end 2005. 67% of drugs were approved, although many with restriction beyond the licence. Decisions were not influenced by budget impact (affordability). Drug utilisation data suggest prompt advice influences prescribing patterns. Benchmarking shows high consistency of advice from NICE (UK) and Australia with that of SMC.

SMC STA shows an open inclusive process, involving clinicians, patients, managers and industry, can produce useful, evidence-based advice to a healthcare system early enough after launch to inform and influence subsequent prescribing patterns.

Key words: health technology, cost-effectiveness

P420090

Protective effects of indole-3-carbinol against ethanol-induced liver injury in precision-cut rat liver slices

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To investigate the effect of indole-3-carbinol (I3C) on ethanol-induced liver injury in precision-cut rat liver slices (PCLS). PCLS were incubated with ethanol or acetaldehyde, and different doses of I3C were added to the medium. ALT release and MDA content were used to estimate hepatotoxin and lipid peroxidation of ethanol and acetaldehyde. The ethanol metabolism pathway was evaluated by the assays of ethanol dehydrogenase (ADH) and aniline hydroxylase (ANH). The content of hydroxyproline (Hyp), transforming growth factor-β1 (TGF-β1) and α-smooth muscle actin (α-SMA) were measured as the status of hepatic stellate cells (HSC) activation. The results showed that I3C decreased leakage of enzymes, lipid peroxidation and content of TGF-β1 in medium, and inhibited the production of Hyp in PCLS. The α-SMA immunohistochemistry expression in PCLS was reduced as such above. These results suggested that I3C can reduce damage in ethanol-induced PCLS injury and this effect may be associated with the modification of ethanol-metabolizing pathway and inhibition of HSC activation. indole-3-carbinol; liver slice; ethanol; hepatic stellate cells.

1 Supported by the National Natural Science Foundation of China, No. 30371666

P420091

Tetrandrine inhibits induction of the mitochondrial permeability transition: a possible mechanism for its protective effect of mitochondria

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This study was designed to evaluate the effect of tetrandrine (Tet) on the function of rat liver mitochondria. Mitochondrial permeability transition pore (MPTP) opening was measured by the permeability to sucrose. Ca^{2+} fluxes were followed with Arsenazo III. GSH level was determined with fluorescence detection with o-phthalaldehyde (OPT). The generation of mitochondrial ROS and the mitochondrial membrane potential ($\triangle\Psi_m$) were determined using DCFH-DA and Rho123. MPTT was inhibited by Tet when induced by various inducers including Ca^{2+} + Pi, the adenine nucleotide translocase (ANT) inhibitor actractyloside, the prooxidant t-butylhydroperoxide (t-BOOH) and RR+FCCP. Calcium efflux induced by high concentration Ca^{2+} was significantly inhibited by Tet. In addition, the release of GSH from mitochondria, ROS generation, NAD(P)H oxidation and $\triangle\Psi_m$ drop were markedly inhibited by Tet. These results suggest that Tet inhibits induction of liver MPT, which may be relative to the modification of the thiol groups on the matrix surface of ANT by Tet.

Key words tetrandrine, MPTP, ANT, oxidative stress

Acknowledgement: Project supported by National Science and Technology Foundation of China "863 project" (No 2004AA2Z3779).

P420092

Effect of Ganyanping on the expression of M1 acetylcholine receptor and β2-adrenoceptor in liver fibrosis of rats

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Objective To study the antifibrotic effect and mechanism of Ganyanping on liver fibrosis of rats. Methods The rats were separated randomly into three groups: Group N、Group G and Group M. The liver changes of pathological histology were observed by HE staining and electron microscope. The expression of M1-AChR and β2-AR in liver tissue were evaluated by immunochemistry and RT-PCR. Results Compared with the group M, the espression of M1-AChR was decressed, while the expression of β2-AR incressed in the liver in the group G. while the expression of β2-AR decressed in the liver in the group M. The difference was significant ($P < 0.05$). and the pathological change of Ganyanping-treating group and were improved. Conclusion Ganyanping could inhibit the expression of M1-AChR and enhanc the expression of β2-AR in the liver fibrosis of rats, which may mediate the effect of neurotransmitter in liver fibrosis.

Key words: Ganyanping; M1 acetylcholine receptor; β2-adrenoceptor; Liver fibrosis.

Nation natural science fund (No.30440088)

P420093

Effect of gestrinone on gene expression in human uterine leiomyoma

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AIM: This paper was to study the effect of gestrinone on gene expression in primary cultured uterine leiomyoma and matched myometrium cell using gene microarray technology. METHODS: Leiomyoma and myometrium cells were cultured in phenol red-free DMEM/F_{12} media containing 10% charcoal-dextran treated bovine serums. Gene expression was analyzed using GEArray Q Series Signal Transduction in Cancer Gene Array. RESULTS: There are marked up-regulation in genes expression in leiomyoma, including IL-4R, IL-4, VEGF, TNFα, ET-1, WNT1, WNT2 Cox-2, c-Fos, CD31, IκBα, and etc. After treatment with gestrinone 0.3μmol/L for 24 hours, there are remarkable down-regulate in gene expressions, including ET-1, IκBα, CD31, IL-4, IL-4R, PR, TNFα, Fra-1, ID2, and etc. CONCLUSIONS: Many signaling pathways were found up-regulation in the development of uterine leiomyoma. Gestrinone could down-regulate the expression of related gene in tumor genesis. The effect of gestrinone involves inhibiting several signaling pathways, such as hormonal, inflammation, survival, STAT, Wnt, Hypoxia and MAP kinase pathways.

Key words: gestrinone, uterine leiomyoma, microarray, gene expression, signaling pathway

P420094

Protection of sodium ferulate on ethanol-induced hepatotoxicity in rat precision-cut liver slices and its mechanism[1]

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To investigate the protection and mechanism of sodium ferulate (SF) on ethanol-induced toxicity in rat precision-cut liver slices (PCLS), PCLS were incubated with SF (0.5-2mM) either co-treated with ethanol or its metabolite acetaldehyde. The releases of glutathione S-transferase (GST) and lactate dehydrogenase (LDH), the activities of aniline hydroxylase (ANH) and alcohol dehydrogenase (ADH) and the content of malondialdehyde (MDA) were monitored. Meanwhile, the contents of hydroxyproline (Hyp), transforming growth factor-β1 (TGF-β1) and α-smooth muscle actin (α-SMA) were detected. The results showed that SF reduce the leakage of enzymes, degrade lipid peroxidation, and turn ANH and ADH to the normal level. Prominent inhibition of HSC activation was achieved with SF against acetaldehyde-induced increase of α-SMA and TGF-β1, and Hyp content showed a decrease tendency. The results demonstrated that SF exerts protective effects on ethanol-induced hepatotoxicity, which attribute to the modification of ethanol-metabolizing pathway and inhibition of HSC activation.

