S13.1

Tocotrienol delays onset and progression of galactose-induced cataract in rat

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Aim: Tocotrienol (T3) is known to have potent antioxidant properties. Since oxidative stress plays a major role in the cataract formation, we hypothesized that T3 delays cataract development. We aimed to investigate effect of T3 eyedrop in delaying onset and progression of galactose-induced cataract. **Methods:** 3 weeks-old Sprague-Dawley rats were divided into 8 groups. Group 1 received normal diet while rest of the groups received 25% galactose diet. Groups 3-8 received one of 6 different doses of microemulsion of T3 ranges from 0.2-0.01% twice daily. Group 2 was similarly treated with vehicle. Pre-treatment was given for 3 weeks and was continued for 4 weeks after starting the galactose diet. Slit lamp examination was done biweekly to assess cataract progression. Cataractous changes were graded from 0-4 according to progression of cortical vacuole formation to nuclear opacity. **Results:** At week 1 of galactose diet, groups 3 and 4 had higher percentage of lenses that progressed to stage 1B compared to group 2 in which progression was less severe, whereas in groups 6 and 7, 10% and 25% lenses respectively remained in stage 0. Groups 3 and 4 continued to show more advanced cataract progression compared to group 2 in the following weeks until end of experimental period. However, cataract progression was delayed in groups 6-8 during these subsequent weeks. **Conclusion:** T3 delayed cataract progression at low doses but enhanced cataract progression at higher doses.

Keywords: tocotrienol; cataract; galactose-induced; rats

Acknowledgements: American River Nutrition.

S13.2

Calcium and magnesium level in cataractous lenses of galactosemic rats topically treated with liposomal formulation of magnesium taurate

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Background: Magnesium (Mg) is critically important in maintaining mineral homeostasis of lens. Imbalances in Mg concentration can occur with intra- and extracellular calcium, sodium and potassium concentration modifications. An altered lens Ca/Mg ratio plays a critical role in triggering the development of cataract. **Aim:** This study aimed to measure Ca^{2+} and Mg^{2+} level in cataractous lenses of galactosemic rats topically treated with magnesium taurate (MgT) liposomal formulation for 28 d. **Methods:** Sprague-Dawley rats, weighing 80–100 g, were divided into **3** groups: normal diet group (ND), 25% galactose diet group (GD) and 25% galactose diet with topical liposomal MgT group (GD-MgT). Cataract progression was evaluated weekly by slit lamp. Animals were sacrificed after 28 d. To measure Ca^{2+} and Mg^{2+} level, lenses were homogenized and centrifuged. Supernatant was separated and was processed according to the Cobas Integra manufacturer's protocol for Ca^{2+} and Mg^{2+} estimation in serum. **Results and Discussion:** The progression of cataract was significantly delayed in GD-MgT group compared to GD. Lens Ca/Mg ratio for ND group was 0.76±0.20 whereas for GD, it was 1.17±0.12. In GD-MgT, the lens Ca/Mg ratio was insignificantly higher than in ND group and lower than in GD group. Results suggested that topical liposomal MgT causes increases lens Mg, thereby tends to delay the onset and progression of galactose-induced cataract in rats. The low intracellular Ca concentration is maintained by calcium-activated, Mg-dependent Ca^{2+}-ATPase. Functions of Mg dependent Na^{+},K^{+}-ATPase are also important for lens ionic balance. Since both Mg-dependent ATPases are important, treatment with MgT may play a role in preventing cataractogenesis by correcting the lens Ca/Mg ratio.

S13.3

Protective effect and mechanism of *L*-carnitine on the rat retinal Müller cells in high-glucose

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Aim: To investigate the protective effect of *L*-carnitine (LC) on cell viability, reactive oxygen species (ROS), mitochondrial membrane potentials (MMP) of cultured rats Müller cells induced by high glucose *in vitro* and the possible mechanism. **Methods:** Müller cells were separated and purified from the retina of 1–3 d SD rats. The cells were identified by immunofluorescence by using glutamine synthetase (GS), glial fibrillary acidic protein (GFAP). Trypan blue staining was used to observe the effect of different concentrations of glucose on the growth and survival status of Müller cells. The Müller cells were randomly divided into control group, high glucose group and the LC-treated group with different concertrations. The cells in LC-treated group were cultured in high glucose medium with 50, 100, and 200 μmol/L LC. Apoptosis rate was measured by Hoechst33342. ROS and MMP were evaluated by flow cytometry. **Results:** Immunofluorescence showed that most cells were positive for anti-GS, anti-GFAP antibody responses, and the purity of Müller cells was above 90%. Cell viability decreased obviously in high glucose (P<0.05), and increased after intervention by LC, the best effect was at 100 μmol/L. ROS level in high glucose group was higher than control group obviously, and decreased after intervention by LC. ROS level in high glucose group was higher than control group obviously (P<0.05), and decreased after intervention by LC (P<0.05). MMP level in high glucose group was lower than control group obviously (P<0.05), and increased after intervention by LC (P<0.05). **Conclusion:** LC could inhibit the apoptosis of Müller cells injuried by high glucose *in vitro*. The decrease in ROS level and increase in MMP level may be one of the mechanisms of its protection.

Keywords: *L*-carnitine; retinal Müller cell; ROS; MMP

S13.4

Endoplasmic reticulum stress in oxidative stress-associated neurogenic hypertension

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Aim: Perturbations of proper functions of the endoplasmic reticulum (ER) cause accumulation of misfolded or unfolded proteins in the cell, creating ER stress. Prolonged ER stress has been implicated in hypertension. Oxidative stress in the rostral ventrolateral medulla (RVLM), where sympathetic premotor neurons reside, plays a pivotal role in neurogenic hypertension. This study aimed to evaluate the contribution of ER stress in RVLM to oxidative stress-associated hypertension. **Results:** Expression of glucose-regulated protein 78 kDa and phosphorylation of protein kinase RNA-like ER kinase-translation initiation factor α, two major protein markers of ER stress, were augmented in RVLM and preceded the development of hypertensive phenotype in spontaneously hypertensive rats (SHR). In RVLM of SHR, stabilizing ER stress by salubrinal promoted antihypertension. Autophagy, as reflected by the expression of lysosome-associated membrane protein-2 and microtubule-associated protein 1 light chain 3-II, was increased in RVLM of SHR, and was abrogated by salubrinal. Inhibition of autophagy in RVLM resulted in antihypertension in SHR. **Conclusion:** These results suggest that redox-sensitive induction of ER stress and activation of autophagy in RVLM contribute to oxidative stress-associated neurogenic hypertension.

Keywords: endoplasmic reticulum stress; autophagy; oxidative stress; neurogenic hypertension

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S13.5

Regulation on gene expression by dietary polyphenols in chronic degenerative diseases and their deleterious effects on human health

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Aim: To summarize the chemical structure and structure-activity relationship, the potential therapeutic effects of dietary polyphenols on chronic degenerative diseases and the potential dangers of individual dietary polyphenols on human health. **Methods:** Significant articles were identified by literature search and selected based on the protective effects of dietary polyphenols on chronic degenerative diseases. **Results:** Accumulating evidence indicates that dietary polyphenols have shown protective effects for chronic degenerative diseases such as cardiovascular diseases, cancers, neurodegenerative diseases and diabetes by regulating genes expression. However, not all kinds of dietary polyphenols are beneficial for human

health. The potential deleterious of several dietary polyphenols have been reported by inducing DNA damage and gene mutants. **Conclusion:** Recent investigations of the pharmacological actions of dietary polyphenols have significantly increased our understanding of their protective function in human various diseases. With further investigation of the structure-activity relationship of polyphenol molecules and the potential dangers to human health, dietary polyphenols could be presented.

Keywords: dietary polyphenols; structure-activity relationship; chronic degenerative diseases; therapeutic effects; potential dangers

S13.6

Preparation of lung-targeted OMT-PLA-MS and its protective effects on lung injury of mice induced by bleomycin

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Aim: To prepare lung targeted oxymatrine-loaded microspheres, the biodegradeable materials, polylactic acid, was used in the study. **Methods:** The optimized preparation conditions of oxymatrine polylactic acid microspheres (OMT-PLA-MS) were acquired through orthogonal test. The surface morphology of the microspheres was observed by scanning electron microscope (SEM). The mean diameter of microspheres, drug release *in vitro*, and tissue distribution after intravenous administration were examined. And the therapeutic effect of the OMT-PLA-MS on pulmonary injury was evaluated in mice induced by bleomycin. **Results:** OMT-PLA-MS microspheres were round and regular in their morphology. The average particle size was 10.9μm. The accumulative release percentage was 86.5% in 12 h. Compared with oxymatrine injective solution, the Emo-PLA-MS were more concentrated in lung tissue. Furthermore, Emo-PLA-MS may improve the pathological changes induced by bleomycin. **Conclusion:** The Emo-PLA-MS show significant sustained release and lung targeting. It also may attenuate the bleomycin-induced pulmonary injury.

Keywords: oxymatrine; lung targeting; lung injury; microspheres

S13.7

Improved anti-nociceptive activities by liposomes-encapsulated diclofenac

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Aim: Liposomes, a promising lipid-based nanoparticulate drug delivery system, was used in present work to enhance the anti-nociceptive activities of a popular non-steroidal anti-inflammatory drug, diclofenac. **Methods:** The liposomal samples were prepared using a simple proliposome technique. Different animal models of nociception was performed to compare the efficacy of free-form and liposomes-encapsulated diclofenac. **Results:** Acetic acid-induced abdominal writhing test showed that liposomes significantly increased the anti-nociceptive effect of diclofenac by as much as 23.08%. In contrast to free-form diclofenac, the liposomes-encapsulated diclofenac significantly inhibited both early and late phases in formalin-induced paw licking test, with percentages of inhibition up to 31.81% and 78.84% in respective phase. A lower dose of liposomes-encapsulated diclofenac was also shown to exhibit significant pain-inhibition effect in the carrageenan-induced mechanical hyperalgesia test. **Conclusion:** In conclusion, liposomes-encapsulated diclofenac produced better anti-nociceptive effects than free-form diclofenac.

Keywords: liposomes; diclofenac; anti-nociception

S13.8

Identification of key genes related to hypoxia preconditioning by gene-chip and gene network analysis

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Aim: To identify the key genes related to hypoxic preconditioning by gene-chip and gene network analysis. **Methods:** The acute and repetitive hypoxic model in mice and microarray analysis are used to identify the differentially expressed genes that are likely to be critical for hypoxic preconditioning. Multiple bioinformatic analysis was conducted to identify the key hub genes, pathways and biological processes. **Results:** 1175 differentially expressed genes in hypoxic preconditioning mice were identified. There were 113 significant function genes up-regulated and 138 significant function genes down-regulated. These differentially genes function in multiple chemical biological processes of cellular processes mainly including signal transduction, synaptic transmission, brain development, neuron differentiation, regulation of cell proliferation and axonogenesis. A subgroup of 21 core genes that participate in hypoxic preconditioning, regulation of transcription and nucleosome assembly was identified by the Dynamic Gene Network analysis. Several critical hub genes that are the key members of significant pathways or gene networks were also identified by our comprehensive analysis. **Conclusion:** Comparisons to other gene array datasets indicate that the global gene expression profiles of hypoxic preconditioning show significant differences between the hypoxic and normal mice, suggesting that the electrotransferation of the key compounds can be considered as a critical chemical biological process in hypoxic preconditioning.

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S13.9

Wound healing activity of stem cell conditioned media in burn wounds in male Wistar rats

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Aim: Burn injury is a major cause of death & disability worldwide. It causes massive defects in the skin. So treating burn wounds has been a continuous challenge for medical practitioners. This study investigated the effects of stem cell conditioned media in healing burn wounds. **Methods:** Three groups-control, standard and test group comprising of six animals each were used for the study. Partial thickness wound was created on the shaven back of the fasting animals. Control group was left untreated. Standard group was treated once daily with silver sulfadiazine ointment and the test group was treated twice daily with stem cell conditioned media. The study was conducted for 21 d. Period of epithelialization was measured for all the three groups. Wound contraction rate was specifically measured on 4th, 8th, 12th, and 16th d of the study. **Results:** Data was analysed by one way ANOVA using SPSS version16. The result of the present study showed that stem cell conditioned media significantly increased wound contraction rate as compared to control group ($P<0.05$). Period of epithelialization in the test group had also decreased as compared to the control group. **Conclusion:** Based on the results it is concluded that, stem cell conditioned media has a role in the process of wound healing. However, further research in this area will help understand the role of stem cell conditioned media in the process of wound healing in a better way.

Keywords: burn wound; healing process; stem cell conditioned media

S13.10

Predictions of BuchE inhibitors using support vector machine (SVM) and naive Bayesian classification techniques

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Aim: Butyrylcholinesterases (BuChE, EC 3.1.1.8) is emerging as an important pharmacological target in Alzheimer's disease (AD) therapy. Due to high cost and laboriousness in experimental tests, it is necessary to develop robust *in silico* methods to predict the activity of BuChE inhibitors. In order to distinguish BuChE inhibitors (BuChEIs) and non-inhibitors, several classification models (SVM models and naive Bayesian models) were built. **Methods:** Each molecule was initially represented by 1235 descriptors from ADRIANA.Code program, 334 descriptors from MOE software and 301 two-dimensional descriptors from Discovery studio. Correlation analysis and stepwise variable selection method were used to find the best reduced set of descriptors for each model. Additionally, LibSVM and Discovery studio were applied to build the SVM models and Bayesian models, respectively. At the same time, fingerprint descriptor was added to improve the predictive ability of models. Performances were measured by cross validation, training set validation including 3200 compounds with 800 BuChEIs and 2400 non-inhibitors, test set validation including 1001 compounds and an external test

set containing 317 diverse chemicals. In order to guard against the possibility of chance correlation, Y-scrambling was also performed. Finally, the best classification models as ligand-based virtual screening tools were used for screening an in-house database. **Results and discussion:** The best two models gave Matthews Correlation Coefficient (MCC) of 0.9551 and 0.955 for the test set, 0.9132 and 0.9221 for the external test set respectively. To demonstrate the practical applicability of the models in virtual screening, we screened an in-house dataset of 3,608 compounds, and finally 35 compound were selected for BuChE inhibition assay. The assay results showed that 17 compounds displayed good BuChE binding affinities ranging from 0.73 to 22.22 μmol/L. To the best of our knowledge, this is the first report for machine learning approaches validated with a successful virtual screening application in identifying cholinesterase (ChE) inhibitors. The study proved that these methods are useful approaches to predict bioactivities or properties of ligands and to find novel lead compounds for drug discovery.

