S12.1

Structural elucidation of metabolites of lapachol in rats by liquid chromatography–tandem mass spectrometry

Lu BAI, Yin HAN, Xiao-rong LI, Pin-xiang XU, Xiao-hui LIU, Ming XUE*. *Department of Pharmacology, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China*

Aim: Drug metabolic profile plays an important role in discovering and developing a novel drug from metabolites possessing pharmacological activities. Lapachol is a major active component isolated and identified from the *Tabebuia avellandedae* herbs, which has been found to possess many biological activities. The aim of our work is to study the metabolite structures. **Methods:** Nine phase I metabolites and four phase II metabolites of lapachol in rat bile were elucidated and identified by a sensitive LC-ESI-MS^n method. **Results:** The molecular structures of the metabolites are presented on the basis of the characteristics of their precursor ions, product ions and chromatographic retention times. The results indicate that the phase I metabolites were mainly biotransformed by three main routes: hydroxylation, hydrogenation, and hydrolysis metabolism. Phase II metabolites were mainly identified as the glucuronidation conjugates which showed a characteristic neutral loss of 176 Da. The biotransformed pathways of lapachol are also proposed on the basis of these metabolite structures. **Conclusion:** This investigation provides the valuable information on lapachol metabolism which is essential for understanding the safety and efficacy of this compound as well as for the development of a novel drug.

Keywords: structural elucidation; lapachol; metabolites; LC-ESI-MS^n

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S12.2

Polymorphisms of CYP1A1 and GSTM1 genotypes and lung cancer susceptibility in Hui nationality

Fu-hou CHANG*, Tu-ya BAI, Lei FAN, Min-jie WANG. *School of Pharmacy, Inner Mongolia Medical College, Hohhot 010059, China*

*Corresponding author, E-mail: changfh@sina.com

Aim: To study the polymorphisms of CYP1A1 and GSTM1 and the susceptibility of lung cancer in Hui nationality of China. **Methods:** ASA and a multiplex PCR were employed to identify the genotypes of CYP1A1 and GSTM1 in a case-control study of 300 lung cancer patients with bronchoscopy diagnosis and 300 matched controls free of malignancy. **Results:** The frequencies of the variant CYP1A1 (Val/Val) genotypes and GSTM1(-) in lung cancer groups were higher than that in control groups (18.3% *vs* 8.1% and 62.32% *vs* 45.16%). The individuals who carried with CYP1A1 (Val/Val) or GSTM1(-) genotype had a significantly higher risk of lung cancer, the OR is 3.12 and 2.10, respectively. Stratified histologically the relative risk increased to 3.2-fold when the patients carried with two valine alleles than the ones carried one valine allele in cases of SCC. GSTM1(-) genotype is the risk factor of SCC (OR=2.18) and AC (OR=1.24). **Conclusion:** The valine allele of CYP1A1 and GSTM1(-) were the risk factors of lung cancer especially for SCC of Hui nationality in China. There may be a synergetic interaction between CYP1A1 valine allele and GSTM1(-) genotypes on the elevated susceptibility of lung cancer.

Keywords: polymorphism; genotype; lung neoplasma; cytochrome P450; glutathione *S*-transferase

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S12.3

Regulation of CYP3A4/CYP2B6/CYP2C9 transcription through nuclear receptor PXR by helicid and its metabolites

Qun CHEN, Hua SUN, Zhi-chen PU, Hua HU, Hai-tang XIE. *Anhui Provincial Center for Drug Clinical Evaluation, Yijishan Hospital, Wuhu 241001, China*

Aim: To establish and validate an *in vitro* system to screen drug inducers of CYP3A4, CYP2B6, and CYP2C9 and study hPXR (human pregnane X receptors)-mediated transcriptional regulation of CYP3A4, CYP2B6, and CYP2C9 by a traditional Chinese medicine helicid and its two metabolites. **Methods:** The promoters of CYP3A4, CYP2B6, CYP2C9 were cloned, which contained the elements that hPXR, a kind of nuclear receptor, can recognize and bind. The trans-element was inserted into the upstream of firefly luciferase reporter gene of the pGL4.17 vectors. The report vector and hPXR expression plasmid were co-ntransfected into HepG2 cell line. After 24 h, the transfected cells were treated with helicid at 0.004, 0.04, and 0.4 μmol/L and its metabolite I and metabolite II (0.0004, 0.004, and 0.04 μmol/L) for 48 h, while rifampin (10 μmol/L) was used as the positive control, 0.1% DMSO as a negative control. Cells were lysed and luciferase activity was determined using a dual-luciferase reporter assay kit. **Results:** Helicid and its metabolites I and II at tested concentrations did not significantly increase promoter activities of CYP3A4, CYP2B6, and CYP2C9 in HepG2 cells transfected with PXR expression plasmid (P>0.05). **Conclusion:** Nuclear receptor PXR-expressed CYP3A4, CYP2B6, and CYP2C9 dual luciferase reporter gene platforms were successfully established, and helicid and its metabolites I and II did not significantly induce the transcription of CYP3A4, CYP2B6, and CYP2C9. The establishment of reporter gene system is an efficient and easy methods for large-scale screening of CYPs inducers.