Key words: sodium ferulate; precision-cut liver slices; ethanol; acetaldehyde.

Supported by the National Natural Science Foundation of China, No. 30371666

P420095

Cytotoxic effect of haloperidol on microglia undergoes apoptosis process

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Schizophrenia is a devastating illness of unknown etiology and its treatment presently relies on antipsychotics. Recently, microglia dysfunction in schizophrenia has been proposed. However, the effects of antipsychotic drugs on microglia have not been reported. Therefore, the present study examined the cytoxicity of haloperidol, a typical antipsychotic drug, on microglia by using mouse microglial cell line N9. Viability of haloperidol on N9 cells was measured by MTT assay. Morphological changes of N9 cells after the drug treatment were observed by fluorescence microscope and transmission electron microscope, respectively. Nucleosomal DNA fragmentation was assayed by agarose gel electrophoresis. The results showed that haloperidol exhibited toxic effect on N9 cells in a dose- and time-dependent fashion. Apoptotic cells were observed by fluorescence and electron microscopic observation. The N9 cells treated with haloperidol showed the characteristic ladder pattern in the DNA ladder assay. In conclusion, the present study demonstrated for the first time that the cytotoxic effect of haloperidol on N9 cells underwent apoptosis process.

Key words: apoptosis; haloperidol; microglia; N9 cells

P420096

Nanopharmacology*

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Nanopharmacology, a new branch of pharmacology is gradually emerging with the development of nanoscience and nanotechnology, which studies the interactions between drugs or nanoscale materials and human body structural materials, such as proteins, DNA and RNA, and cells, tissues or physiological systems at nanoscale level. Considering nanopharmacology a new branch of pharmacology is mainly because: (1) it uses nanotechniques such as atomic force microscopy; (2) it studies nanostructures and particulate drugs, not only those in the mode of molecules. The pharmacological effects of the particulate drugs are different from that of drug molecules because the effects of the former include not only general chemical effects but also special pharmacological effects produced by nanometer sizes, highly proportional surfaces and quantum scale effects and micro-mechanical effects; (4) the mature nanopharmacology will be able to assemble drug molecules with atoms one by one. Such drug molecules will remove pathological moiety from biological macromolecules or repair them in situ. In present paper we summarize our practice in nanopharmacology.

Supported by National Science Foundation of China, 90406024-7.

P420097

PAI-1 and tPA Modulating Activity and Thrombolytic Effects of Cytochalasine D

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Cytochalasine D (extracted from Engleromycetes gotzii) was investigated on thrombolytic effect as well as modulating activity of type 1 plasminogen activator inhibitor (PAI-1) and tissue-type plasminogen activator (tPA). Charlton's and Tomihisa's methods were modified to investigate the thrombolytic effect of intravenous cytochalasine D. The activity of PAI-1/tPA in rat plasma was assayed by use of chromogenic substrate. The results showed that intravenous cytochalasine D (2, 4, and 8 mg/kg) had a dose-dependent thrombolytic effect in rats. Cytochalasine D significantly inhibited PAI-1 activity in rat plasma or platelet-released substances while elevated plasma tPA activity, in a concentration-dependent manner. It is indicated that cytochalasine D inhibited PAI-1 activity and increased tPA activity, and this property of cytochalasine D is assigned to be responsible for the thrombolytic effect.

Key words: cytochalasine D; thrombolysis; tPA; PAI-1

Acknowledgement: This project was supported by the United Cultivation Base of Yunnan Province for Innovative Talents of Medicine & Biotechnology and Pharmacological Innovative Group Foundation of Kunming Medical College.

P420098

Effects of Rhynchophylline on the Amphetamine Dependence in Rats

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The purpose of this study was to investigated the effects of rhychophylline (Rhy), an active component from the Chinese herbal medicine, on amphetamine (AM)-induced conditioned place preference (CPP) in rats. 50 SD rats were randomly divided into 6 groups: normal control group, AM-dependent model group, Rhy high (60 mg/kg), middle (20 mg/kg) and low (10 mg/kg) dose groups. A model of CPP induced by AM in rats was established. Immunohistochemistry and in situ hybridization were used to examined NR2B positive cells and NR2B mRNA expression in nucleus accumbens (NAc) and amygdaloid (Amy) of rat brain. After treated with AM for 8 days, the rat staying time in the AM-paired compartment was significantly longer, which indicated that the rats have produced a strong CPP effect. The staying times in three dose groups of Rhy were obviously shorter than that of model group. NR2B positive cells and NR2B mRNA expression in NAc and Amy of model group were significantly increased. In middle- and high-dose groups of Rhy, the numerical density of NR2B and NR2B mRNA expression were obviously decreased. The findings indicated that Rhy could suppress the acquisition of CPP induced by AM in rats and inhibit expression of NR2B in NAc and Amy after rats were treated with AM.

Key Words: Rhynchophylline; Amphetamine

The research was supported by National Natural Science Funds of China, No. 3031773

P420099

Hypertension in the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS2)

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Background: Treatment of hypertension reduces cardiovascular events. There is a need to identify hypertension in the community. Method: 1944 subjects (901 men and 1043 women; age 52 ±12 yrs) of the Hong Kong Cardiovascular Risk Factor Prevalence Survey were recruited in 1995-6 and were followed up in 2000-4. The prevalence of hypertension in the cohort and the factors related to its development were determined. Results: In 2000-4, the prevalence of hypertension was 23.5% in men and 17.8% in women. In those age ≥64 years, it was 55.3 ± 3.5 % in men and 50.6 ±3.7 % in women. In men <55 years, the prevalence of hypertension had increased since 1995-6. Among 1602 subjects normotensive at baseline, there were 258 cases of new hypertension after a median interval of 6.4 years. In multivariate analysis, age and baseline systolic blood pressure were significant predictors in both sexes. In men, BMI and plasma triglycerides were significant predictors, but in women, HDL was the predctor instead. Conclusions: Hypertension is common, especially in the elderly. As its development is related to metabolic factors, diet and exercise may prevent or delay its onset, or reduce the need for drug therapy.

P420100

THE MECHANISMS OF ACTION OF ERGOT ALKALOIDS AND SEMISYNTHETIC DERIVATIVES ON THE UTERUS

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Our purpose is to investigate the mechanisms of action of ergot alkaloids induced contractions on the isolated uterus. The experiments were carried out on uterine strips obtained from pregnant Swiss Albino mice (n=42). After the application of vehicles waited for incubation for 30 min in control groups. Same procedure was carried out for antagonist drugs ketanserine, indomethacine, prazosine, yohimbine and losartan. At the end of the incubation period methylergonovine was applied cumulatively at 10^{-9}--10^{-4} M concentrations. Frequency, amplitüde and area under the curve (AUC) of methylergonovine induced contractions were reduced significantly after incubation with ketanserine. After incubation with indomethacine, amplitüde and AUC of methylergonovine induced contractions were reduced significantly but the frequency was not affected. Prazosine, yohimbine, losartan did not affect the methylergonovine induced contractions. It was concluded that methylergonovine contracts mice uterus through the agonistic action at 5-HT2 serotonergic receptors. Additionally, we thought that the oxytocic prostaglandins may also have a role in methylergonovine induced contractions.