Keywords: butyrylcholinesterases; Alzheimer's disease; SVM; Bayesian; virtual screening.

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S13.11

Identification of a novel spinal dorsal horn astroglial D-amino acid oxidase-hydrogen peroxide pathway involved in morphine analgesic tolerance

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Aim: This study investigates the potential role of the spinal astroglial D-amino acid oxidase (DAAO)-hydrogen peroxide pathway in the development of morphine analgesic tolerance. DAAO is a FAD-dependent peroxisomal flavoenzyme and catalyzes the oxidation of D-amino acids to hydrogen peroxide. **Methods and Results:** Pharmacologic blockade of DAAO by inhibitors CBIO, AS057278, and sodium benzoate dose-dependently and completely prevented and reversed morphine analgesic tolerance in the formalin test, and the hot-plate and tail-flick tests, with a positive correlation to their DAAO inhibitory activities. MK-801 prevented morphine tolerance but not reversion of established tolerance. Genetic ablation by siRNA/DAAO and shRNA/DAAO almost fully prevented morphine tolerance. CBIO and siRNA/DAAO completely prevented increased spinal hydrogen peroxide levels in morphine tolerant rats. The hydrogen peroxide scavenger PBN and catalyst catalase also abolished established morphine tolerance. Double immunohistochemical staining revealed that DAAO specifically expressed in astrocytes in the spinal dorsal horn was significantly upregulated accompanying astrocyte hypertrophy following morphine tolerance. **Conclusion:** Our results identify a novel spinal astroglial DAAO-hydrogen peroxide pathway that is critically involved in the initiation and maintenance of morphine analgesic tolerance, and suggest that this pathway is of potential utility for the management of morphine tolerance and chronic pain.

Keywords: D-amino acid oxidase; morphine analgesic tolerance; hydrogen peroxide; spinal astrocytes

S13.12

Brief discuss on experimental guinea pigs feeding and management

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Aim: To reduce stress, reduce the morbidity and mortality of guinea pigs, this article discusses the optimization of the various methods of feeding and management. **Methods:** Through literature review, combined with author's experience, we summarized the influencing factors such as rearing methods, temperature, humidity, vitamin C, crude fiber on feeding and management of guinea pigs. **Results:** (1) Plastic box (930 mm×615 mm× 245 mm) with padding material, which can hold eight to ten guinea pigs, meet the need for hi-activities and gregarious, keep good insulation. It is more conducive to recover the stress state to normal conditions. (2) Guinea pig is sensitive to temperature, humidity and air due to its poor thermoregulation. Therefore, in our GLP center, we control the rearing conditions as follows: temperature between 19 to 28 °C, relative humidity of 40% to 70%, the maximum daily temperature ≤4 °C, minimum ventilation 8 times/h or more, the noise less than 60dB, the ammonia concentration below 14 mg/m^3, padding material was replaced every two days. (3) Guinea pigs are characterized by developed chewing muscles and cecum, thin stomach wall, crude fiber digestion ability, lack of vitamin C synthase. Therefore, grass or hay with high crude fiber content or 10%–15% crude fiber content should be fed daily; drinking water with vitamin C dissolved should be freshly prepared. **Conclusion:** Large plastic box keepers, an appropriate temperature, humidity, air, an appropriate vitamin C and crude fiber feeding, are essential to the health of guinea pigs.

Keywords: guinea pigs; feeding; management

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S13.13

Cannabinoid receptor 2 protects against acute experimental sepsis in mice

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Aim: The effects of cannabinoid receptor 2 (CB2R) on the sepsis still remains undefined. Our study is to explore the role and mechanism of CB2R in acute sepsis model of mice. **Methods:** Survival rate was observed to evaluate the role of CB2R in LPS-induced acute sepsis in mice. ELISA was used to detect protein level of mice serum or cell supernatants. Cell Count Kit-8 (CCK-8) was used to measure cell proliferation. Western blot was used to detect protein expression and immune-fluorescence was used to track the translocation of NF-κB p65 protein. **Results:** Mice were more vulnerable for LPS-induced death and inflammation after CB2R deletion. CB2R agonist, GW405833, could significantly extend the survival rate and decrease serum pro-inflammatory cytokines in LPS-treated mice. GW405833 dose-dependently inhibit pro-inflammatory cytokines release in splenocytes and peritoneal macrophages as well as splenocytes proliferation, and these effects were partly abolished in $CB2R^{-/-}$ splenocytes but completely abolished in $CB2R^{-/-}$ peritoneal macrophages. Further studies found GW405833 inhibits LPS-induced phosphorylation of ERK1/2 and STAT3, and blocks IκBα degradation and NF-κB p65 nuclear translocation in macrophages. **Conclusion:** CB2R provides a protection and is a potential therapeutic target for the sepsis.

Keywords: cannabinoid receptor 2; sepsis; pro-inflammtory factor; proliferation; macrophage; T cells

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S13.14

Strategy and methods for assessment of drug-induced mitochondrial toxicity

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Mitochondria are the main sites of energy metabolism as well as one of the main sources of cellular reactive oxygen species (ROS). Numerous studies have implicated that mitochondria are important targets for drug-induced toxicity. In fact, mitochondrial toxicity is one of the most important limiting factors leading to drug development failure or restricted clinical usage. Here we summarize the strategy and methods for assessment of drug-induced mitochondrial toxicity. According to the characteristic and possible mechanism of drug-induced mitochondrial toxicity in targeted organs, the assessment of drug-induced mitochondrial toxicity includes: first, high throughput screening of mitochondrial toxicity in the early phase of drug discovery, particularly for targeted screening-based on multiple mechanisms of mitochondrial injury, which may involve the application of many special *in vitro* models of cell culture, high content screening, and computational modeling. Secondly, animal model-based mitochondrial toxicity studies in drug discovery or preclinical study. Third, predictive study of drug-induced mitochondrial toxicity in human including human originated cell/tissue-based studies and related biological/computational/extrapolative modeling. Recently, by application of these strategies and many modern advanced technologies like high content screening, gene silencing and overexpression, we have investigated the mitochondrial toxicity of doxorubicin, acetaminophen in

human cardiomyocytes and hepatocytes, focusing on intervention of two critical toxicity pathways, peroxisome proliferator receptor γ coactivator (PGC 1a) and the nuclear factor-E2-related factor 2 (Nrf 2) respectively for regulating mitochondrial biogenesis and oxidative stress. Moreover, many transgenic mouse models have been used for assessing *in vivo* mitochondrial toxicity. Based on these studies, we have established many sensitive, rapid, and economic methods for assessments of drug induced mitochondrial toxicity in drug discovery and preclinical study. Selection of appropriate methods for assessing mitochondrial toxicity is particularly important for discovering the potential risk of mitochondrial toxicity as early as possible, which is of great significance for a successful drug discovery and development.

Keywords: mitochondrial toxicity; drug discovery; preclinical evaluation; target; drug screening

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S13.15

Critical amino acids in the transmembrane 6 of human organic anion transporting polypeptide 1B1

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Aim: Organic anion transporting polypeptides (OATPs) are a group of membrane transporting proteins that mediate the transport of a wide spectrum of structurally independent compounds. Because of their broad substrate specificity, wide tissue distribution and the involvement of drug-drug interaction, OATPs have been extensively recognized as key determinants of absorption, distribution, metabolism and excretion (ADME) of various drugs, xenobiotics and toxins. As an important structure in membrane proteins, transmembrane domains (TM) have been found to be crucial for properly targeting the protein to cell membrane as well as carrying out transport functions in transporters. Putative TM6 of OATP1B1 contains part of the OATP family signature and is a relatively conserved TM in OATPs, the role of this TM in proper transport function of OATPs, however, remains unclear. **Methods:** In the present study, site-direct mutagenesis was used to individually mutate the amino acids on putative TM6 of human OATP1B1 into alanine and uptake activity of OATP prototypic substrate estrone-3-sulfate was measured. **Results:** Some mutants were found to partially or totally lost transport functions while others retained compatible activity compared with the wild-type. Two mutants, W258A and W259A seemed to cause the most significant effect. Further study demonstrated that these two tryptophan residues play different roles in substrate transport of OATP1B1. **Conclusion:** Two amino acids, *ie*, Trp258 and Trp259, were identified as critical amino acids within TM6 of OATP1B1.

Keywords: OATP1B1; transport function; transmember domains

S13.16

A validation safety pharmacology study of the central nervous system, employing a functional observational battery (FOB) in female Cynomolgus monkey

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Aim: The objective of this study was to establish and validate a functional observational battery (FOB) at the Center for Drug Safety Evaluation & Research (CDSER) that provides a basic neurological and behavioral assessment in Cynomolgus Monkey. **Methods:** Four female cynomolgus monkeys were assigned to this study and were administered the vehicle and substances (chlorpromazine, (+)-MK-801 or caffeine) in 4 separated days (a 7-d wash-out period between each drug treatment). On each dosing day, 4 animals were staggered for dosing. A FOB was performed and recorded by two independent observers for each animal at designated time points. Discordances between two observers were evaluated and actions were taken to minimize the subjective biases. **Results:** The administration of 5 mg/kg chlorpromazine resulted in sedative effect between 0.5 and 3 h post dose and decreased body temperature at 1 h post dose. The administration of (+)-MK-801 (0.1, 0.5, 1.25 and 2.5 mg/kg) resulted in suppressed behavior (low arousal, infrequent vocalization, hypoactive, etc), autonomic responses (salivation, decreased respiration and rectal temperature, etc) and sensorimotor/ neuromuscular alternations (unsteady, un-coordinated, lack of auditory response, tremor and convulsion, *etc*) at all doses, and monkeys becoming unconscious and/or pupil dilation at high dose levels (1.25 and 2.5 mg/kg). The observations were mainly recorded between 0.5 and 3 h post dose. Caffeine resulted in stimulant behavioral changes (high arousal, hyperactivity and/or intermittent circling behavior) in one monkey at a dose of 30 mg/kg (between 0.5 and 4.5 h post dose) or one of two monkeys at a dose of 45 mg/kg (between 0.5 and 1 h post dose). At higher doses (one monkey dosed at 45 mg/kg and one at 60 mg/kg), caffeine resulted in decreased arousal and hypoactive between 0.5 and 3 h post dose. Autonomic alteration caused by caffeine was evidenced by vomiting/retching seen in the 45 mg/kg monkey at 0.5 h post dose and in the 60 mg/kg monkey between 0.5 and 3 h post dose. **Conclusion:** The observations recorded in this study were consistent with expected pharmacological effects of the neurosedative or neurostimulant reference substances under the conditions of the study. A FOB in cynomolgus monkeys has been successfully established at CDSER. This standard FOB can now be conducted in safety pharmacology and/or toxicology studies to support regulatory submission.

Keywords: FOB; cynomolgus; autonomic alteration; safety pharmacology

S13.17

Hydrogen sulfide releasing aspirin, ACS14, attenuates methylglyoxal and high glucose-induced oxidative stress

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Aim: ACS14 is a novel aspirin analogue which releases hydrogen sulfide (H_2S). Methylglyoxal (MG) is a reactive byproduct of glucose metabolism. High MG levels and oxidative stress are associated with diabetes and some other diseases. Our aim of this study is to investigate the effect of ACS14 on cellular MG levels and reactive oxygen species (ROS) production in cultured vascular smooth muscle (A10) cells in a comparison with aspirin and a H_2S donor, NaHS. **Methods:** A10 cells were incubated for 24 h with high glucose (25 mmol/L) or MG (30 μmol/L) with or without ACS14 (100 μmol/L), aspirin (100 μmol/L) or NaHS (90 μmol/L). Cellular MG levels were measured by a specific and sensitive HPLC method. Peroxynitrite was determined by dicholorofluorescin (DCFH) assay. Nitrite plus nitrate was assayed with a fluorimetric assay kit. NADPH oxidase 4 (NOX4) was determined by Western blotting. **Results:** ACS14 and NaHS, but not aspirin significantly decreased MG levels in cells treated with MG or high glucose. ACS14 and NaHS significantly reduced peroxynitrite and nitrite plus nitrate formation induced by MG or high glucose. Co-treatment with ACS14 attenuated MG- induced NOX4 up-regulation. **Conclusion:** ACS14 shows an ability to scavenge cellular MG and reduce oxidative stress, which is significantly greater than that of aspirin. Due to the beneficial effects of H_2S on vascular relaxation, ACS14 can be useful in cardiovascular complications of diabetes thanks to its potential ability to reduce high glucose-induced MG and oxidative stress.

Keywords: aspirin; hydrogen sulfide; methylglyoxal; glucose; oxidative stress

S13.18

Peroxisome: a new player in redox homeostasis in the kidney

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Oxidative stress defined as an excessive production of reactive oxygen species (ROS) surpassing existing antioxidative defense mechanisms plays a critical role in decline of renal function. Overproduction of ROS in the kidney is both a direct consequence of metabolic stressor (high plasma glucose and free fatty acid) and an indirect consequence through mediators such as cytokines and growth factors. Mammalian peroxisomes are redox organelles possessing a comprehensive enzymatic apparatus of more than 50 enzymes. Peroxisomal fatty acid β-oxidation generates hydrogen peroxides which are effectively decomposed by catalase in peroxisome. We have recently reported that fatty acid-induced peroxisomal dysfunction exacerbates diabetic renal injury and that endogenous catalase plays an important role in maintaining peroxisomal and mitochondrial fitness. Autophagic degradation of peroxisomes, pexophagy, appears to be essential in functional maintenance of peroxisome during endotoxic injury as well as metabolic stress. Further studies elucidating the pharmacological and molecular methods to restore peroxisomal fitness will provide new insight into the therapeutic strategy against renal injury.