Key words: Ergot, Mice, Methylergonovine, Uterus

P420101

Effect of simulated microgravity conditions on rat intestinal transit

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Exposure to weightlessness and modeled microgravity leads to modfications of several physiological processes whose mechanisms are not clearly understood. The modification or the loss of the gravitational force vector strongly affects many fundamental cellular functions (1). The aim of the present study has been to investigate the effects of modeled microgravity conditions, using a three dimensional clinostat (Random Positioning Machine, RPM) on rat intestinal transit and on the expression of inducible isoforms of nitric oxide synthase (iNOS) and cicloxygenase (COX-2). Our data indicated that RPM significantly reduced rat intestinal transit giving raise to 31% inhibition compared to control animals and with lower (11%) and not significant inhibition if compared to ground control animals. To

further elucidate the mechanism by which RPM modifies rat intestinal transit time we performed Western blot analysis on rat colon and stomach to assess whether the weightlessness could influence the expression of COX-2 and iNOS. These results showed that RPM reduced rat intestinal transit and influences the iNOS and COX-2 expression.

Key words: simulated microgravity, iNOS, COX-2, intestinal transit

P420102

Somatosensory Evoked Potentials of Experimental Rat's Cervical Spondylotic Radiculopathy Model and Effects of Ibuprofen

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Objective: To establish a new kind of cervical spondylotic radiculopathy model and monitor its somatosensory evoked potentials' (SEP) change. Method By using nylon suture inserted into vertebral canal to making cervical dorsal nerve root continuously compressed, we established a cervical spondylotic radiculopathy model on rats. Median nerve SEPs were recorded by hypodermal stainless steel needle electrodes on ipsilateral Erb's point, C6 interspinal ligament and contralateral parietal somatosensory cortex. Used SCT(subtraction of C6 and Erb's latency) and CCT(subtraction of cortex and Erb's) to estimate sensory nerve's conductibility. Results: 3 days and 7 days after the operation, operational side's SCT and CCT are all significantly prolonged compared with uninjured side's values. Whereas the phenomena disappeared on 14 days. Using Ibuprofen orally can accelerate the operational side's SCT and CCT recovery significantly. Conclusion: using nylon suture insert method, rats' SEP latencies prolonged. Oral administration of Ibuprofen induced significant peripheral and central SEP abnormalities in such model.

Key words: Cervical spondylotic radiculopathy; SEP; Ibuprofen

P420103

Enhanced nuclear delivery and improved cytotoxic effect of hydroxycamptothecin by oil-in-water emulsions in HeLa cells

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Hydroxycamptothecin-Emulsions (HCPT-E), being a promising for intravenous applications, were prepared and assessed for intracellular distribution and cytotoxic potential as compared to HCPT-Injections (HCPT-I). Two formulations (containing 5 mg/ml HCPT) incubated with HeLa cells, and then drug amounts of nuclei and cytoplasm were quantified by HPLC. The drug amounts of nuclei were higher than cytoplasm in cells of HCPT-E incubation, just the reverse these of HCPT-I. The drug amounts of nuclei and cytoplasm in cells exposed to HCPT-E were pronounced high, about 18 to 33 times, than those of HCPT-I. MTT results showed that cytotoxicity of HCPT-E was higher than HCPT-I and IC_{50} values of HCPT-E were more lower, about 4 and 7 times, than those of HCPT-I. After HeLa cells incubated with two HCPT formulations for 4 h, cells treated with HCPT-E displayed morphological characteristic of apoptotic cell death at 72 h. The results suggested that HCPT-E enhanced intracellular drug amount and changed its intracellular distribution in favor of a targeting effect towards nuclei, and showed significant cytotoxicity against HeLa cells.

Key words: HCPT; intracellular distribution; emulsions; cytotoxicity

This study was supported in part by the Specialized Research Fund for the Doctoral Program of Higher Education of China (SRFDP No.20050335044), the National Natural Science Foundation of China (NSFC No.30572270) and the project supported by the MInistry of Personnel of the P.R.China.

P420104

Effect of novel isoquindine derivatives on the reversal of multidrug resistance

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To study the effect and mechanism of novel isoquinoline derivatives, CPUB2、CPUB3、CPUC1 on the reversal of MDR in adriamycin-induced multidurg-resistance K562 cells. We use the methods of MTT assay, flow cytometry and RT-PCR. These isoquinoline derivatives increased the cytotoxity of ADM and VCR in a concentration-dependent manner and enhanced the apoptosis induced by VCR in K562/A02 cells. But they have little effect on the cytotoxity of ADM and VCR in K562 cells. They strongly inhibited the function of Pgp and increased the intracellular accumulation of RH123 and ADM, and also decreased the efflux of ADM in a concentration-dependent manner in K562/A02 cells. The effect of increasing the intracellular accumulation of RH123 is CPUB2 > CPUB3 > CPUC1 > VER, and the effect on ADM accumulation is CPUB3 > CPUB2 > CPUC1 > VER. The reversal effect of MDR is CPUB3 > CPUC1 ≈ CPUB2 > verapamil (VER). CPUB2、CPUB3 decreased Pgp expression in mRNA level and protein level after 72h exposure while CPUC1 had no effect on Pgp expression in K562/A02 cells. CPUB2、CPUB3、CPUC1 exhibited a strong inhibitory effect on the activity of P-gp in K562/A02 cells.

Key words: Multidrug resistance; P-glycoprotein; MDR1 gene; Isoquinoline;

P420105

PROTECTIVE EFFECTS OF FLAVONE FROM IPOMOEA BATATAS POIR.CV. ON MICE THYMOCYTES IRRADIATED BY ^{60}Co γ RAY

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Objective: To study the protective effects of flavone from Ipomoea batatas poir. cv. on thymocytes of Qunming mice irradiated by ^{60}Co γ ray (3650μl/cm^2). Methods: The cells were randomly divided into six groups: control group, ^{60}Co groups (model, 0.625g/L、1.250g/L、2.500g/L of flavone and 1g/L VitC). The intracellular free calcium, mitochondria membrane potential and apoptosis rate of thymocytes were tested using flow cytometry (FCM). The expressions of p53 proteins and p21 were examined by immunocytochemistry and in situ hybridization respectively. Results: The concentration of intracellular free calcium and apoptosis rate were decreased by flavone. It also decreased the expression of p53. Furthermore the mRNA expressions of p21 decreased in flavone treatment groups. Conclusion: Flavone has the protective ability on damages of thymocytes caused by the Co60. The mechanisms might be its decreasing intracellular free calcium and the expressions of p53 and p21 gene, stabilizing the mitochondria membrane potential.