Keywords: autophagy; catalase; diabetic nephropathy; peroxisome; redox homeostasis

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S13.19

Effect of magnesium salts on oxidative protein modification in rats fed low magnesium diet

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Aim: The study was to compare level of protein carbonyls (PC) as biomarker of oxidative stress in hypomagnesiemic rats before and after treatment with different magnesium (Mg) salts. **Methods:** Experiments were carried out on 60 Wistar male rats weighing 300–330 g. Initially rats were divided into 2 groups. The first group received low Mg diet (LMD) (Mg content ≤15 mg/kg) and demineralized water during 2 months. The other group was fed a basal control diet (Mg content≈500 mg/kg) and water (Mg content 20 mg/L) for equal duration. Two month of LMD led to decrease of Mg concentration in plasma and red blood cells by 45% and 50% compared with group fed with control diet. Afterwards Mg sulfate, Mg chloride, Mg L-aspartate, Mg oxybutirate and Mg *N*-acetyltaurate (50 mg elementary Mg per kg of body weight) were administered to part of LMD rats per tube during 2 weeks. Mg level were assayed utilizing the method based on staining reaction of Mg and thiazole yellow. Level of PC was measured using reaction with 2,4-dinitrophenylhydrazine. **Results and Conclusion:** Hypomagnesemia led to 1.4 fold rise of PC content compared to control group (P<0.05). During 2 weeks of treatment, Mg sulfate, Mg chloride, Mg oxybutirate and Mg N-acetyltaurate replenished Mg level in plasma and red blood cells to normal level, but did not change PC significantly as compared with LMD group. Mg L-aspartate the only magnesium salt that reversed PC level to control values (4.87±0.58 μmol·L^{-1}·g^{-1} protein *vs* 3.82±0.10 μmol·L^{-1}·g^{-1} protein in control group, P>0.05).

Keywords: magnesium deficiency; carbonyl products of protein oxidation; magnesium chloride; magnesium sulfate; magnesium chloride; magnesium L-aspartate; magnesium oxybutirate and magnesium *N*-acetyltaurate

S13.20

Comparison of 24-h ocular hypotensive effect of single drop application of enalaprilat and losartan in steroid-induced ocular hypertensive rat model

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Aim: Angiotensin converting enzyme inhibitors and angiotensin II receptor blockers are currently attractive as potential drugs to reduce elevated intraocular pressure (IOP). The purpose of the present study was to compare 24-h ocular hypotensive effect of single drop application of enalaprilat and losartan in steroid-induced ocular hypertensive rat model. **Methods:** Ocular hypertension was developed in normotensive Sprague Dawley rats by topical instillation of dexamethasone 0.1% 2 times daily for 36 days. The rats that developed more than 25% rise in IOP were divided in 2 groups and were unilaterally treated with single drop (10 μL) of either 1.00% enalaprilat dehydrate (n=7) or 2.00% losartan potassium (n=7) in test eyes (TE). The contralateral eyes served as control (CE). IOP measurements were done by applanation tonometry at 0 h (baseline), hourly for the first 12 h post-instillation and subsequently 4 hourly till the 24 h. **Results:** Both groups showed a significant IOP reduction in the first 11 h post-treatment. In the enalaprilat-treated group, the maximum IOP reduction in the TE was 34.9% from baseline. The maximum mean IOP reduction in the TE for the losartan group was 36.7% from baseline. **Conclusion:** Single drop application of enalaprilat and losartan in steroid-induced ocular hypertensive rat model caused significant IOP reduction in first 11 h post-treatment. Comparison of maximum IOP reduction among the groups did not show significant difference.

Keywords: enalaprilat; losartan; intraocular pressure; steroid-induced ocular hypertension

S13.21

***In vitro* anti-influenza virus activity of stilbene derivatives**

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Aim: Neuraminidase (NA) inhibitors (NAIs) are currently the only antivirals effective against influenza infections due to widespread resistance to M2 inhibitors. This study is to evaluate *in vitro* anti-influenza viral activities of stilbene derivatives to investigate the prevention and treatment effects for influenza infections. **Methods:** Neuraminidase activity assay was used to examine NA inhibitory activity of 18 stilbene derivatives through fluorometric assay, and influenza virus induced cytopathic effect (CPE) inhibition assay was used to verify their anti-influenza viral activities *in vitro*. **Results:** The assay results showed that all stilbene derivatives displayed relatively high NA inhibitory activities. IC_{50} of compounds J35885, J35888, J35889, and J35896 are less than 2 nmol, while Oseltamivir was 0.8–2 nmol as reported. Also J35887 with diphenyl azo scaffold exhibited obvious *in vitro* anti-viral activity in CPE experiment with IC_{50} value of 7.94 μg/mL and Ribavirin was 11.35 μg/mL as control. **Conclusion:** It can be concluded that most stilbene derivatives possessed NA inhibitory activity, but among them, one with novel scafold displayed significant *in vitro* anti-influenza viral activities, and exhibited better activity than Ribavirin. These results will provide important information for drug design and drug development.

Keywords: influenza virus; neuraminidase (NA); cytopathic effect (CPE); stilbene derivatives; *in vitro* antiviral activity

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S13.22

3-Hydroxyphthalic anhydride-modified human serum albumin as a microbicide candidate for preventing sexually transmitted diseases

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Aim: Female-controlled topical microbicides for prevention of sexually transmitted diseases (STDs) are a diverse group of active ingredients that are capable of affecting microorganisms' growth in various stages, including human immunodeficiency virus (HIV) and herpes simplex virus-2 (HSV-2). We recently demonstrated that 3-hydroxyphthalic anhydride modified-HSA (HP-HSA) is a HIV entry inhibitor with strong anti-HIV-1 activities. Here we further investigated the efficacy of HP-HSA on HSV-2, HIV-2 and SIV infection *in vitro* and the safety and stability of HP-HSA as a microbicide. **Methods:** The *in vitro* anti-HSV-2 activities of HP-HSA were detected by measuring MTT assay. The anti-HIV-2 and SIV infection were tested by luciferase activity. The cytotoxicities of HP-HSA on target cells were evaluated by MTT assay. The effects of HP-HSA on susceptibility of vaginal lactobacillus strains were evaluated in MRS broth in an anaerobic chamber. The diversities of anti-HIV-1 activities of HP-HSA in the presence of seminal plasma (SP) and vaginal fluid simulant (VFS) were assessed by p24 measurement. **Results:** We found that HP-HSA exhibited potent antiviral activity on infection by HSV-2, HIV-2 and SIV strains. It displayed no or low cytotoxicity on the cells used for testing viral infectivity. HP-HSA had not produced significant inhibitory effect on 17 strains of vaginal lactobacilli (MIC>15.000 μmol/L). The MICs of positive control ampicillin on different strains were from 0.841 to 6.730 μmol/L. The anti-HIV-1 activities of HP-HSA had no significant diversification in presence or absence of SP and VFS. **Conclusion:** Because of its broad and potent antiviral activity and the safety and the stability, HP-HSA has exhibited the potential required for further development as a microbicide to prevent the sexual transmission.

Keywords: 3-hydroxyphthalic anhydride human serum albumin; microbicide; HIV-1 sexual transmission; herpes simplex virus-2

S13.23

XLHX-124-50 as a potent HIV-1 entry inhibitor targeting on HIV-1 gp41 envelope

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Aim: Our group has screened hundreds of small molecular compounds for detecting the inhibition on HIV-1 entry. We have identified several effective compounds, such as Theaflavin and ADS-J1. Here, we tested a new small-molecule compound, XLHX-124-50, and investigated its potential activity as a HIV-1 entry inhibitor. **Methods:** The *in vitro* anti-HIV-1 activities of XLHX-124-50 were determined by pseudotyped virus, cell-cell fusion and HIV-1 lab-adapted virus

assay. Flow cytometric analysis, cell-based ELISA, a sandwich ELISA, N-PAGE and CD spectroscopy were used to confirm whether XLHX-124-50 targeted on 6-Helix Bindle (6-HB) formation. The key residue of gp41 was detected by site-directed mutagenesis analysis. The cytotoxicities on the target cells were evaluated by XTT assay. **Results:** XLHX-124-50 has the strong anti-HIV-1 activity on both HIV-1 pseudotyped virus and HIV-1 lab-adapted virus. Cell-cell fusion assay showed that it could interfere on the first step of HIV-1 virus entering to the target cells. The results suggested that XLHX-124-50 could inhibit 6-HB formation of HIV-1 gp41 envelope by a sandwich ELISA, N-PAGE, and CD spectroscopy assay. The results also were confirmed that XLHX-124-50 could not inhibit the binding of gp120 with CD4 receptor or co-receptors by flow cytometric analysis and cell-based ELISA. Furthermore, the results of site-directed mutagenesis analysis suggested the positively charged residue (K574) located in gp41 pocket region was important for the binding of XLHX-124-50 to gp41 envelope. In addition, XLHX-124-50 in high concentration has no significant cytotoxicities on the HIV target cells. **Conclusion:** XLHX-124-50 inhibited the 6-HB formation of HIV-1 gp41 envelope by targeting on the key residue K574 on the gp41 pocket region. Therefore, XLHX-124-50 inhibited HIV-1-mediated membrane fusion and blocked HIV-1 entry. Those findings suggested that XLHX-124-50 could serve as a lead compound for the development of novel HIV-1 entry inhibitors.

Keywords: HIV-1 entry inhibitor; gp41 envelope; 6-helix bindle; cell-cell fusion

S13.24

The auxiliary GABA(B) receptor subunits KCTD12 and KCTD16 differentially modulate receptor function

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Aim: KCTD12 and KCTD16 were recently identified as $GABA_B$ receptor auxiliary subunits, yet their functional significance has yet to be clearly elucidated. Here we characterised the impact of human KCTD12 and KCTD16 on $GABA_B$ receptor kinetics and pharmacology *in vitro*. **Methods:** Using a *Xenopus laevis* oocyte based automated two-electrode voltage clamp assay, $GABA_B$ receptor activated GIRK current was used to characterize the effects of the $GABA_B$ receptor agonist baclofen or the positive allosteric modulator CGP7930 on receptor kinetics in the absence and presence of KCTD subunits. **Results:** Co-expression of KCTD12 with $GABA_B$ receptor accelerated the kinetics of response to agonist application (20%–80% rise time from 2.8 s to 1.3 s) and the kinetics of subsequent receptor desensitization (increased from 3.9% to 54.7%, compared with $GABA_B$ receptor only expression, n>7 oocytes). In contrast, KCTD16 co-expression did not significantly alter $GABA_B$ receptor kinetics. Analysis of baclofen dose-response curves showed that neither KCTD12 nor KCTD16 co-expression altered the EC_{50} or Hill slope (n>10 oocytes at each agonist concentration). However, the potentiating effect of CGP7930 on an EC_{20} concentration of GABA was enhanced in the presence of either KCTD12 or KCTD16 (from 11.5% to 20.3% and 24.4% respectively, n>33 oocytes). **Conclusion:** KCTD12 and KCTD16 differentially modulate $GABA_B$ receptor kinetics and pharmacology, raising the possibility of selective targeting of interactions between KCTDs and $GABA_B$ receptors.

Keywords: $GABA_B$ receptor; KCTD

S13.25

Investigation on potential mutagenicity of piperphentonamine hydrochloride

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Aim: To study the potential mutagenicity of piperphentonamine hydrochloride. **Methods:** Ames, aicronucleus assay and chromosomal aberration test were performed. **Results:** The results of Ames, Micronucleus assay and chromosomal aberration test are all negative. **Conclusion:** Piperphentonamine hydrochloride demonstrated no mutagenicity in this experimental system.

Keywords: piperphentonamine hydrochloride; mutagenicity; ames; micronucleus assay; chromosomal aberration test

S13.26

Calcium/calmodulin kinase II and protein kinase C in dorsal raphe nucleus involved in sleep regulation

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Aim: The dorsal raphe nucleus (DRN) is critically involved in sleep-wake regulation, but our understanding of the mechanisms of this regulation remains incomplete. The present study was designed to determine the role of DRN intracellular calcium/calmodulin kinase II (CaMKII) and protein kinase C (PKC) signaling in the regulation of wake and sleep. **Methods:** CaMKII inhibitor KN-93 (2.5 or 10.0 nmol), or PKC inhibitor chelerythrine (CHEL, 5.0 or 10.0 nmol) was microinjected (500 nl) into the DRN of freely moving rats, respectively, and the sleep parameters were investigated by electroencephalogram (EEG). **Results:** Microinjection of KN-93 (2.5 or 10.0 nmol) resulted in increased total sleep, mainly due to augmentation in non-rapid eye movement (NREM) sleep. Microinjection of CHEL (5.0 or 10.0 nmol) significantly reduced wake (W) time, due to reduction in the duration of W episodes; enhanced NREM sleep, particularly light sleep (LS), due to increased LS bouts. **Conclusion:** These data provide direct evidence that activation of intracellular CaMKII and PKC signaling in the DRN promotes W and suppresses sleep. These findings are relevant for designing a drug that could treat excessive sleepiness by promoting alertness.

Keywords: calcium/calmodulin kinase II; protein kinase C; DRN; sleep

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S13.27

Autophagy mediates the cytoprotective mechanism of polyphenols

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Autophagy is an evolutionarily conserved catabolic mechanism used by most kinds of mammalian cells that involves the sequestration, transport, bulk degradation and recycling of cytoplasmic components, such as long-lived proteins and organelles. In mammalian cells, autophagy acts predominantly as a prosurvival pathway, protecting the cells from oxidative stress. Polyphenols such as resveratrol, catechin, quercetin, and curcumin for a variety of diseases has beneficial effects. In recent years, the role of autophagy mediated the cytoprotective effects of polyphynols have been studied.

We found that curcumin could protect human endothelial cells from the damage caused by oxidative stress via autophagy. Pretreatment with curcumin remarkably improves the survival of HUVECs from H_2O_2-induced viability loss, which specifically evokes an autophagic response. Curcumin-pretreated up-regulated the level of LC3-II and increased the number of autophagosomes in HUVECs. Curcumin promoted BECN1 expression and inhibited the phosphatidylinositol 3-kinase (PtdIns3K)-AKT-mechanistic target of rapamycin (MTOR) signaling pathway. Curcumin also reversed the FOXO1 (a mediator of autophagy) nuclear localization along with causing an elevated level of cytoplasmic acetylation of FOXO1 under the circumstance of oxidative stress. These results suggest curcumin may have the potential for use as an autophagic-related antioxidant for prevention and treatment of oxidative stress.