Key words: flavone, Ipomoea batatas poir. cv.; ^{60}Co irradiation; thymocytes; mice

P420106

Detection and Quantitation of PS20, a phosphorothioate oligodeoxynucleotides in tissue homogenanate

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AIM: To establish the method for quantitation of the phosphorothioate oligodeoxynucleotides (S-ODNs) in tissue homogenate. METHODS: After incubating with protease K over night, two solid-phase extraction columns combined with a strong anion-exchange column were utilized to remove proteins and lipids in homogenanate after extraction by the mixture of the phenol and the chloroform (w/w 1:1), and the salts were removed by a reverse-phase column followed by the method of dialysis with a 2500 Da-cutoff membrane. The concentration of the tested S-ODNs, PS20, and its metabolites extracted from the tissue homogenanate were determined by the method of non-gel sieving capillary electrophoresis (NGCE) with diode array detection in the presence of internal standard (IS). RESULTS: The validity study showed the method was with good base number specificity, RSD% of both intra and inter assay were all less than 15%, the total mean recovery was about 87%. The methodology was successfully used to determine the distribution of an anti-tumor antisense S-ODNs in rat and identify the metabolites with single base difference. CONCLUSION: The combined method of solid-phase extraction and NGCE could be used to study the distribution of S-ODNs, and the main parameters of the methodology met the requirement of distribution study.

KEY WORDS: oligonucleotides; tissue homogenate; extraction; NGCE

Aknowledgement: Project supported by the National Natural Science Foundation of China (No 39870878) and the National high-tech R & D plan (No. 2003AA2Z347B)

P420107

The Effect on Lipid in Serum and Liver of Fatty Liver Rats with Hyperlipermia by KangLing decoction

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Objective: To investigate the effects of kangling decoction (KLD) on the content of lipid serum and liver of fatty liver rats with hyperlipemia, and provide a new therapeutic method to hyperlipemia fatty liver. Methods: To feed the rats with high fat diet and duplicate the model of fatty liver for four weeks. Rats were randomly divided into six groups (normal group, model group, KLD group [high dose, middle dose, low dose at 6g·kg^{-1}、12g·kg^{-1}、24g·kg^{-1} respectively], dongbaogantai group). Blood lipid, hepatic lipid, hepatic index, hepatic function, and liver were assayed respectively before and after therapy with KLD. Results: KLD can remarkably decrease the content of total cholesterin (TC), triglyceride (TG), low density lipoprotein (LDL-c) ($P<0.05$ or $P<0.01$), in the serum of the model rats apo-A1in. the serum can remarkably raise ($P<0.05$) and has a dose-dependent manner; the levels of high density lipoprotein (HDL-c) and apo-B were insignificant compared with the hyperlipemia model group but had the decreasing tendency. KLD can lower the contents of AST、ALT in serum and lipid in liver and heighten the activity of SOD so that KLD can protect the function of the liver. Each KLD group is better then model group and middle-KLD group、low-KLD group are better then dongbaogantai group through tissue section. Conclusion: KLD can lower the contents of TC, TG, LDL in serum and liver and heighten the activity of SOD and protect the function of the liver.

Key words: Kangling decoction Serum lipid Hepatic lipid Liver protect

P420108

Therapeutic effect for anti-fibrosis of the extract from Scirpus yagara Ohwi in hepatic fibrosis rats

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Aim: to study therapeutic effect of Scirpus yagara Ohwi extract (PHS) on hepatic fibrosis. Methodes: PHS was extracted from plant materimals of Scirpus yagara Ohwi Rhizome with ethyl acetate. hepatic fibrosis rats was induced by CCl4 for 8 weeks. In curative treatment extract (equivalent to 3, 6, 12g crude materials/kg, p.o) was given for 6 weeks after the establishment of fibrosis for 8 weeks. After then, Animals were examined for serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), hyaluronic acid (HA), laminin (LN), hepatic hydroxyproline (Hyp), malondialdehyde (MDA), superoxide dismutase (SOD) and tissue morphology. Results: The hepatic fibrosis model rats treated with the extract, its serume HA and liver Hyp, hepatic MDA content were remarkably decreased. Histopathological changes of hepatic lesions induced by CCl_4 were improved by treatment with PHS. Conclusion: Our results suggest that the PHS could inhibit peroxidation, improve liver function and reduce liver fibrosis in hepatic fibrosis rats.

Key words: Scirpus yagara Ohwi; hepatic fibrosis rats; therapeutic effect

P420109

Induction of oxidative stress in chronic exposure to aluminum

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The physiological role of aluminum (Al) is not yet known. Exposure to Al may cause many human disorders. This study is aimed at providing further information on how occupational human exposure to Al might affect the body oxidative stress. The relation between Al toxicity and oxidative stress was studied in blood samples obtained from 45 primary Al production workers, with a minimum work history of 5 years in the age range of 28 to 52 years. They were evaluated for oxidative stress markers including thiobarbituric acid reactive substances (TBARS) indicator of lipid peroxidation (LPO), ferric reducing ability of plasma (FRAP) indicator of total antioxidant capacity, total thiol groups, and Al level in blood. The results showed that workers have significantly higher blood Al levels, and concomitant lower blood FRAP and total thiol groups in comparison to controls. No significant statistical correlation between oxidative stress markers and Al level, history of disease, history of work, smoking, and education were found. It is concluded that Al induces oxidative stress and supplementation of antioxidant vitamins may have benefical effects.

Key words: aluminum, oxidative stress, workers, lipid peroxidation, total antioxidant capacity

P420110

Improvement effect of melatonin on elderly mood disfunction

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Objective: To study the influence of melatonin (MT) on elderly persons with mood dysfunction of anxiety and depression. Methods: 224 aged 60～79 years cases of sub-health and the patients with non-acute stage cardiac and/or cerebral vascular diseases were carried on the multi-centers, randomized, double blinded and placebo paralleled comparison clinical research. The patients were divided into two groups: the MT group (115 examples) and the placebo group (109 examples). The MT group had taken MT capsule 1-2 grain (3-6mg)/per evening orally, the placebo group was given the capsule with same contour containing the starch in the same time. The taking MT lasted out for 24 weeks continuously. Before research started (0 week), and 4, 12, 24 weeks after taking MT or placebo, the receivers filled out the Zung test scales of despondent (SDS) and anxious (SAS) scores. Results: the average values of anxious (SAS) and despondent (SDS) grades in the MT group from 0 week to 4, 12, 24 weeks, gradually decreased, which compared with the values of the placebo group, having the statistical significant differences ($P<0.05$～0.0001). The effectiveness of anxious mood reduction was 69.4% In 24 weeks after taking MT, when the despondent mood improvement effectiveness is 67.6%. Conclusions: Senior citizens taking MT have remarkably improved their anxious and despondent mood.

Key words: melatonin; mood barrier; the elderly

P420111

The Clinical research of melatonin administration on the elderly blood pressure and serum MAO-B activity

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Objective: To observe the influences of the Brain Platinum capsule (Melatonin, MT) administration on the blood pressure and the serum monoaminoxidase-B (MAO-B) in elderly persons. Methods: The 222 old testee aged from 60～79 years, who included the 100 sub-healthy senior citizens and the 122 patients with non-acute cardiac and/or cerebral vascular diseases, were carried into the clinical research. which was conducted in multi-centers, by randomized, double-blinded and placebo paralleled comparison process. The receivers were divided into two groups: the MT group (114 cases) and the placebo group (108 cases). The MT group had taken MT capsule 1-2 grain (3-6mg)/per evening orally, the placebo group was given the capsule with same contour containing the starch in the same time. The taking MT lasted out for 24 weeks continuously. The blood pressure and The blood serum monoaminoxidase (MAO-B) activeness of all receivers were measured. before and after research per-month. The data were analyzed by SPSS 10 statistics software. Results: the average values of the systolic and diastolic blood pressures in the MT group after taking 3, 4, 5, 6 months were gradually decreased, which compared with the values of the placebo group, having the statistical significant differences ($P<0.05$～0.001). The blood serum monoaminoxidase (MAO-B) activeness of MT group had remarkably reduced, comparing with the placebo group's MAO-B activeness. After 3 months administration, in MT group, the reduction values of the diastolic blood pressure and serum MAO-B activeness existed the significant positive relationship ($P<0.05$). The placebo group didn't show this relationship. Conclusions: In the elderly persons and patients, long-term (beyond 3 months) taking MT might remarkably reduce the blood pressure and the serum MAO-B activeness, thus possibly slow down the senile step.

Key words: melatonin; blood pressure; MAO-B activity; the elderly

WORKSHOP

W1.1

Lessons from the UK Pharma-CAL-ogy project

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More than £1M was obtained from the UK government between 1993 and 2001 for a group of pharmacologists, led by Prof. Ian Hughes, to develop teaching resources. The group produced 35 computer-aided learning (CAL) programs covering most areas of basic pharmacology, 5 videos and 19 workbooks to aid their incorporation into courses. Extensive evaluation of content and process was performed during and after development (Dewhurst, DG & Norris, BEE-j 1, 2003, 1-6). Ownership of the materials was transferred to the British Pharmacological Society (http://www.pharmacology.com). Over 4,100 CAL programs and 230 workbooks have been sold to 28 countries. Development was very time consuming and technical developments have required significant program updating. The project worked well as it brought together enthusiastic academics with a shared interest in teaching pharmacology. It markedly aided the understanding and use of learning technology in pharmacology teaching in the UK. Such a project would be very different today due to the development of the web, the realisation of the value of virtual learning objects and increased pressures on academic staff time.

Computer-aided learning. Pharmacalogy

W1.2

The use of technology for distance learning in pharmacology

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Technology-based learning revolutionized teaching and learning in several ways, while it has not necessarily improved accessibility and quality in all cases. Distance learning in particular benefitted from improved and faster communication, access to electronic databases and information on the Web and electronic student support. Computer software that enhance and facilitate independent, asynchonous learning have some value for the distant learner.

We will discuss and demonstrate several ways that technology may enhance the learner support and learning process for the distant learner, as well as student perceptions of the quality, appropriateness and success of these technologies. In addition we will discuss new guidelines/criteria for ensuring the quality of E-learning in South African higher education, as recently derived from case-studies and discussed at a workshop on the topic. These guidelines may eventually be adopted by the Council for Higher Education as regulatory framework and measuring instument.

Key Words: technology-based; distance; e-learning; pharmacology

W1.3

Internet as learning tool among the medical students in the public and private medical schools. A preliminary experience from Indonesia.

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Interaction between students and lecturers is enriched by "virtual" meetings via internet. Some of well-established e-learning portals, such as Blackboard, Web-CT, etc., are getting popular among the scholars. In medical area, educational materials are, nowadays, available via internet. Pictures, graphs and presentations of numerous topics from many sites are at our own disposal to download. The demand of using internet as learning media has pushed many faculties in developing countries, like Indonesia, to design and launch their own e-learning portals by using public websites, like Yahoo! A cross-sectional survey on the usage of the internet among the 3rd-4th grade medical students was conducted in two schools of medicine in Jakarta. The survey was designed to identify the experiences, comments and critics of students on internet facility available in their respective schools. The results of the survey show that most of the students found that internet is very useful especially for e-mail and tapping new information, although access to internet is still limited and slow. Students also showed their eagerness to utilise the internet, even though it is not yet officially used for learning.

W1.4

ReCAL-preserving traditional computer-based learning materials in pharmacology

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Since 1993 the UK government has invested over £3m in the development of multimedia CBL programs in pharmacology. These were developed in ways which intrinsically tied the content and educational approach to the run-time environment so that, as the underlying technology changes, they require significant rewrites to avoid becoming redundant. The RECAL project is developing a methodology based on abstracting the content and sequencing of a program and separating this from the runtime environment such that all of a program's assets (text, images, animations, sequencing, assessments) are stored separately in a simple learning object repository and catalogued. The process encodes the program's structure, sequencing and pedagogical design using IMS Simple Sequencing and makes these available as XML files. To run the new version of a program a generic run-time shell (currently Macromedia Flash but Web server-based application planned) is set up and launched. This sequentially loads the basic program parameters followed by its presentation and sequencing parameters and the media assets. Changing any component allows the program to be easily adapted to meet user needs (e.g. different language versions).

W2.1

QUALITY CONTROL OF HERBAL MEDICINE: CHEMICAL AND BIOLOGICAL FINGERPRINTS

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Modern medicine should have evidence-based therapeutic claims, safety concerns addressed, preparation consistency, as well as provide insights into mechanisms of action and potential interactions with other drugs. The major challenge in transforming traditional medicine into modern medicine is preparation consistency. The logical methodology in assessing preparation consistency is through animal models. This approach however is not feasible in the absence of a good animal model and it is also impractical during manufacturing. The solution is chemical and biological fingerprints of the preparation using modern multiplex and information rich technologies: LC-MS assesses chemical fingerprints. Cells as sensors monitoring cellular RNA alterations or signal transduction pathways and in vitro activities of relevant enzymes or receptor assays establish in vitro biological fingerprints. PHY906, a traditional Chinese Medicine under investigation in a US phase II clinical trial for the treatment of hepatocellular carcinoma, will be the example for this presentation.