Keywords: autophagy; polyphenols; curcumin

S13.28

RhoA/ROCK pathway regulates canonical transient receptor potential channels in h ypoxic pulmonary vasoconstriction

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Aim: Hypoxic pulmonary vasoconstriction (HPV) can be modulated through either elevation of $[Ca^{2+}]_i$ by Ca^{2+}-dependent mechanism or inhibition of MLCP by Ca^{2+}-independent mechanism. TRPC channels constitute the major Ca^{2+} pathways for the elevated $[Ca^{2+}]_i$, which is considered a classic trigger for HPV. However, it is generally believed that RhoA/ROCK pathway can modulate Ca^{2+} sensitivity to influence HPV by regulating MLC phosphorylation and dephosphorylation. The present study investigated the role of RhoA/ROCK pathway in the regulation of vascular function via Ca^{2+}-dependent mechanism in HPV. **Methods:** Mice were exposed to either normoxia or hypoxia. PASMCs were digested as previously described. Muscle tension was measured by PowerLab system. $[Ca^{2+}]$i was measured using fluorescence imaging system. **Results:** After treatment with RhoA/ROCK inhibitor fasudil, the expression of TRPC1 and 6 were significantly decreased compared with hypoxia mice. Contraction of pulmonary arteries and Ca^{2+} influx

in PASMCs elicited by store depletion using CPA were dramatically augmented in hypoxia group. However, RhoA/ROCK inhibitor Y-27632 obviously attenuated these changes. **Conclusion:** Inhibition of RhoA/ROCK pathway can attenuate the expression and function of TRPC channels, in turn alleviate Ca^{2+} influx through TRPC channels in HPV. These data demonstrate that RhoA/ROCK pathway plays the crucial role in the regulation of HPV via Ca^{2+}-dependent mechanism.

Keywords: HPV; RhoA; Rho-kinase; TRPC channels

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S13.29

Inhibitory effect and mechanism of calcitonin gene-related peptide on bleomycin-induced lung fibrosis

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Aim: Calcitonin gene-related peptide (CGRP) is a predominant neurotransmitter in sensory nerves. It has been reported that the expression of CGRP in lung was decreased in PF patients evoked by crystalline silica and crocidolite and CGRP significantly inhibited vascular remodeling in hypoxia-induced PAH. Therefore, in the present study, we tested whether the inhibitory regulation of CGRP on lung fibroblasts proliferation and pulmonary fibrosis was through inhibition of eukaryotic initiation factor 3a (eIF3a) expression. **Methods:** Bleomycin (5 mg/kg) was used to induce pulmonary fibrosis rat model, and capsaicin (50 mg/kg, sc) was used to deplete endogenous CGRP. Proliferation of cultured fibroblasts was determined by BrdU incorporation method and flow cytometry. The expression/level of CGRP, TGF-β_1, eIF3a, p-ERK1/2, α-SMA, and collagen I and III was analysed by radioimmunoassay, immunohistochemisty, real-time PCR or Western blot. **Results:** The expression of CGRP was significantly decreased in lung tissues in rats treated with bleomycin. However, pre-treatment with capsaicin to deplete endogenous CGRP further aggravated fibrosis, and up-regulated expression of TGF-β_1, eIF3a, p-ERK1/2, α-SMA, and collagen I and III. Exogenous application of CGRP significantly inhibited proliferation of fibroblasts, and markedly decreased the expression of eIF3a, p-ERK1/2, α-SMA, and collagen I and III induced by TGF-β_1. The effects of CGRP were abolished in the presence of $CGRP_{8\text{-}37}$. **Conclusion:** Endogenous CGRP is related to the development of pulmonary fibrosis induced by bleomycin, and the inhibitory effect of CGRP on proliferation of lung fibroblasts involves the ERK1/2/ eIF3a signaling pathway.

Keywords: calcitonin gene-related peptide; ERK1/2; eukaryotic initiation factor 3a; pulmonary fibrosis

S13.30

H_2S protects endothelium cell against Hcy-induced apoptosis and promotes cystathionine-γ-lyase expression

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Aim: To investigate the role of the H_2S effect in atherosclerosis (AS). **Methods:** The HUVECs were divided into the following groups: (1) the control group; (2) the DL-Homocysteine(Hcy) 10, 20, 40, and 200 μmol/Lgroups; (3) the sodium hydrosulfide (NaHS) 10, 60, 120, 240, and 300 μmol/L groups; and (4) the Hcy-NaHS 10, 20, 40, and 200 μmol/L Hcy+300 μmol/L NaHS groups. Each of the groups was further divided into 24 h and 48 h subgroups. After being treated with various amounts of the above mentioned, the status of HUVECs proliferation were detected by CCK-8 assay. The mRNA levels of CSE expression were detected by semi-quantitative RT-PCR analysis. Morphological changes in the HUVECs were observed by microscopy. **Results:** Hcy markedly inhibited HUVECs proliferation in a dose-dependent manner compared with the control group. HUVECs proliferation were markedly increased following 24 h treatment with NaHS at 300 μmol/L compared with the control group, but it was further strengthened and stabilized following 48 h treatment with NaHS at 300 μmol/L (P<0.01). The Hcy-NaHS group markedly increased HUVECs proliferation compared with the Hcy group. Furthermore, the Hcy-NaHS promote CSE expression in HUVECs at transcriptional level. **Conclusion:** The H_2S may inhibit Hcy-induced endothelium cell apoptosis. And it has a potent promotion effect on the expression of CSE, which is the key enzyme involved in the synthesis of H_2S in endothelium cell. It is a potential anti-atherosclerosis candidate for the treatment and prevention of atherosclerosis.

Keywords: atherosclerosis; homocysteine; cystathionine-γ-lyase; hydrogen sulfide (H_2S); human umbilical vein endothelial cells

S13.31

***Bis*-(2-ethylhexyl) phthalate suppresses myogenic differentiation and promotes adipogenic differentiation of skeletal muscle progenitor cells**

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Aim: *Bis*-(2-ethylhexyl) phthalate (DEHP) is a manufactured chemical that makes plastic more flexible. Exposure to DEHP has recently been shown to possess a potential health hazard in premature infants with low birth weight and as an obesogen in child development. The mechanism of DEHP on the skeletal myogenic and adipogenic differentiations still remain unclear. Here, we investigated the effects of DEHP on myogenic and adipogenic differentiations from myogenic progenitor cells. **Methods:** Both mouse myoblast progenitor cell C_2C_{12} and primary human skeletal muscle progenitor cells (HSMPCs) were exposed to 1 100 μmol/L DEHP in the differentiation media for 4 and 25 d to differentiate into myotubes and adipocytes, respectively. Morphology was assayed by hematoxylin and eosin staining and oil-red O staining. The associated-protein expressions during differentiation were analyzed by Western blotting. **Results:** DEHP significantly inhibited the myotube formation and myosin heavy chain expression after 4 d of differentiation in C_2C_{12} cells and HSMPCs in a dose-dependent manner. DEHP could also obviously promote adipogenic differentiation by activating PPAR-γ expression. Exposure to DEHP enhanced Aryl hydrocarbon receptor (AhR) expression during myogenesis and adipogenesis. AhR-antagonist α-naphthoflavone efficiently reversed the inhibition of myogenesis and enhancement of adipogenesis by DEHP. **Conclusion:** These findings provide the evidence that exposure of DEHP intervenes the myogenesis and promotes adipogenesis through an AhR activation-related signaling pathway.

Keywords: *bis*-(2-ethylhexyl) phthalate; myogenesis; adipogenesis; skeletal muscle progenitor cells

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S13.32

Discovery of a potent and orally-available *D*-amino acid oxidase inhibitor for the management of chronic pain

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Aim: It has been shown that spinal D-amino acid oxidase (DAAO) might be a potential molecular target for the treatment of chronic pain and morphine tolerance to analgesia. This study evaluated the *in vitro* and *in vivo* effects of Compound 15, a newly discovered DAAO inhibitor. **Methods:** The pig, rat and human DAAO enzymatic activities were determined according to the keto acid method. The rat and mouse formalin test, and rat spinal nerve ligation model of peripheral neuropathy were performed. The morphine analgesic tolerance was also established. **Results:** Compound 15 concentration-dependently inhibited pig, human and rat DAAO enzymatic activity, with ED_{50} values of 0.18, 0.21, and 0.14 μmol/L, respectively. Intrathecal injection of Compound 15 dose-dependently blocked formalin-induced tonic pain but not acute nociception in rats, with an ED_{50} value of 0.3 μg and maximum inhibition of 77.3%. Subcutaneous injection of Compound 15 prevented formalin-induced tonic pain with an ED_{50} of 1.8 mg/kg, while gavage of Compound 15 was also effective in inhibiting formalin-induced tonic pain. Intrathecal injection of Compound 15 effectively relieved spinal nerve ligation induced mechanical allodynia and heat hyperalgesia in neuropathic rats. Co-administrations of Compound 15 not only significantly prevented but also reversed morphine analgesic tolerance. **Conclusion:** These results characterize Compound 15 as a novel and potent human and rat DAAO inhibitor, and an orally-available painkiller, which may have potential for further development.

Keywords: DAAO inhibitor; Compound 15; morphine tolerance; neuropathic pain; formalin-induced pain

S13.33

The effects of high-purine diet on the blood pressure and kidney function of mice

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Aim: Referring to the relation between hypertension and long-term high-purine diet, we established the hypertension model mice by feeding with high-purine food, which is similar to human diseases. **Methods:** ICR mice were divided into 2 groups according to their blood pressure. The first group were fed with high-purine food (yeast extract and adenine were mixed in a certain ratio) and normal tap water. The second group was used as normal control group, received standard diet and water. The whole experiment lasted 32 weeks. In the 0th week, 5th week, 13th week, 24th week and 32nd week, heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MBP) were measured by intelligent non-invasive blood pressure monitor (BP-2010A). In the 32nd week, blood samples were gathered from eyeball to measure alanine aminotransferase (ALT), aspartate aminotransaminase (AST), uric acid (UA), blood urea nitrogen (BUN), creatinine (Cr), xanthine oxidase (XOD), adenosine deaminase (ADA) and nitrogen monoxidum (NO) levels. The mice was dissected, and the heart, liver, spleen, lung and kidney were picked up and weighed to calculate the viscera index. The XOD, ADA activity in liver were also measured. **Results:** After feeding with high-purine food for 13 weeks, the blood pressure of ICR mice started to rise significantly. 32 weeks later, in the first group, UA, Cr, and BUN levels of serum were obviously increased; NO level was reduced obviously; XOD and ADA activities in serum and liver uniform plasma had great ascending trends; kidney somatic index significantly decreased while lung somatic index had a contrary trend. In addition, biochemical criterion of renal function was abnormal and kidney was atrophied seriously. **Conclusion:** The blood pressure of ICR mice could increase by feeding with high-purine food for 13 weeks. The blood pressure reached mild and moderate level of hypertension. In the 32nd week, serious kidney damage were observed in ICR mice.

Keywords: hypertension; model; mice; renal function; high purine

S13.34

Adenoviral construction of histone lysine specific demethylase 1 gene

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Aim: Histone lysine specific demethylase 1 (LSD1) plays an important role in epigenetic modification of gene activation and repression. The current study is designed to generate a recombinant adenovirus vector containing LSD1 gene, using the AdEasy adenoviral vector system. **Methods:** The LSD1 gene was amplified by PCR and inserted into the shuttle vector, pShuttle-IRES-hrGFP-2, which would be used to transform into the competent *E coli* BJ5183, containing adenovirus backbone plasmid pAd Easy-1. The homologous recombinant adenoviral plasmid was extracted and verified by sequence analysis and was transfected into the *E coli* XL-10 Gold cells to be further amplified. Then, the adenoviral plasmid was digested by *Pac* I and infected into HEK293 cells for final virus resembling and amplification. The recombinant LSD1 adenovirus was propagated by 3–5 generations of repeat infection into HEK293 cells, then the virus particles were counted and the purity and titer were determined by commercial available kit. **Results:** PCR amplification, restriction analysis and sequencing identified that both recombinant shuttle plasmid pShuttle-IRES-hrGFP-2-LSD1 and the homologous recombinant adenovirus vector were correctly constructed. The LSD1 adenovirus vector was proved with the expression of GFP under fluorescent microscope. **Conclusion:** The LSD1 adenovirus vector was successfully constructed, which laid a foundation for further study regarding the LSD1 function.

Keywords: LSD1; adenovirus; GFP; HEK293

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S13.35

GLP-1 receptor promotes osteogenic differentiation of bone marrow mesenchymal stem cells by improving Wnt signaling pathway

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Aim: The coordinated balance between osteoblastic bone formation and osteoclastic bone resorption is a kind of key target of anti-osteoporotic agents. Now among therapeutics currently used to treat osteoporosis, the vast majority is anti-resorptive agents, and anabolic agents are rare. In the present study, we evaluated the effects of the GLP-1 receptor agonist on bone formation both *in vivo* and *in vitro* and underlying molecular mechanism. **Methods:** Tail-suspended rats were administered GLP-1 receptor agonist exendin-4 for 4 weeks, and then bone characters and related biomarkers were analyzed. Bone mesenchymal stem cells (BMSCs) were cultured in to modeled microgravity and treated by exendin-4, and then BMSCs function was evaluated by real-time PCR, Western blotting, alizarin red stain and oil red O staining. **Results:** In tail-suspended rat model exendin-4 prevented the loss of bone mass, enhanced the bone strength and prevented the deterioration of trabecular microarchitecture by increasing osteoblast number and decreasing adipocyte number. Moreover, exendin-4 increased serum ALP, OC and P1NP, key biochemical markers of bone formation. In rotary cell culture model exendin-4 increased the expressions of OC and Runx2 mRNA and decreased the expressions of PPARγ and LPL mRNA by promoting osteogenic differentiation and inhibiting adipogenic differentiation of BMSCs. Exendin-4 activated Wnt/β-catenin signaling pathway, playing important roles in regulating differentiation of BMSCs, through triggering cAMP signaling pathway. **Conclusion:** GLP-1 receptor is important for regulating the differentiation of BMSCs and exendin-4 might be a potential candidate for treatment of osteoporosis as anabolic agents.

Keywords: GLP-1 receptors; Exendin-4; bone mesenchymal stem cells (BMSCs); osteoporosis; Wnt/β-catenin signaling pathway

S13.36

Severe adverse reactions of vitamin K1 injection are anaphylactoid reaction, not anaphylaxis

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Aim: Adverse reactions of vitamin K_1 (VK_1) injection are considered as anaphylaxis. The study is to clarify a hypothesis that it is an anaphylactoid reaction. **Methods:** Beagle dogs were administered intravenously to observe behavioral responses. Plasma histamine and IgE were assayed by ELISA. Blood pressure was recorded via biological functional system. Histamine and β-hexosaminidase releases of cells were assayed by spectrophotometry. The apoptosis was analyzed using microscope and flow cytometry. **Results:** Dogs appeared multisystemic symptoms after first administration of VK_1 injection. The plasma histamine concentration increased, and the blood pressure decreased sharply. Dogs stimulated by VK_1 injection performed the same degree of symptoms as sensitization. However, when the dogs sensitized by VK_1 injection were stimulated by VK_1-fat emulsion, abnormal behaviors did not appear. There was no significant change of IgE concentration in VK_1 injection group. Histamine andβ-hexosaminidase releases increased and apoptosis rate was elevated in a concentration-dependent manner with VK_1 injection in RBL-2H3 cells. In Tween-80, dogs had multisystemic symptoms and degranulation and apoptosis of RBL-2H3 cells sharply increased. However, the dogs in VK_1-fat emulsion group had not any behavioral abnormality and significant change of plasma histamine. There was no degranulation and apoptosis in RBL-2H3 cells. **Conclusion:** Adverse reactions induced by VK_1 injection are anaphylactoid reaction, not anaphylaxis. VK_1 injection induces anaphylactoid reaction via non-IgE-mediated immune pathway, in which the trigger may be solubilizer.

Keywords: vitamin K_1; anaphylactoid reactions; anaphylaxis

S13.37

IOP lowering effect of losartan and latanoprost in oculohypertensive rats: A comparative evaluation

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Aim: To evaluate the intraocular pressure (IOP) lowering effect of losartan potassium in rats with steroid-induced ocular hypertension and compare the IOP lowering effects with that of latanoprost. **Methods:** Ocular hypertension was induced in Sprague Dawley rats weighing 100 to 120 g by topical application of 5 μL dexamethasone 0.1% twice daily for 36 d. Rats with IOP increment of 25% or more from baseline were used for further study and were divided into 2 groups (n=7). Following baseline IOP estimation single drop (10 μL) of losartan 2% in group 1 or latanoprost 0.005% in group 2 was applied topically to one of the randomly chosen eyes as test eye. Contralateral eyes received vehicle and served as control. The IOP was measured hourly thereafter for 12 h. **Results:** Losartan potassium caused significant IOP reduction (P<0.01) at 1-h post-instillation which lasted until the eleventh hour while the onset of significant IOP lowering and during 12th hour post-instillation the IOP returned back to baseline. In latanoprost treated group onset of significant IOP lowering was observed at 3-h post-instillation. The maximum reduction of IOP after treatment with losartan was observed at 8-h post-instillation

and it amounted to 36.73% from baseline which was comparable to that caused by latanoprost. **Conclusion:** Losartan potassium 2% caused significant IOP reduction in the steroid-induced oculohypertensive rats. As compared to latanoprost the peak IOP reduction was comparable, although the duration of IOP lowering was shorter.
Keywords: IOP; steroid-induced; rats; losartan; latanoprost

S13.38

Emerging artemisinin resistance in Southeast Asia?

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Malaria remains one of the major global public health problems. The major problem which limits the control of this infection is resistance of *Plasmodium falciparum* to most of the available antimalarial drugs. Artemisinin-based combination therapies (ACTs) have become the counterpiece of global malaria control to halt the impending epidemics of drug resistant malaria. They are now the recommended first-line treatment for uncomplicated *P falciparum* in all malaria endemic countries to improve efficacy and delay development and selection of drug-resistant parasites. Despite the precautionary measure however, artemisinin resistant *P falciparum* malaria has recently been reported in western Cambodia and the bordering areas of Thailand, the well recognized hotspot of MDR *P falciparum*. Until comprehensive coverage with effective malaria vaccine is available, and with only a handful of effective antimalarial drugs available, the development of artemisinin resistance by malarial parasites would be a challenge for the current global malaria control programs. The situation accentuates the importance of closer surveillance and containment in parallel with effective malaria control program to avoid the emergence of new foci of resistance and to limit the spread of the resistant parasites to other areas. Intense efforts are being pursued worldwide to confirm, characterize, and contain resistance to artemisinins. The urgency of these efforts is increased by the paucity of alternative antimalarials should artemisinins fail.

S13.39

Pharmacological study of a novel urea transporter inhibitor PU-48 as a diuretic

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Aim: The purpose of this study is to determine the feasibility of urea transporters (UT) as novel diuretic drug targets and to discover urea transporter inhibitors with diuretic activity. **Methods:** *In vitro* erythrocyte lysis model was used to get the potential urea transporter inhibitors. Inhibition activity of hits on UT-A was assayed in an MDCK cell line that stably expresses rat UT-A1. Cytotoxicity was assayed with MDCK cell viability after incubation with compounds for 72 h by cell counting kit-8 (CCK-8) assay. Diuretic activity of the compounds was determined in rats. Urine output, blood and urinary osmolarity and urea concentration were measured to evaluate the diuretic role of compounds. **Results:** Structure-activity analysis indicated that the most potent compound, thienoquinolin PU-48, reversibly inhibited UT-B-facilitated urea transport in human, rabbit, rat and mouse erythrocytes with IC_{50}s of 0.18, 0.68, 0.35, and 0.77 μmol/L, respectively. PU-48 did not affect urea transport in UT-B null mouse erythrocytes. PU-48 showed no significant cellular toxicity at concentrations up to 80 μmol/L. Subcutaneous delivery of PU-48 (at 3.12, 12.5, and 50 mg/kg) to rats produced an increase of urine output and a decrease of the urine urea concentration and osmolarities without electrolyte disturbance and liver or renal damages, which indicates that PU-48 has significant diuretic effect by urea-selective diuresis. Moreover, there was no significant difference in UT-A1-A3, UT-B and AQP1-4 protein abundance in the medulla between PU-48 treated rats and control. **Conclusion:** These results indicate that PU-48 or its analogues might be developed as a new diuretic to increase renal fluid clearance in diseases associated with water retention without causing electrolyte imbalance, as well as a tool drug to establish 'chemical knockout' analysis of the physiological functions of UTs.
Keywords: diuretics; urea transporter; urine concentrating mechanism; drug discovery

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S13.40

Establishment of mitochondrial toxicity model in $HepG_2$ cell

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Aim: In recent years, marketed drugs withdrawn and termination of new drug study in pre-clinical/clinical stage due to mitochondrial-related side effects were uncommon. In this project, we established a fast and efficient evaluating model of mitochondrial damage in HepG2 cell. And it was applied to evaluate mitochondrial toxicity of nucleoside analogues. **Methods:** HepG2 cells incubated with mitochondrial inhibitors or nucleoside analogues in different medium, glucose or galactose. After 24 h and 48 h, ATP was detected by CytoTox-Glo™ Cytotoxicity Assay kit. **Results:** Rotenone 1 μmol/L, oligomycin 10 μmol/L, CCCP 10 μmol/L showed obvious mitochondrial toxicity after incubation for 24 h in glucose medium. While in galactose medium, mitochondrial inhibitors (0.001 μmol/L) obviously damaged mitochondrial function. After 48 h, damage in galactose medium increased more and more obviously. After incubation with nucleoside analogues for 48 h, elvucitabine, adefovir, adefovir dipivoxil, ganciclovir, penciclovir, acyclovir showed evident mitochondrial toxicity in galactose medium. However, all the other nucleoside analogues in addition to adefovir dipivoxil did not show evident mitochondrial toxicity in glucose medium. **Conclusion:** Cells cultured in glucose medium present Crabtree effect, and ATP production mainly depends on glycolysis, supplemented to mitochondrial oxidative phosphorylation. When galactose instead of glucose as cell growth energy, ATP production mainly depends on mitochondrial oxidative phosphorylation, mitochondrial toxicity is more likely to appear.
Keywords: mitochondrial toxicity; ATP; glucose medium; galactose medium

S13.41

The protective effects of *Hovenia acerba* Lindl on ethanol-induced liver injury in mice

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Aim: To study the protective effects of compound preparation of *Hovenia acerba* Lindl (CPH) with different ratio compatibility on acute ethanol-induced liver injury in mice. **Methods:** The acute liver injury animal model was established in mice by ethanol gavages (56% at 5 g/kg). The levels of ALT, AST, and AFP in serum and levels of MDA, SOD and GSH in liver tissue were measured and hepatic histological changes were observed by optical microscope in order to evaluate the protective effects of CPH. **Results:** Compared with model group, the levels of ALT, AST, and MDA content were significantly down-regulated, while SOD activity and GSH-Px content in liver tissue of acute alcoholism mice were up-regulated by CPH, but CPH could not affect the level of AFP effectively. Histological results showed that CPH could ameliorate histological damages induced by ethanol in liver. **Conclusion:** CPH has the protective effects on acute ethanol-induced liver injury in mice, and the mechanism may be associated with the elimination of free radicals and anti-lipid peroxidation. All of these may provide significant evidence for developing CPH to be safe and effective functional foods.
Keywords: CPH; ethanol; liver injury; anti-lipid peroxidation

S13.42

Study on the effect of active carbon to treat acute gastric ulcer in rates

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Aim: Activated carbon is a black powder or granular amorphous carbon. In this study, the effects of activated carbon against ethanol induced acute gastric ulcers injury were investigated. **Methods:** Acute gastric ulcers were induced by gavage of ethanol, with ig administration by 0.6 mL/100g dosage in SD SPF grade rats, after 6 h, rats with ethanol ulcer were treated with continuous ig administration of activated carbon once a day for a total of seven times at the dose of 3.3, 10, and 30

mg/kg, respectively. Ulcer index was assayed by Guth standard. **Results:** Under activated carbon conditions, the Guth ulcers index in rats was markedly decreased. **Conclusion:** It is suggested that activated carbon has a significant anti-ulcer effect.
Keywords: active carbon; ethanol; antiulcer agents; acute gastric ulcer

S13.43

Comparative proteomics changes in neuropeptide Y-induced apoptosis in human hepatocytes

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Aim: In human adult liver, NPY-ergic nerves are the most abundant peptidergic nerve fibers. These nerve fibers are in close contact with the entire hepatic lobule. However, the role of NPY in hepatocyte function is largely unexplored. **Methods:** The viability of different concentrations of NPY on L-02 cells was measured by MTT. The apoptosis markers such as Bax, Bcl-2, Bad, and caspase-3 were detected by Western blot. The proteomic approach based on 2D-nano-LC-MS/MS was applied to uncover protein alterations after NPY treatment of L-02 cells and discovered the signaling of NPY induced L-02 cells apoptosis. The inhibitor of mTOR rapamycin was used to observe the role of mTOR in NPY induced apoptosis. **Results:** NPY significantly decreased the viability of L-02 cells. Bcl-2 was down-regulationed, Bax, Bad, and 17 kDa cleaved caspase-3 were up-regulated. Through the proteomic approach, 317 proteins were identified with NPY/CON. The altered intensity of mTOR, S6K detected by Western blot matched well with the differences observed in 2D-nano-LC-MS/MS based proteomic analysis. And rapamycin blocked the effect of NPY induced L-02 cells apoptosis. **Conclusion:** NPY induced the L-02 cells apoptosis in a dose-dependent manner. mTOR/S6K/BAD, might be one important apoptotic pathway involved in NPY induced apoptosis.
Keywords: neuropeptide Y; liver; apoptosis; 2D-nano-LC-MS/MS; proteomics

S13.44

Rolipram rescues oxidative stress-induced premature phenotypes by SIRT6-dependent NF-κB inhibition

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Aim: Cyclic adenosine monophosphate (cyclic AMP) is a key mediator of metabolic regulation and its signalling pathway is highly complex. However, whether cyclic AMP itself could also rescue aging-related functional declines remains unknown. Intracellular cyclic AMP is mainly degraded by type 4 cyclic adenosine monophosphate phosphodiesterase (PDE4), whose selective inhibitor, rolipram, has been proved to facilitate the establishment of long-term potentiation and suppresses the expression of pro-inflammatory cytokines. Both cyclic AMP and rolipram have been developed into clinical drugs, which had positive inotropic and anti-depression effects, respectively. However, the comprehensive effects and underlying molecular mechanisms of these drugs on aging mammalian physical metabolism and motor functions are still unclear. We suppose that cyclic AMP may also contribute to other Sirtuin family members. **Methods:** We investigated the physiological and metabolic changes in oxidative stress-induced premature mice, which were treated with experimental galactosemia and developed glycometabolism dysfunction. **Results:** Here, we demonstrated that rolipram, the specific PDE4 inhibitor, could increase cyclic AMP level to rescue the pathophysiology in premature mice. The mice treated with rolipram showed increased SIRT6 and reduced acetylated NF-kappaB protein level. However, cyclic AMP lost its inhibition effect on NF-kappaB when SIRT6 was knocked down. **Conclusion:** Therefore, administration of rolipram may protect against premature phenotypes in galactosemia or other glycometabolism dysfunction.
Keywords: rolipram; premature; cAMP; SIRT6; NF-kappaB
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S13.45

Important role of PKA pathway in endothelial cell migration induced by VEGF and modulation by norisoboldine

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Aim: To investigate the role of PKA pathway in VEGF-induced endothelial cell migration and modulation by norisoboldine (NOR). **Methods:** Fibrin bead assay, RT-PCR, Western blot, coimmunoprecipitation, immunofluorescence, EMSA and ELISA were performed. **Results:** PKA inhibitor H-89 significantly inhibited VEGF-induced endothelial cell migration and sprouting. NOR suppressed VEGF-induced cAMP production, and activation of PKA, src, VASP and eNOS. NOR reduced IκBα phosphorylation and disruption of p65-IκBα complex, and inhibited the expression of p-p65 (ser276) but not p-p65 (ser536) and PKAc in endothelial cells. It prevented the formation of PKAc/p65 complex and p65 binding to DNA. Furthermore, H-89 improved Notch1 activation but Notch inhibitor DAPT failed to affect PKA activation. **Conclusion:** PKA was crucial in VEGF-induced endothelial cell migration, and NOR inhibited the migration by suppressing the activation of cAMP-PKA-NF-κB/Notch1 pathway.
Keywords: PKA pathway; VEGF; endothelial cell migration; norisoboldine
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S13.46

Respiratory syncytial virus induces glucocorticoid-insensitivity in airway epithelial cells