W2.2

Quality Control of Traditional Chinese Medicine and Natural Products

Xinsheng Yao, Academician of Chinese Academy of Engineering; Shenyang Pharmaceutical University; Honor Dean of School of Pharmacy and Director of the Institute of TCM & Natural Products, Jinan University, China.

Quality Contrcl is not only the guarantee for the continuous development of Traditional Chinese Medicine(TCM), but also the premier for its globalization. The original purpose of Quality Control of TCM/Natural Products is to keep the biological equality (the efficacy and adverse reaction) of the same product. However, for the difficulties in application, it turns to the chemical methods now. The active component in TCM that play critical roles in prevention and treatment of diseases is usually selected as a marker for Quality Control of TCM. However, the action of TCM may be regulated by several active compounds, not solely by one marker component. So the chemical fingerprint spectrum of TCM is considered as an important supplement to quantifying the marker component. But generally speaking, the chemical fingerprint spectrum can only prove the chemical equality of the same product with different production code, not the biological equality. To answer this question, the 'spectrum-efficacy' theory is proposed. Of course, the principle of this theory is to clarify the genuine active component in this product. All these should be included to consideration when dealing with Quality Control.

W2.3

Quality assurance and authentication of herbal and traditional medicines - the Australian experience

Hans Wohlmuth (1), David Leach (2), Ashley Dowell (2); (1) Department of Natural & Complementary Medicine, Southern Cross University, Lismore NSW, Australia; (2) Centre for Phytochemistry & Pharmacology, Southern Cross University, Lismore, Australia

Following an overview of the regulatory framework for herbal and traditional medicines in Australia, this presentation will focus on the challenges of raw materials authentication and the methodologies employed to meet these challenges.

In Australia, natural and traditional medicines are regulated by a federal agency, the Therapeutic Goods Administration (TGA), as a separate category of therapeutic goods. Authentication of raw materials is an often complex procedure involving taxonomy, morphology, histology and analytical chemistry. In Australia many herbal medicines are used for which a pharmacopoeial monograph does not exist. Such medicines can be authenticated by comparison with authentic reference material, which is linked to a voucher specimen. Southern Cross University is a TGA-accredited centre for herbal authentication in Australia, providing a service to industry and government agencies. Authentication is integrated with the Medicinal Plant Herbarium and Gardens. The authentication process will be illustrated by several case studies.

Key words: herbal authentication, quality control

W2.4

Anti-inflammatory Medicinal Plants - An Ethnopharmacological Approach

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Medicinal plants used to treat inflammation are well documented in the Chinese Pharmacopoeia but less so in traditional Australian Aboriginal medicines. Using Chinese ethnobotanical and ethnopharmacoligcal data a targetted approach to select and investigated closely related Australian plant families, genera and species was undertaken. A hit rate (>60%) was observed using a combination of in vitro anti-inflammatory assays. Bioassay-guided fractionation on three of eight plant extracts that exhibited 70-100% inhibition of cyclooxygenase-1 activity was completed. Several compounds were identified as active constituents, one from Ficus racemosa, racemosic acid (1), was novel with an IC_{50} of 109uM. The scope of anti-inflammatory assays and their applications to plant extracts as used by the Centre will be discussed.

Key words: anti-inflammatory, ethnopharmacology, racemosic acid

W2.5

In vivo methods for assessing Interactions: The Challenges for Future Research

Brian Tomlinson, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong SAR.

The use of herbal medicines is increasing worldwide so there is a considerable risk for herb-drug interactions. Some interactions may be predicted by in vitro studies with liver microsomes or cells such as the Caco-2 cell model of human intestinal transport. However, it is usually necessary to perform in vivo studies to elucidate the in vitro findings. Complete pharmacokinetic interaction studies may be required with some drugs with critical dosage requirements such as digoxin, but in many circumstances a probe-drug cocktail screening approach can be employed to provide real-time assessment of the CYP or other drug-metabolizing enzyme activities. The "Pittsburgh cocktail" was developed as a five-drug approach and the "Cooperstown cocktail" was used for simultaneous phenotyping of 6 drug-metabolizing enzymes. Furthermore, interactions may be genotype-dependant as we found with Ginkgo biloba and omeprazole (Yin OQ et al. Pharmacogenetics 2004; 14: 841-50) so it may be necessary to assess interactions in subjects with different genotypes for enzymes which show common polymorphisms. There is considerable scope to perfect such techniques to provide more comprehensive data in this developing area.

W2.6

Request for chemical & biological fingerprints: Dual-seal of botanical products quality

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It is well known that an herb contains not only one bioactive component, most bioactive components have several bioactivities, and drug interactions will occur in-or/and out-side organism. Previously, we demonstrated that (1) quercetin and probucol did not affect the β-amyloid induced neurotoxicity, however, they potentiate the protective effect of apigenin; (2) non-major ginsenosides display the most potent relaxing activity on rabbit corpus cavernosum; (3) the mechanism of anti-inflammatory, cardiac protective, antihypertensive and antiarrhythmic effects of partial purified extract of Radix Stephaniae tetrandrae; (4) Evodia rutaecarpa (E.R.) protects circulation failure and organ dysfunction in endotoxaemic rats, better than their major bioactive components, respectively. Recently, we found the lack of corelation between vascular smooth muscle relaxing effects and four major bioactive components, evodiamine, dehydroevodiamine, rutaecarpine, and synephrine of E.R. Thus, even whole or partial purified extracts of herb to be more economical and more effective than solitary isolates, but both the chemical and biological fingerprints should be considered to ensure the quality of botanical products.

W3.1

Activity-based proteomics

Benjamin F. Cravatt, The Skaggs Institute for Chemical Biology and Departments of Cell Biology and Chemistry, The Scripps Research Institute

The field of proteomics aims to characterize dynamics in protein function on a global scale. However, several classes of enzymes are regulated by posttranslational mechanisms, limiting the utility of conventional proteomics techniques for the characterization of these proteins. Our research group has initiated a program aimed at generating chemical probes that interrogate the state of enzyme active sites in whole proteomes, thereby facilitating the simultaneous activity-based profiling of many enzymes in samples of high complexity. Progress towards the generation and utilization of active site-directed chemical probes for the proteomic characterization of several enzyme classes will be described. These enzyme classes fall into two general categories: 1) enzymes for which active site-directed affinity agents have been well-defined, and 2) enzymes for which active site-directed affinity agents have been lacking. The application of activity-based protein profiling to the functional characterization of enzyme activities that vary in models of human cancer and primary tumor specimens will be highlighted, as will be the use of this strategy as a screen to discover potent and selective reversible enzyme inhibitor

W3.2

MODULATION OF PROTEIN-PROTEIN INTERACTIONS IN HCV

A. Donny Strosberg, Smitha Kota and Carlos Coito; Department of Infectology, The Scripps Research Institute-Florida; 5353 Parkside Drive, RF-2, Jupiter, Florida 33458, USA

Protein-protein interactions are increasingly recognized as important contributors to the diversity of action of proteins in cells. Interfering with these interactions in order to modify cellular mechanisms has been the goal of many studies. While generally successful when using antibodies for this purpose, initial efforts have mostly been disappointing when using small peptides or other types of small molecules. Recent work done with novel libraries of compounds increasingly suggest however the feasibility of this approach.