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Aim: Respiratory syncytial virus (RSV) is a major cause of acute respiratory, which responds inadequately to anti-inflammatory actions of glucocorticoids (GC). RSV triggers expression and activity of cytokines, including transforming growth factor-β (TGF-β). We identified that TGF-β induces profound impairment of GC transactivation in human epithelial cells. We now explore the contribution of TGF-β to RSV-induced GC insensitivity. **Methods:** Cells were inoculated with RSV at a multiplicity of infection of 0.1 for 1 h, and incubated for 48 h. Cells were transiently transfected with a GC Response Element (GRE)-SEAP reporter to measure budesonide-induced GRE activity. Nuclear localization of GC receptor α (GRα) was ascertained by subcellular fractionation. Endogenous GC-responsive gene expression was measured by RT-qPCR. **Results:** RSV infection profoundly inhibited budesonide-induced GRE activation, and attenuated the budesonide-induced nuclear translocation of GRα in epithelial cells. RSV infection also impaired budesonide-induced expression of GILZ and ENaCα in epithelial cells. The impairment of gene expression was attenuated by the selective ALK5 (TGFβRI) inhibitor, SB431542 and prevented by the therapeutics, tranilast or pirfenidone, which each reduce TGF-β activity. **Conclusion:** RSV infection-induced GC insensitivity is partially mediated by endogenous TGF-β. Establishing the effectiveness of tranilast and pirfenidone in viral infection supports the use of these drugs in prevention/treatment of asthma and COPD exacerbations.
Keywords: GCs; virus; asthma exacerbation

S13.47

Exenatide given intrathecally produces efficacious diuretic effect

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Aim: Exenatide is an exogenous peptide agonist of glucogan-like peptide-1 (GLP-1) receptors and stimulates guanylate cyclase/PKA and its subsequent signal transduction. Exeantide has been used for the treatment of type II diabetes by systemic administration. This study aimed to investigate the diuretic effect of exenatide via the intrathecal injection, in a comparison with endogenous GLP-1 (7–36) and the DPP-4 inhibitor sitagliptin, as well as two small molecule GLP-1 receptor agonists. **Methods:** Exenatide was administered intrathecally in conscious rats and urine was collected. Urine volume was gauged and electrolytes were measured by commercial kits. **Results:** Intrathecal exenatide caused a dose-dependent diuretic effect, with a high efficacy comparable to that of furosemide. Urine was nearly neutral and hypisotonic. GLP-1 (7–36) and the DPP-4 inhibitor sitagliptin caused similar diuretic effects, but two small molecule GLP-1 receptor agonists were not effective in producing diuretics. Furthermore, intrathecal single injection of the selective GLP-1 receptor antagonist exendin (9–39) and intrathecal multi-daily injections of gene silencer siRNA/GLP-1 receptor for 7 d did not significantly affect the spinal diuretic effect of exenatide. Intrathecal administration of the adenylate cyclase inhibitor 2′,5′-dideoxyadenosine and PKA inhibitor H89 were also not effective in blockade of spinal diuretic effect of exenatide. **Conclusion:** Our results indicate that exenatide given intrathecally exhibits efficacious diuretic effect and suggest the spinal cord may be an important organ for modulation of water balance in the body. The mechanism underlying the diuretics of exenatide is not understood, but might not be related to the activation of classical GLP-1

receptors in the spinal cord.

Keywords: exenatide; GLP-1 receptors; diuretics; spinal cord

S13.48

Experimental study of long-term toxicity of human umbilical cord mesenchymal stem cells in rats

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Aim: To observe the long-term toxicity and severity occurred after administration of human umbilical cord mesenchymal stem cells (HUCMSCs) in rats. **Methods:** Sprague-Dawley rats were randomly divided into 4 groups. Each group was intravenously injected HUCMSCs at dose of 3.0×10^5, 1.5×10^6, and $7.5\times10^6\cdot kg^{-1}$ Every three days for up to 90 d, respectively. Control group were given the same bulk of saline. After the withdrawal of HUCMSCs, 2 more weeks were observed. The rats' body weight, food intake, hematology, blood biochemistry analysis, and organ coefficients were measured, and the histopathological changes were also observed. **Results:** The outer appearance and behavior, body weights, organ coefficients, the indexes of hematology and biochemistry analysis in rats of HUCMSCs groups showed no significant difference from those of control group. Pathological examination discovered no obvious pathological changes related to HUCMSCs toxicity and no delayed toxicity reaction after HUCMSCs withdrawal. **Conclusion:** HUCMSCs had no obvious toxicity in rats for long-term administration.

Keywords: human umbilical cord mesenchymal stem cells; long-term toxicity

S13.49

The mechanism of retinal damage induced by NMDA

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Aim: The present study was designed to investigate the influence of cyclooxygenase-2 (COX-2) expression on the rat retinal damage induced by *N*-methyl-*D*-aspartate (NMDA). **Methods:** Twenty-five Sprague-Dawley rats were divided into five groups: control, NMDA and NS-398 (a selective inhibitor of COX-2; 1, 10, and 100 ng/mL) groups. Rats in the control group were given saline over the eyes. Rats in NMDA and NS-398 groups were given an intravitreal injection (5 μL) of NMDA (40 nmol/L) or NMDA plus concentrations of 1, 10, and 100 ng/mL of NS-398, respectively. The neuron numbers of retinal ganglion cell layer (RGCL) were counted, the apoptosis of the RGCL cells was determined by TUNEL assay; the expressions of both caspase-3 and COX-2 in the rats' retinas were admeasured by using an immunohistochemical staining. **Results:** Compared with NMDA group (47%±1.7% of control), the neuron numbers of RGCL of NMDA plus NS-398 groups were greatly increased (71%±1%, 88%±1%, 65%±2% of control, respectively). The expressions of both caspase-3 (278%±1%, 176%±1%, 199%±0% of control respectively) and COX-2 (428%±0%, 398%±1%, 459%±1% of control, respectively) in the NMDA plus NS398 groups were significantly lower as compared with that in NMDA group (410%±1%, 520%±0% of control, respectively). **Conclusion:** NMDA may evoke the excessive expression of COX-2 in the rats' retinas, and then cause the apoptosis of RGCL, also result in damage to the retina.

Keywords: Retina; N-methyl-D-aspartate; COX-2; inhibitor

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S13.50

Circadian- and sex-variations of gene expressions associated with bile acid synthesis and transport in mice

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Aim: Bile acids play important roles in physiology and are important signaling molecules. Bile acid homeostasis is also a target of drugs. This study examined diurnal- and sex-variations in expression of bile acid synthesis and transport genes in mice. **Methods:** Adult Kunming mice were housed in SPF-grade facilities, with controlled light/dark cycles (light on 8:00–20:00) and free access to standard rodent chow. Livers were collected at 6:00, 10:00, 14:00, 18:00, 22:00, and 2:00 during a 24-h period. Hepatic total RNA was isolated, purified, and subjected to real-time RT-PCR analysis. **Results:** The bile acid synthesis rate-limiting enzyme Cyp7a1 showed diurnal rhythm, peaked at 18:00 with a peak/nadir ratio of 10-fold, while Cyp27a1 had a peak/nadir ratio of 3-fold. The nuclear receptor farnesoid X receptor (FXR) and small heterodimer partner (SHP) also showed similar circadian variation, and peaked during the dark-phase. Hepatic bile acid transporter Na^+-taurocholate co-transporting polypeptide (Ntcp, 3-fold) and bile salt export pump (Bsep, 5-fold) also showed circadian rhythm, with a peak around 14:00. The expression of clock genes *Clock* and *Nr1d1* showed typical circadian patterns. Furthermore, expression of Cyp7b1 is male dominant, while females have higher expression of Cyp7a1, Cyp27a1, FXR, and Bsep. **Conclusion:** The bile acid synthesis genes and transporters have diurnal rhythms and sex-difference, which could influence drug efficacy at different times of the day.

Keywords: bile acid homeostasis; circadian variation and sex difference; Cyp7a1 and Cyp7b1; FXR and SHP; Ntcp and Bsep

S13.51

Efficient discrimination of three hotspot mutations associated with aminoglycoside antibiotics induced deafness in the mitochondrial 12S rRNA by on/off switch

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Aim: The 961delT, C1494T and A1555G mutations in the mitochondrial 12S rRNA are the hotspot mutations associated with aminoglycoside antibiotics induced deafness (AAID). The objective of this study was to apply the "on/off" switch consisting of 3′ phosphorothioate-modified allele specific primers and Exo^+ polymerase in single base discrimination of the three hotspot point mutations rapidly. **Methods:** The wild-type pMD19-T vector templates were prepared by primer extension performed using polymerases without 3′ exonuclease activity, and the mutation-type templates harboring 961delT, C1494T, and A1555G three mutations were prepared by overlap extension (SOE) PCR respectively. Allelic specific primers targeting wild-type and mutation-type templates were designed with 3′ terminal phosphorothioate modification and 5′ terminal different fluorescence label and a extinction signal. Real-time PCR was performed using Exo^+ polymerases. **Results:** Allelic specific primers perfectly matching wild/mutation-type templates were extended and have the fluorescence signals consistent with 5′ terminal fluorescence label, while no products or fluorescence signals were produced from primers mismatching mutation/wild-type templates. **Conclusion:** The "on/off" switch combined with real-time PCR is reliable in the diagnosis of monogenic diseases and have enormous application in screening of the 12S rRNA 961delT, C1494T and A1555G mutations simultaneously.

Keywords: Exo^+ polymerase; phosphorothioate modification; mitochondrial 12S rRNA; point mutation; real-time PCR

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S13.52

Comparative characterization of muscarinic receptor binding activity of fesoterodine, its active metabolite and tolterodine for treatment of overactive bladder

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Aim: Fesoterodine is one of the most novel antimuscarinic agents approved for the treatment of overactive bladder. Fesoterodine is rapidly and extensively converted by ubiquitous nonspecific esterase to its active metabolite, 5-hydroxymethyl tolterodine (5-HMT), which is also active metabolite of tolterodine. Both agents may contribute significantly to the pharmacological effects in the bladder after oral administration of fesoterodine. Fesoterodine displayed about 20 times higher affinity to M_2 and M_5 subtype than M_3 subtype in membranes prepared from CHO cells expressing human M_1 to M_5 receptors. However, the muscarinic receptor binding properties of fesoterodine and 5-HMT in the bladder and other tissues have not been comparatively examined. Thus, the current study was aimed to examine comparratively the muscarinic receptor binding activity of fesoterodine, 5-HMT and tolterodine in nine different tissues from rats, by using radioligand binding assay. **Methods:** The tissues (bladder urothelium and detrusor, submaxillary gland, lung, heart, ileum, colon, brain) from rats were dissected and homogenized for the preparation of crude membranes by suspension and centrifugation. The muscarinic receptors in tissue homogenates were measured by a radioligand binding assay using [*N*-methyl-^{3}H]scopolamine methyl chloride ([^{3}H]NMS), a selective radioligand of muscarinic receptors as previously described. The inhibition constant (Ki) for the inhibitory effects of fesoterodine and 5-HMT in rat tissues was estimated. **Results:** Fesoterodine inhibited concentration-dependently specific [^{3}H]NMS binding in the bladder urothelium and detrusor, submaxillary gland, lung, heart, ileum, colon, prostate, and brain from rats. Similarly, 5-HMT

and tolterodine inhibited specific [^{3}H]NMS binding in these tissues. Based on K_i values, the rank order of binding affinity of each agent in rat tissues was 5-HMT >>tolterodine>fesoterodine. Notably, the binding affinity of 5-HMT was about 20 times higher than that of fesoterodine in tissues except the prostate and cerebral cortex which showed about 140 and 350 times respectively, higher affinity. 5-HMT displayed about 2 times higher affinity to muscarinic receptors in the bladder than the submaxillary gland. There was no significant difference among nine tissues of rats in the muscarinic receptor binding affinity of tolterodine. The Hill coefficients for the inhibition of [^{3}H]NMS binding by fesoterodine and 5-HMT in each tissue were close to unity. **Conclusion:** Fesoterodine and 5-HMT bind to the muscarinic receptors in the bladder and other tissues, and the affinity of 5-HMT is significantly higher than that of fesoterodine.

Keywords: overactive bladder; antimuscarinics; bladder; muscarinic receptors

S13.53

Trisomy chromosome R, a novel chromosomal aneuploidy involved in azole resistance in *Candida albicans*

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Aim: Genome plasticity is a hallmark in *Candida albicans*. This study was to examine the potential cause at the genome level of a variant strain CaLY188 in which the complete deletion of *SRD1* has been confirmed by PCR while the re-introduction of *SRD1* did not restore the decreased azole sensitivity to the wild-type level. **Methods:** The entire coding sequence of *SRD1* was deleted from the parental SN152 by two-step homologous recombination. The spot assay, the minimal inhibitory concentration and the time-killing curve assays were performed to confirm the azole sensitivity in the variant CaLY188. Array based comparative genomic hybridization technology (aCGH) and whole genome expression profiling were then used to understand the mechanism of the resistance to azoles in this strain. **Results:** Two alleles of *SRD1* were confirmed being deleted by genomic PCR in the variant CaLY188 that was significantly resistant to azoles. Array CGH revealed DNA copy number amplification of the whole chromosome R in the strain. A total of 250 differentially expressed genes were identified when comparing CaLY188 to its parental strain. Among them, 76 genes were located in Chr R, which might result from the amplification at the genomic DNA level. *ERG25*, encoding a C-4 sterol methyl oxidase, was the only gene involved in ergosterol biosynthesis in Chr R that was up-regulated 2.23 fold in the CaLY188. **Conclusion:** Our results indicated that transcriptional induction of *ERG25* resulting from the trisomy of chromosome R contributed to azole resistance in this variant strain.

Keywords: *Candida albicans;* aneuploidy; antifungal drug resistance

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S13.54

Mechanisms of action of K^+ channel openers and nicorandil to treat vasospastic angina

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The primary hyperreactivity of coronary artery smooth muscle cells (SMCs) has been shown to be involved in the pathogenesis of the vasospastic angina that refers to a sudden, intense vasoconstriction of an epicardial coronary artery that causes vessel occlusion or near occlusion. Thus, it is important to examine the effects of various stimuli or agonists on the reactivity of SMCs owing to intracellular signal transduction. While the contractile proteins responsible for generation of force reside in the cell interior, most of the upstream signals generated upon G-protein coupled receptors stimulation, including Rho kinase (RhoK) and protein kinase C (PKC), are regulated at the plasma membrane. The hyperpolarization induced by K_{ATP} channel openers (KCOs) has been shown remarkable influences on VSMC tonus. KCOs induce hyperpolarization to result in deactivation of Cav channels, inhibit the production of inositol triphosphate (IP_3) by the agonists and reduce the Ca^{2+} sensitivity of contractile proteins, probably through inhibiting RhoK and PKC signaling. Although Cav channel blockers (CCBs) are effective to treat the vasospastic angina, nicorandil that acts as a hybrid of nitrate (NO/cGMP generation) and KCO should be the first choice for the treatment, since KCOs have a broader spectrum of relaxation mechanisms compared with CCBs. The recent scope of the relaxation mechanisms of cGMP will also be discussed.

Keywords: coronary artery; smooth muscle cells; hyperpolarization; nicorandil; Ca^{2+} sensitivity of contractile proteins

S13.55

Effect of reactive oxygen species on hypersensitivity of lung vagal C fibers induced by intermittent hypoxia in rats

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Aim: Obstructive sleep apnea, manifested by intermittent hypoxia (IH) and excess production of reactive oxygen species (ROS) in the airways, is associated with hyperreactive airway diseases. Sensitization of lung vagal C fibers (LVCFs) contributes to the airway hypersensitivity. In the present study, we examined whether 24 consecutive hours of IH exposure enhances the LVCF sensitivity and, if so, by what mechanisms. **Methods:** Conscious rats were exposed to repetitive 1.25 min cycles (30 s of N_2+45 s of 21% O_2) of IH or room air (RA) for 24 consecutive hours. Subsequently, the LVCF-mediated pulmonary chemoreflex or single-unit LVCF activity to chemical stimulants was recorded. **Results:** Right atrial injection of capsaicin, phenylbiguanide or α,β-methylene-ATP evoked an augmented apneic response in IH-exposed rats, compared with the RA-exposed rats. The reflex apnea evoked by chemical stimulants was abolished by bilateral vagotomy or perineural capsaicin treatment of both cervical vagi, suggesting the involvement of LVCFs. The IH-augmented apneic response to these chemical stimulants was prevented by pretreatment with *N*-acetyl-*L*-cysteine (an antioxidant). Electrophysiological study revealed the afferent responses of LVCFs to these chemical stimulants were augmented by IH, and this sensitization of LVCFs was prevented by *N*-acetyl-*L*-cysteine. **Conclusion:** ROS are essential for IH-induced LVCF hypersensitivity, which may contribute to exaggerated airway reflexogenic responses.

Keywords: intermittent hypoxia; ROS; lung vagal C fibers; hypersensitivity

S13.56

New strategy for fabrication nanodrugs of poorly solubility with high drug content

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Aim: Despite nanodrugs have great potential application in diagnosis and treatment for cancer, the poorly solubility of drugs, the PEGylated method, and the structure of the nanodrugs are still problems in fabrication and performance of drugs. A new structure of nanodrugs of poorly solubility with high drug content was designed for resolving those problems. **Methods:** The nanoparticles of poorly solubility drugs were fabricated by nano-emulsion method with diameter of 140 nm. Amino acid was added to the system, adsorbing in the surface of NPs. On the condition of catalysis of EDAC and NHS, the carboxyl and amino group of the amino acid condensation polymerize to form a layer coating on the NPs. The PEG-COOH could be covalently linked to the poly amino acid coated NPs via the reaction with the surplus amino or pyrole group of the layer and finally form a stable layer of PEG. **Results:** The nanodrugs of poorly solubility with high drug content were successfully prepared on the assistant of poly amino acid layer. **Conclusion:** Poorly solubility anti-cancer drugs were fabricated to nanoparticles by nano-emulsion method, which enhance the treatment effect remarkably. Assisted by amino acid polymerized to form a middle layer, we have successful synthesis PEGylated NPs, which improve the drug NPs surface properties for passive target effect and enhance the effect of drug slow release. Furthermore, the new structure of nanodrugs avoids the defects that bring by the carries and have advantages of high drug content *etc.*

Keywords: nanoparticles; coating; poorly solubility; amino acid

S13.57

The mechanism of LKB1-dependent signaling pathways in liver fibrosis

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Aim: Liver Kinase B1 (LKB1) proteins serve as a master regulator of metabolism

that inhibits anabolic processes and stimulates catabolic processes. In the present study, we investigated the roles of LKB1 in activated human hepatic stellate cells. **Methods:** Human hepatic stellate cells, LX-2 were incubated with different stimulating factor at various times. The protein and mRNA extracted from cells were analyzed by Western blotting analysis and Reverse Transcription Polymerase Chain Reaction (PCR). **Results:** The results show that ethanol and TGF-β activated the expressions of LKB1 and AMP-activated protein kinase (AMPK) phosphorylation. PD98059 (ERK inhibitor) and LY294002 (PI3K inhibitor) reduced LKB1 and AMPK phosphorylation. Furthermore, PD98059 and LY294002 influenced the expressions of α-smooth muscle actin (α-SMA) and collagen I. **Conclusion:** Our data demonstrate that cell signals influenced liver fibrosis via LKB1 and ERK signaling pathways in activated hepatic stellate cells.

Keywords: liver fibrosis; hepatic stellate cells; LKB1; AMPK

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S13.58

Over-expression of SIRT1 regulates differentiation of human visceral adipose-derived stem cells

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Aim: Human adipose tissue-derived stem cells (hADSCs) have ability to self-renew and differentiate into multiple tissues. Recent studies reveal that NAD-dependent protein deacetylase SIRT1 is required for long-term growth of hADSCs, and it also participates in brown remodeling of mouse subcutaneous white adipose tissue. In this study, we investigate the effects of SIRT1 on human visceral adipose tissue-derived stem cells (hVADSCs). **Methods:** Human adipose tissue from abdominoplasty procedures is obtained, and hVADSCs are isolated from the tissue. Human SIRT1 gene is cloned into lentiviral vector pGCSIL-GFP, and then transfected into hVADSCs. **Results:** Over-expression of hSIRT1 significantly inhibits white fat specific marker resistin mRNA expression, and reduces lipid deposit in hVADSCs, which indicates that SIRT1 might restrain differentiation of hVADSCs to white adipose tissue. We also observe that brown adipose differentiation-related genes C/EBPbeta. UCP1 are down-regulated by hSIRT1 over-expression, while expression of PRDM16 mRNA shows no significant change. **Conclusion:** SIRT1 gene participates in regulating differentiation fate of hVADSCs, and over-expression of hSIRT1 might inhibit conversion of hVADSCs to both white and brown adipose tissue.

Keywords: SIRT1; human adipose tissue-derived stem cells; white fat; brown adipose tissue

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S13.59

Methyl palmitate inhibits cell proliferation but promotes cell migration in human mesenchymal stem cells

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Aim: To investigate the effects of the methyl palmitate (PAME) on the proliferation and migration of human bone marrow mesenchymal stem cells (hMSCs). **Methods:** The hMSCs were incubated with various concentrations of PAME for 48 h or 12 h. Cell proliferation was subsequently measured by the MTT assay and analyzed the cell cycle progression by PI staining using flow cytometry. The cell migration capability after PAME treatment was performed by wound healing assay. **Results:** The results of MTT showed that PAME can inhibit the proliferation of hMSCs in a dose-dependent manner (10, 30, 50, and 100 μmol/L) (P<0.05) after 48 h of treatment. We also observed that G_2/M phase arrest in hMSCs after PAME treatments at different concentrations. Furthermore, We found that 50 μmol/L PAME caused maximal increase in cell migration over untreated cells after 12 h. Additionally, our MTT assays showed that treatment with 50 μmol/L PAME for 12 h did not noticeably increase the proliferation of hMSCs. In addition, medium containing 0.5% bovine serum albumin (BSA, 0.5%), which binds fatty acids, can inhibit the actions of the PAME in the hMSCs. **Conclusion:** Our results show that the PAME inhibits the proliferation of hMSC in a dose-dependent manner through G_2/M cell cycle arrest but promotes cell migration.

Keywords: mesenchymal stem cells; methyl palmitate (PAME); proliferation; migration

S13.60

Effects of magnesium taurate in liposomal formulation on the onset and progression of cataract in galactose-fed rats

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Aim: In our earlier studies topically applied solution of magnesium taurate (MT) delayed the development of cataract in rats. However, *in vitro* studies showed more pronounced effects. This study investigated the anticataract effects of liposomal formulation of topical MT in galactose-fed rats. **Methods:** Sprague Dawley rats (80–100 g) were divided into 3 groups (n=25). Group 1 received normal diet, while groups 2 and 3 received 25% galactose diet. Group 2 was treated with vehicle topically twice daily and group 3 similarly received 1% liposomal form of MT. Diet and treatment continued for 28 d. Cataract progression was accessed by slit lamp and was graded from stages 1–4 based on progression from cortical to nuclear cataract. **Results:** At the end of week 1, 40% and 4% lenses progressed to stage 1b and 1c respectively in group 2 but majority of lenses in group 3 remained in stage 1a. At the end of week 2, in group 2, half of the lenses progressed to stage 2b and more than one-fourth in stage 3 compared to group 3 with none in stage 3. At the end of 3rd week, half of the lenses progressed to either stage 3 or 4 in group 2. At the same time point in group 3 upto 56% of lenses were still in stage 2b. At the end of the experimental period, group 3 had 14% and 30% lenses with stage 2b and 3 respectively and 56% in stage 4 cataract compared to group 2 with 90% lenses in stage 4. **Conclusion:** MT in liposomal formulation showed significant delay in the onset and progression of cataract in galactose-fed rats.

Keywords: magnesium taurate; taurine; liposomes; cataract; galactose-induced

S13.61

Oculohypotensive effect of topical losartan potassium in ocular normotensive and steroid-induced ocular hypertensive rat models

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Aim: Systemically administered angiotensin II receptor blocker, losartan, has been shown to reduce intraocular pressure (IOP) in animals and human. The aim of the current study was to compare the ocular hypotensive effect of topical losartan in normotensive and steroid-induced ocular hypertensive rats. **Methods:** Ocular hypertension was developed in normotensive Sprague Dawley rats by topical instillation of dexamethasone 0.1%, 2 times daily for 36 d. The rats that developed more than 25% rise in IOP were considered ocular hypertensive. Ocular normotensive (n=7) and hypertensive (n=7) rats were treated with single drop (10 μL) of losartan potassium 2.00% in test eye and of vehicle in control eye. IOP estimations were done using calibrated tonometer (TonoLab) at baseline and then hourly and 4 hourly in the first and second 12 h respectively. **Results:** Losartan treatment resulted in IOP lowering in ocular normotensive rats for 8 h post-instillation with peak IOP reduction of 13.3% from baseline at 3 h post-instillation. In ocular hypertensive rats losartan showed IOP reduction for the 11 h post-treatment with maximum IOP reduction of 36.7% at 8 h post-instillation. **Conclusion:** Topical losartan produced significant ocular hypotensive effect in both ocular normotensive and hypertensive model. However, in ocular hypertensive rats the extent of losartan-induced ocular hypotension was significantly greater and the duration of IOP lowering was longer compared to ocular normotensive rats.

Keywords: losartan; intraocular pressure; steroid-induced ocular hypertension

S13.62

Epigallocatechin-3-gallate inhibits homocysteine-induced apoptosis of endothelial cells by demethylation of DDAH2 gene

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Aim: Our previous study showed hypermethylation of dimethylarginine dimethylaminohydrolase 2 (DDAH2) contributes to homocysteine (Hcy)-induced apoptosis of endothelial cells (ECs). Epigallocatechin-3-gallate (EGCG) is a green tea-derived phenol which has beneficial on atherosclerosis. It was demonstrated that EGCG inhibits DNA methyltransferase (DNMT) activity and reactivate methylation-silenced genes in cancer cells. The aim of this study was to address whether EGCG could induce DNA demethylation of DDAH2 gene, contributing to preventing ECs from apoptosis induced by Hcy. **Methods:** ECs were treated with Hcy (1 mmol/L) for 48 h with or without EGCG (20 μmol/L) or 5-Aza (DNMT inhibitor, 5 μmol/L). Apoptosis rate of ECs was assayed by flow cytometry. The mRNA expression level of DDAH2 was detected by real-time PCR. DNA methylation level of DDAH2 was assayed by Methylation Specific PCR. **Results:** The apoptosis rate was decreased significantly in ECs treated with Hcy and EGCG or 5-Aza compared with ECs treated with only Hcy. Furthermore, the mRNA level of DDAH2 was up-regulated, DNA hypermethylation of DDAH2 gene promoter was inhibited and DNMT1 protein level was decreased in ECs treated with Hcy and EGCG or 5-aza compared with ECs treated with only Hcy. **Conclusion:** EGCG exerted protective effects on Hcy-induced apoptosis of ECs by inhibiting promoter hypermethylation of DDAH2 gene and inducing DDAH2 expression.

Keywords: EGCG; DNA methylation; DDAH2
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S13.63

Gelsemine, a principle alkaloid from *Gelsemium sempervirens* Ait, exhibits potent and specific analgesia in chronic pain by acting at spinal α3 glycine receptors

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Aim: To examine the analgesic effects of gelsemine, a principle alkaloid from *Gelsemium sempervirens* Ait. **Methods:** Analgesic effects of gelsemine were determined in formalin-induced nociception, bone cancer-induced mechanical allodynia and spinal nerve ligation-induced painful neuropathy. The relation between gelsemine-induced analgesia and glycine receptor was also determined. **Results:** Single bolus intrathecal injection of gelsemine produced potent and specific analgesia with maximal inhibition of 50%–60% and ED_{50} values of 0.5–0.6 μg. Multiple daily intrathecal injections of gelsemine for 7 d induced no tolerance to analgesia in the rat model of bone cancer pain. The specific analgesia of gelsemine in chronic pain was dose-dependently blocked by the glycine receptor (GlyR) antagonist strychnine with an apparent ID_{50} value of 2.2 μg. Gelsemine concentration-dependently displaced [^{3}H]-strychnine binding to the membrane fraction of rat spinal cord homogenates, with 100% inhibition and a K_i of 21.9 μmol/L. Gene ablation of the GlyR α3 subunit (α3 GlyR) but not α1 GlyR, by a 7-d intrathecal injection of siRNA targeting α3 GlyR or α1 GlyR, nearly completely prevented gelsemine-induced analgesia in neuropathic pain. **Conclusion:** Our results demonstrate that gelsemine produces potent and specific analgesia in chronic pain states without induction of apparent tolerance to analgesia. The results also suggest that gelsemine produces analgesia by activation of spinal α3 GlyRs, and support the notion that spinal α3 GlyRs are a potential therapeutic target for the management of chronic pain.