To better understand the functional role of interactions between Hepatitis C viral proteins we have set out to evaluate the effects of inhibitors on viral assembly, replication and infectivity. Interactions between several HCV protein domains have been identified by a variety of methods including two hybrid in yeast, co-precipitation using antibodies or other capture proteins etc … Inhibition screening assays are now being developed for four distinct pairs of interacting domains derived from several structural and non-structural HCV proteins. Peptides and small molecule inhibitors are now evaluated for their capacity to affect replication of HCV grown in hepatoma cells.

W3.3

Comparative study of the effects of Liuewei and Bawei Dihuang decoction with proteomic techniques

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Liuwei Dihuang decoction (LW) and Bawei Dihuang decoction (BW) are two classical traditional Chinese medicinal prescriptions. In this study, the effects of LW and BW on the protein profiles in senescence-accelerated mice (SAM) were studied with comparative proteomics techniques. The results showed that compared with that of SAMR1, 49 protein spots were up-regulated and 47 were down-regulated in the serum, 27 were up-regulated and 7 were down-regulated in the hippocampus of SAMP8. LW and BW were found to regulate the abnormal protein expressions of SAMP8 both in serum and hippocampus. There were commonness and differences between the proteins LW and BW affected. Some responded to both LW and BW, some only changed expressions toward LW or BW, and some others showed no responses to both of them. The results suggested that LW and BW may have common and specific reactive proteins, and the specific reactive proteins of LW or BW may be related to their differential pharmacological effects.

Key Words: Proteomics; Liuwei Dihuang decoction; Bawei Dihuang decoction; SAMP8

Acknowledgement: This work was supported by the 973 Project (2004CB518907) and the National Natural Science foundation of China (30200367)

W3.4

Design and Application of Protein Chip and Compound Array for Bioactive Substances Test and Drug Discovery

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Three different protein chips have been designed and prepared for testing bioactive substances and drug discovery. The nuclear receptor protein chip, membrane receptor protein chip and enzyme protein chip have been used for drug discovery with the compound array. Also, the protein chip based on receptor binding assays to measure bioavailable serum sex hormone levels (BSSHL). 224 aging healthy Chinese were investigated to get the referenced values of BSSHL for the first time. In the assays recombined sex hormone receptor proteins were jointed to polysaccharide coated slides to make protein chip, and the dose-dependence curve of sex hormone on chip were prepared. The data showed that this method had good precision (CV < 16%) and accuracy (Bias < 10%), and the sensitivity can reach 1 pM. The bioavailable serum androgen level of men was 52-112 pmol/l, women's was 3-70 pmol/l and the whole group was 41.9-81.4 pmol/l. The bioavailable serum estrogen level of men was 0.8-3 pmol/l, women's was 1.2-2.5 pmol/l and the whole group was 0.6-2.64 pmol/l. The milti-receptors protein chip, estrogen receptor-β and androgen receptor LBD for anabolic steroids, opioid receptor-μ for narcotic analgesics and adrenergic receptor-β2 for β-adrenergic blockers was prepared for testing the propranolol in 3%BSA sample solution with IC50 value of 0.22 nM and Ki value of 0.12 nM. In the same manner, the Ki values of estradiol, diethylstilbestrol, naloxone, estosterone propionate in samples were determined 1.46, 0.92, 1.49, 0.85 nM. It is believed that the receptor microarrays should be a rapid, economical, non-hazardous and multifunctional assay method for doping detection in the future. In order to find inhibitors of elastase, the enzyme chip and chemical arrays were combined together on glass slides. After the enzyme catalyzes reaction for two hours, the enzymatic activity by detecting color change of spots. By this method, more than 10 000 compounds have been screened and 2 active compounds have been found. Also, the receptor protein chip used for drug discovery with the compounds array. The techniques of protein chips with compound array are efficacious methods for drug discovery.

Keywords: protein Chip, Compound array, drug discovery

This work was supported by the Natural Sciences Foundation of China and National High Technique project(863).

W3.5

RNAi library for Potential Dug Target discovery

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siRNA-directed RNAi has rapidly become a powerful research maneuver in drug target discovery and validation. It also holds promise as a new therapeutic approach. In an attempt to expand the potentials of RNAi, we have recently developed a novel technology named EPRIL (Enzymatic Production of RNA Interference Library). In EPRIL, cDNAs of interest are used as a starting material from which an RNAi library, a large pool consisting of various siRNA sequences, is produced. The complexity of RNAi library is high enough to cover the entire region of target sequences. Then we can find out the most effective siRNA constructs for efficient knocking down. The selected siRNA constructs should be useful for biological experiments as well as for development of siRNA-based drugs. Furthermore, EPRIL can be applied to production of an RNAi library from a complex mixture of various cDNAs such as a cDNA library, providing a new strategy for drug target discovery based on phenotypic screening of genes. Thus EPRIL technology greatly expands the potentials of RNAi for drug development in various manners.

W4.1

IN VITRO APPROACHES FOR PREDICTION OF HUMAN DRUG CLEARANCE AND DRUG-DRUG INTERACTION

J. Brian Houston, University of Manchester, Manchester, UK

Expectations are high that in vitro kinetic studies will provide quick and reliable prediction of human in vivo drug clearance and CYP inhibition potential. Principles of scaling and modelling in vitro parameters have been validated using animal tissue and methodologies have advanced to provide a range of experimental tools. However several challenges remain before routine success can be assured for human predction, including the prevalence of CYP3A4 and interindividual variability. The success of the in vivo predictions, particularly for drug-drug interactions, has been mixed and a comprehensive scaling strategy has yet to be widely accepted. In principle, the scaling of an in vitro inhibition effect may be achieved from the inhibition constants (Ki) towards particular CYPs, provided that the concentration of the inhibitor in vivo at the enzyme site (I) and the role of the particular CYPs in the metabolic clearance (fmCYP) of the drug in question is known. For inhibitors of CYP3A4, obtaining an in vitro Ki is problematic requiring multiple binding sites and models with interaction factors to describe cooperativity creates a level of complexity that further confounds the prediction process.