Keywords: gelsemine; chronic pain; glycine receptor (GlyR); strychnine; GlyR α3 subunit (α3 GlyR)
Acknowledgements: We thank Dr Pei-zhuo ZHANG at GenePharma Inc (Shanghai, China) for synthesis of the gene silencers targeting α1 GlyRs and α3 GlyRs and their corresponding nonspecific oligoneucleotide.

S13.64

Interlaboratory transferability study of the *Pig-a* mutation assay with immunomagnetic enrichment

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Aim: *N*-Ethyl-*N*-nitrosourea (ENU) is a potent mutagen that has been studied as part of an international trial that continues to evaluate the reproducibility and transferability of *Pig-a* mutation assay. The current report extends this line of investigation using an updated method that utilizes immunomagnetic separation prior to flow cytometric scoring. **Methods:** Groups of male Sprague-Dawley rats were given 3 daily doses of ENU at 0, 10, 20, or 40 mg/kg. Bloods collected on days -1, 14 and 30 were evaluated for *Pig-a* mutant frequencies in total peripheral blood erythrocytes and reticulocytes (RETs). Day 4 samples were scored for micronucleated reticulocyte frequencies (%MN-RET). **Results:** While ENU-induced *Pig-a* mutant frequencies were consistent with previously reported results, analysis rates and number of cells evaluated were dramatically increased. ENU also induced significant increases in %MN-RET. **Conclusion:** The results indicate that the new *Pig-a* scoring methodology is reliable and transferable, and support the concept that *Pig-a* mutation and MN analyses can be readily combined into one study.

Keywords: *Pig-a* mutation; immunomagnetic separation; micronuclei
Acknowledgments: The authors thank our collaborators at Litron Labs for including us in these trials and supplying *Pig-a* mutation kits.

S13.65

Protective effect of fibrauretin against lipopolysaccharide-induced acute lung injury in mice

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Aim: In this study, the protective effect of fibrautetin against lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice was investigated. **Methods:** Kunming mice were injected intratracheally with LPS at 5 μg/10 g body weight to induce acute lung injury. Before treatment with LPS, fibrautetin was given intragastrically to mice at 2.25 mg/10 g body weight twice a day for three days. At 12 h after treatment with LPS, the death rate of mice was observed, the protein content in BALF of mice was determined by Lowry method, and the number of PMN in BALF of mice was counted under microscope. In addition, the pathological observation was performed by an experienced pathologic doctor. **Results:** Pretreatment with fibrautetin significantly decreased the death rate of ALI mice at 12 h after treatment with LPS (from 52% to 30%). Fibrautetin decreased the protein content in BALF of ALI mice at 12 h after treatment with LPS (from 0.289±0.014 to 0.217±0.018 μg/mL). Fibrautetin also reduced the percentage of PMN in BALF of ALI mice at 12 h after treatment with LPS (from 21.83%±1.94% to 15.17%±1.17 %). Typical pathological inflammation changes in the lung were observed in ALI mice, including alveolar congestion, hemorrhage, edema, infiltration of neutrophils in the airspace or vessel wall, thickness of the alveolar wall, and hyaline membrane formation. When fibrautetin was pretreated before LPS administration, the inflammatory cell infiltration, the thickness of the alveolar wall were markedly attenuated and alveolar edema was reduced. **Conclusion:** Fibrautetin can decrease the death rate and alleviate acute lung injury induced by LPS in mice.

Keywords: fibrautetin; lipopolysaccharide; acute lung injury; inflammation; mice

S13.66

Necrosis factor-alpha (TNF-α) alters differently to alcohol, paracetamol and tert-Butyl hydroperoxide (t-BHP) induced human hepatoma HepG2 cell injury

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Drug- or alcohol-related hepatotoxicity is the most common cause of liver failure, which has been associated with the induction of oxidant stress and inflammatory stress. The human hepatoma cell line HepG2 is frequently used in *in vitro* models for human biomedical research. In this work we evaluated the suitability of this model to predict hepatotoxicity of alcohol, paracetamol (drug) and tert-Butyl hydroperoxide (t-BHP, oxidizing agent), as well as whether the pro-inflammatory cytokine necrosis factor-alpha (TNF-α) alters these damage. HepG2 cells were pre-incubated with or without TNF-α, and then treated with ethanol (10-100 mmol/L, 24 or 48 h), acetaldehyde (1–10 mmol/L, 24 h or 48 h), paracetamol (1–30 mmol/L, 24 h) and t-BHP (10–200 μmol/L, 3 h), respectively. Cell viability and alanine aminotransferase (ALT) release were measured by MTT assay and fluorometric assay, respectively. We found that, HepG2 cells are highly resistant toward ethanol and acetaldehyde, treatment with ethanol (100 mmol/L) or acetaldehyde (10 mmol/L) for 48 h only caused 10%–15% decrease of cell proliferation and <30% increase of ALT level, although their cytotoxicity can be enhanced by 10%-20% through TNF-α treatment. HepG2 cells were sensitive to paracetamol and t-BHP that both caused time- and dose-dependent cytotoxicity. Pre-treatment with TNF-α resulted in 50%–60% increase in ALT release by paracetamol, while no significant difference was observed in the presence or absence of TNF-α in t-BHP treated cells. These results show that HepG2 cells are a suitable tool for some oxidants and drug metabolism research, cytokine TNF-α affect differently in different substances induced hepatotoxicity. For alcohol-related hepatotoxicity studies, primary hepatocytes or transfected HepG2 cells might be the better choices.

Keywords: HepG2 cells; ethanol; acetaldehyde; paracetamol; t-BHP; TNF-α

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Effects of Mg^{2+}, Ca^{2+}, and Mn^{2+} on mitochondrial oxygen consumption rate

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Aim: Mitochondria are closely related to ischemic disease and neurodegenerative diseases, both of which involve ion dyshomeostasis, mitochondrial dysfunction and oxidative stress. Ca^{2+}, Mg^{2+}, and Mn^{2+} are important participants in these processes. The aim of this article is to study the impact of the three divalent metal cations on the oxygen consumption rate of mitochondria and presume their possible pathophysiological significances. **Methods:** The Clark oxygen electrode was used to detect the oxygen consumption rate of mitochondria isolated from rat brain and heart. Some pharmacological agents were used to explore the possible pathophysiological significances of the effects of Ca^{2+}, Mg^{2+}, and Mn^{2+}. **Results:** Above the concentration of 100 μmol/L Ca^{2+} can inhibit oxidative phosphorylation. Mg^{2+} and Mn^{2+}, in the concentration range of 10–500 μmol/L, can accelerate brain and cardiac mitochondrial oxygen consumption rate in a concentration-dependent manner, and these processes depend on the presence of ATP and respiratory chain oxidative substrate and be inhibited by rotenone, antimycin A and oligomycin. **Conclusion:** Inhibitory effects of Ca^{2+} on oxidative phosphorylation may be associated with the opening of mitochondrial permeability transition pore. The process of rapid mitochondrial oxygen consumption induced by Mg^{2+} and Mn^{2+} may involve the cycle between ATP and ADP. So we infer that Mg^{2+} and Mn^{2+} may protect brain and heart by reducing acidosis and the generation of ROS, and accelerating the oxidation of glutamate, leading to reduced excitotoxicity. We are verifying this hypothesis.

Keywords: mitochondria; oxygen consumption rate; Ca^{2+}; Mg^{2+}; Mn^{2+}

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Catecholamine inputs to expiratory laryngeal motoneurons in rat

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Aim: Many motoneurons and respiratory neurons are demonstrated to receive catecholamine inputs, but the extent and distribution of these inputs to expiratory laryngeal motoneurons (ELM) in the caudal nucleus ambiguus (NA) is unknown. In the present study, we aim to: 1) examine whether or not close appositions exist between tyrosine hydroxylase immunoreactive (TH-ir, *ie*, catecholaminergic) boutons and ELM by using intracellular labeling combined with immunohistochemistry, and, if so, identify the origins of the inputs, and, if yes, 2) identify the potential origins of the catecholamine inputs by using tract-tracing study. **Methods:** we examined catecholamine inputs to ELM using a combination of intracellular labeling and immunohistochemistry in adult Sprague-Dawley rat. ELM were identified *in vivo* by their post-inspiratory firing pattern and antidromic responses to electrical stimulation of the recurrent laryngeal nerve. Further tract-tracing study was carried by injecting cholera toxin β (CTB) into the caudal NA. The retrogradely labeled neurons and the TH-ir structures were revealed by dual fluorescence immunohistochemistry. **Results:** TH-ir close appositions were found on all ELMs, with an average number of 18±5 per neuron (n=7, mean±SD). Close appositions were commonly observed on the distal dendrites rather than on proximal ones, but none on somata or axons. Further tract-tracing study revealed that a large number of CTB labeled neurons in the ipsilateral nucleus tractus solitarii (NTS) and area postrema (AP) were catecholaminergic, and the highest density appeared at 0.2–0.4 mm caudal to the obex. **Conclusion:** We demonstrated for the first time that the ELM receive TH-ir close appositions, suggesting that the catecholamine inputs may play a role in the activity of ELM. The afferents of the superior laryngeal nerve (SLN) project to the NTS neurons at the same longitudinal level, our further tract-tracing study suggests that catecholaminergic neurons located in the NTS at the obex level may play an important role in control of the SLN-mediated airway protective reflexes.

Keywords: laryngeal motoneurons; tyrosine hydroxylase; nucleus ambiguus; nucleus tractus solitarii; intracellular recording

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A simplified genotyping strategy reducing the detection cost of individualized medicine

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Aim: Compared to gel-based Sanger sequencing method, pyrosequencing is much more suitable for individualized medicine due to the straightforward readout and easy protocol. However, template preparation prior to pyrosequencing is costly. To allow individualized medicine at a low cost, the protocol for pyrosequencing should be further simplified. As the production of templates for pyrosequencing is the most tedious and costly step, the main purpose of this paper is to improve PCR process. **Methods:** An efficient asymmetric amplification method termed as LATE-PCR was proposed to generate ssDNA amplicons directly for pyrosequencing. In addition, a novel multiplex PCR with a primer consisted of a target-specific sequence and a tagged sequence for supplying a universal priming site was developed. **Results:** As a-proof-of-concept, 7 SNPs in the genes encoding cytochrome P450 2D6 and sulphotransferase 1A1 were employed for the investigation. These SNPs are related to the levels of the active metabolite of tamoxifen, which is used for the treatment of breast cancer. By a simple optimization of multiplex PCR condition, only a 4-plex and a 3-plex PCR are required for typing the 7 SNPs with pyrosequencing. **Conclusion:** As a single multiplex-PCR and a single step of ssDNA preparation are performed for multiple targets, the whole process for multiplex SNP typing becomes time-saving, labor-saving, sample-saving, and cost-saving. By coupling PCR with whole blood as starting material, the protocol was greatly simplified, and the cost is 3-times reduced in comparison with conventional pyrosequencing protocol; thus, more patients can afford the individualized medicine.

Keywords: pyrosequencing; genotyping; multiplex-PCR;tamoxifen; individualized medicine

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Hepatic bile acids and bile acid-related gene expression in pregnant and lactating rats

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Aim: Intrahepatic cholestasis of pregnancy (ICP) is a liver disease which may occur in the third trimester of pregnancy. The etiology and pathogenesis of ICP is thought to be closely related to bile acid metabolism. The objective of this study was to examine the regulation of bile acid metabolism in normal pregnant and lactating rats. **Methods:** Livers from timely pregnant SD rats were collected on gestational days (GD) 10, 14, and 19, and postnatal days (PND) 1, 7, 14, and 21. Total bile acids were determined by the enzymatic method, total RNA was isolated and subjected to real time RT-PCR analysis. Liver protein was extracted for Western blot. **Results:** Under normal physiological conditions hepatic bile acids was not elevated during pregnancy but was increased during lactation. Bile acid synthesis enzyme Cyp7a1 was slightly reduced on GD19, but increased on PND14 to 19 at both mRNA and protein levels. The small heterodimer partner (SHP) was highly expressed at GD19 (3-fold higher than controls), and farnesoid X receptor (FXR) was increased at postpartum. Bile acid uptake transporters Ntcp, Oatp2 and efflux transporters Bsep, Mrp3 were elevated more than 2-fold during lactation. **Conclusion:** Under normal physiological conditions, hepatic bile acids are not elevated during pregnancy, probably due to the high expression of SHP, but during lactation Cyp7a1 is increased, together with increased liver bile acid and the expression of bile acid transporters.

Keywords: pregnancy and lactating rats; liver bile acids; Cyp7a1 and SHP; Ntcp and Bsep

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A new method for preparation of mouse germ cell slides

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Aim: To explore a new quick method for preparation of mouse germ cell slides for immunofluorescence assay. **Methods:** Different developing stage mice (16, 21, 28, and 120 d) were treated with colchicine by intraperitoneal injection and testes were removed 3 h later. Seminiferous tubules were separated and were minced in cold PBS. Germ cells were filtered and collected by centrifugation. After 3 times wash, germs cells were treated with hypotonic and were fixed to prepare slides. Immunofluorescence testes were performed to assay the quality of the slides. **Results:** Different developing stage mice (16 d, 21 d, 28 d and 120 d) germ cells were successfully prepared on slides. After stained with DAPI, various germ cells were observed and the background was clear on each slides. On these slides, abundant of spermatocytes were dispersed on 16, 21, and 28 d slides and meiotic stage germ cells were clearly observed, but spermatogoniam and spermatocytes were rare on 120 d slides. Round spermatids, various developing elongated spermatid and spermatozoa were abundantly on 120 d slides, but not on 16 d and 21 d slides. The slides of 21 d mice were used in immunofluorescence testes and the result revealed that the target proteins were detected and the background was clear. **Conclusion:** A quick method for preparation of mouse germ cells was successfully established, providing the foundation for the study of the process of spermatogenesis.

Keywords: mouse testis;cell slides; hypotonic; spermatogenesis