W4.2

Predicting the role of transporters in drug

Yuichi Sugiyama, Department of Molecular Pharmacokinetics, Graduate School of; Pharmaceutical Sciences, The University of Toky

Drug transporters are expressed in many tissues and play key roles in drug absorption, distribution and excretion. The information on the functional characteristics of drug transporters provides important information to allow improvements in drug delivery or drug design. In this presentation, I will summarize the significant role played by drug transporters in drug disposition, focusing particularly on their potential use during the drug discovery and development process. The use of transporter function offers the possibility of delivering a drug to the target organ, avoiding distribution to other organs, controlling the elimination process, and/or improving oral bioavailability. It is useful to select a lead compound that may or may not interact with transporters, depending on whether such an interaction is desirable. The changes in pharmacokinetics due to genetic polymorphisms and drug-drug interactions might be predcted based on appropriate in vitro transport data, some examples of which will be provided in my presentation.

W4.3

Drug Binding and Metabolism by Cytochromes P450: Virtual and in vitro Prediction and Screening

Nico P.E. Vermeulen*, B. Chris Oostenbrink and Jan N.M. Commandeur; LACDR-Division of Molecular Toxicology, Department of Chemistry and Pharmacochemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam.

Firstly, a brief survey is given on the role of Cytochromes P450 (P450s) in drug disposition and (de-)toxication. Secondly, a 'structure-based' computational approach is presented to rationalize and predict drug binding and metabolism. The emphasis is on integration of 'ligand-based', 'protein-based' and 'protein-ligand interaction-based' methods as are being applied to P450s. P450 2D6, in this regard a model, is an important enzyme, for example genetically polymorphic and thus contributing to inter-individual differences in drug response and in susceptibility to toxicity. Thirdly, a new in vitro technology to screen individual components in metabolic mixtures or in libraries of compounds for affinities to Cyt P450s is presented. This so-called High-Resolution Screening (HRS)-technology, developed in co-operation with Kiadis BV (NL), is based on (automated) gradient-HPLC, connected to a new P450-bioaffinity detection system. Interestingly, a novel P450-bioreactor unit could be integrated on-line in this HRS-system. Finally, the relevance of combining in silico and in vitro technologies is stressed for the prediction of drug binding and metabolism by Cyt P450s, e.g. for drug discovery and development.

W4.4

The Increased Emphasis of ADME Properties in Hit-to-Lead Drug Discovery

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Parallel chemistry, a new approach to identify and optimize drug leads, has been successful in synthesizing large libraries of compounds for novel therapeutic targets. As part of the lead generation process, it becomes crucial for the hit to lead (HTL) molecules to have good ADME (absorption, distribution, metabolism and excretion) and PK (pharmacokinetics) properties as well as good physicochemical properties for their clinical success. Even before the optimization process begins, potential issues in ADME area need to be identified so that they can be addressed in parallel with the more traditional aspect of potency. Consequently, in silico (computational) prediction of ADME properties is required in drug design due to its ability of handling multiple chemical series, saving time and cost compared to routine laboratory work. In this presentation, several examples will be discussed to demonstrate how ADME strategies can be applied to early drug discovery to enable rapid progression of high quality hits into leads. These strategies include classical ADME tools, physicochemical properties, computational approaches and data visualization tools.

Key Words: ADME, in silico, HTL

W4.5

In vitro and in silico approaches for the prediction of drug glucuronidation parameters: Promises and pitfalls

John O. Miners, Department of Clinical Pharmacology, Flinders University and Flinders Medical Centre, Adelaide, Australia.

UDP-Glucuronosyltransferase (UGT) comprises an enzyme superfamily involved in the metabolism of drugs, environmental chemicals and endogenous compounds. Identification of the UGT(s) involved in the metabolism of a given compound ('reaction phenotyping') currently relies on multiple confirmatory approaches, which may be confounded by the dependence of UGT activity on enzyme source, incubation conditions, and the occurrence of atypical glucuronidation kinetics. While the feasibility of computational prediction of UGT substrate selectivity has been demonstrated, the development of easily interpretable and generalisable models requires further improvement in the datasets available for analysis. Quantitative prediction of the hepatic clearance of glucuronidated drugs and the magnitude of inhibitory interactions based on in vitro kinetic data also remains problematic. Intrinsic clearance values generated using human liver microsomes under-predict in vivo hepatic clearance, typically by an order of magnitude. In vivo clearances of glucuronidated drugs are also generally under-predicted by hepatocellular intrinsic clearance, but to a lesser extent than observed with the microsomal model.

W5.1

Evaluaiton of drug effects on cognitive function

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There are no clear guidelines for evaluating potential adverse drug effects on cognition. The term cognition includes notions such as learning, memory and attention. The tests employed should attempt to differentiate drug effects on each aspect to ensure that the novel substance is devoid of impairing effects thereon. A hierarchical approach is proposed whereby simpler tests are used initially to screen for potential impairment followed by more complex tests for closer identification of the functions implicated. A first test would be the one-trial passive avoidance procedure in the rat, followed by the Morris water maze and the food-reinforced radial maze tasks, whereby drug effects on short- and long-term memory can be distinguished. More complex tasks in the rat using operant conditioning techniques can, in addition, assess drug effects on attention. Operant tasks have the advantage of being transposable to primates and even to man.

W5.2

Drug abuse and dependence

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Behavioral procedures in non-humans are used widely to assess new compounds because of their validity in predicting abuse and dependence in humans. Two primary objectives of these preclinical studies are to determine: 1) whether a drug has positive reinforcing effects that could promote or maintain drug seeking and drug taking; and 2) whether repeated administration of a drug leads to the development of physical dependence that might also contribute to drug seeking and drug taking. One hallmark of these studies is a direct comparison of new compounds to reference substances that are known to be abused. Preclinical assays that are used most often to assess abuse liability include drug discrimination, self administration and conditioned place preference. Basic methodologies for evaluating physical dependence potential will be discussed along with the weaknesses and strengths of each approach with regard to their value in predicting abuse. Examples of how each of these procedures can contribute to an overall abuse liability profile will be discussed and critiqued.

Key words: abuse, dependence, animal model, withdrawal

Acknowledgement: USPHS Grants DA05018, DA09157, DA14684, DA17918;

W5.3

ICHS7A requirements for core battery studies on nervous system function: a critical view

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For assessing effects on nervous system function in vivo, the ICHS7A safety pharmacology guidelines specify that "a functional observational battery (FOB), modified Irwin's, or other appropriate test can be used". The Irwin test was originally developed as a screen for psychotropic activity in mice, whereas the FOB originated from the chemical industry for neurotoxicity evaluation in rats. These are both 'first-tier' tests, in that any effects detected may be investigated further in more specific studies. However, they may constitute the only evaluation undertaken of effects on nervous system function, particularly for non-CNS targeted compounds, and therefore need to be robust. Whereas both tests evaluate a wide range of nervous system functions, some functions (e.g. special senses, cognition, anxiety) are not addressed. Individual companies have to decide on a case-by-case basis whether to plug this gap with additional studies, after considering the known pharmacology and pharmacokinetics of the compound. The costs of getting it wrong are potentially high: up to 10% of all drug withdrawals from the market are due to neurological side effects.