Keywords: helicid; human pregnane X receptors; CYP3A4; CYP2B6; CYP2C9

S12.4

Liquid chromatography-mass spectrometry based metabonomic investigation into the potential biomarkers for acute repetitive hypoxia

Hai-ting CHENG, Ming-ming WANG, Su SU, Pin-xiang XU, Ming XUE*. *Department of Pharmacology, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China*

Aim: To identify and character the biomarkers in mice suffered the repetitive hypoxia. **Methods:** A metabonomic method utilizing LC-ESI-MS^n and multivariate statistical technique has been successfully applied to investigate the metabolic profiles of hypoxic preconditioning in mice by the model of acute repetitive hypoxia. Both the brain and heart tissues were collected for studying the potential biomarkers via comparing the tissues from the normal mice and the hypoxic preconditioning mice. The tissue samples were analyzed by LC-ESI-MS^n. After de-noise, peak detection and peak alignment, the data of the metabolites were fed to partial least squares discriminate analysis (PLS-DA) and pattern recognition to find the potential biomarkers. The candidate molecules of the potential biomarkers were searched from the Human Metabonomic Database (HMDB) and METLIN databases. **Results**: According to the corresponding the accurate molecular weight, retention time and tandem mass results, several potential biomarkers and their levels in the tissues were identified. The results indicated that phenylalanine, valine, proline, leucine and glutamine were increased, while creatine was decreased, both in brain and heart of the hypoxia group. In addition, γ-aminobutyric acid was markedly decreased in brain of the hypoxia group. **Conclusion:** The biological significance of the present study was discussed by referring to the relevant literature and databases.

Keywords: metabonomics; biomarker; acute repetitive hypoxia; brain; LC-ESI-MS

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S12.5

Assay of plasma protein binding rate of puerarin with microdialysis *in vitro* tests

Jia-yi CHENG[1], Wen-bin DONG[2], Ai-ying CHEN[2], Gao-li ZHENG[2, *]. *[1]School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310013, China; [2]Institute of Materia Medica, Zhejiang Academy of Medical Sciences, Hangzhou 310013, China*

Aim: To study the plasma protein binding rate of puerarin. **Methods:** The microdialysis-HPLC method was applied to determine the plasma protein binding rate of puerarin. **Results:** The plasma protein binding rate of puerarin with rate plasma at the concentration of 20, 60, and 200 μg/mL were 34.86%, 33.82%, and 25.09%, respectively, while the plasma protein binding rate of puerarin with normal human plasma at the above concentrations were 31.69%, 31.02%, and 22.90%, respectively. **Conclusion:** Puerarin belongs to low protein bounding drugs.

Keywords: puerarin; high protein bounding; microdialysis; zero-net flux; HPLC

S12.6

Contribution of baicalin on the plasma protein binding displacement and CYP3A activity inhibition to the pharmacokinetic changes of nifedipine in rats *in vivo* and *in vitro*

Zhen-yu CHENG, Xin TIAN, Jie GAO, Hong-meng LI, Hai-ling QIAO*. *Department of Clinical Pharmacology, School of Medicine, Zhengzhou University, Zhengzhou 450052, China*

*To whom correspondence should be addressed.
E-mail: qiaohl@zzu.edu.cn

Aim: Baicalin purified from the root of *Radix scutellariae* has been widely used in clinical practices. This study aimed to evaluate the effect of baicalin on the pharmacokinetics of nifedipine, a CYP3A probe substrate, in rats *in vivo* and *in vitro*. **Methods:** Nifedipine (2 mg/kg) was administered to rats immediately following the injection of baicalin (0.225 and 0.45 g/kg) or saline in a Latin-Square design. The nifedipine concentrations in rat plasma or liver microsomes (RLMs) were determined by HPLC. **Results:** Significant changes in pharmacokinetics of nifedipine were observed after treatment with baicalin in rats. The maximum concentrations (C_{max}) of total nifedipine decreased by about 40% and 65% (P<0.01), area under plasma concentration–time curve ($AUC_{0-\infty}$) decreased by about 41% and 63% (P<0.01), apparent volume of distribution (V_d) increased by about 85% and 224% (P<0.01), clearance (CL) increased by about 97% and 242% (P<0.01), in 0.225 and 0.45 g/kg baicalin-treated groups, respectively. *In vitro* results indicated that baicalin was a competitive displacer of nifedipine from plasma proteins. Baicalin could competitively inhibit CYP3A activity in RLMs in a concentration-dependent manner. **Conclusion:** The pharmacokinetic changes of nifedipine may be caused by the inhibitory effects of baicalin on its plasma protein binding and CYP3A–mediated metabolism.

Keywords: baicalin; nifedipine; pharmacokinetics; rats; plasma protein binding

S12.7

HZ08 inhibits the multi-drug resistance on multiple sites as the substrate of p-glycoprotein

Yi-dong FENG, Wei-rong FANG, Yun-man LI*
Department of Physiology, School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China
*To whom correspondence should be addressed.

Aim: Over expression of p-glycoprotein (p-gp) leads to the production of multi-drug resistance (MDR) which could discharge various anti-tumor chemicals with structural heterogeneity. HZ08, a novel tetrahydroisoquinoline derivate, was discovered to modulate the MDR. What was confirmed is its definite inhibition of multi-drug resistance caused by P-gp and its promotion for the intracellular cytotoxins accumulation in the previous study. We aimed explore whether HZ08 is the substrate of P-gp and on which sites it exerts its function. **Methods**: RNAi to *mdr1* was introduced and the interaction between HZ08 and some classic agents (verapamil, rhodamine 123) with clearly binding sites were also investigated. **Results**: Experimental results revealed that HZ08 is the most probable the substrate of P-gp and may share the same modulation sites located at the p-gp with verapamil. Data obtained also indicated that there is a common binding site shared by rhodamine 123 and HZ08, but negative competition showed between HZ08 and adriamycin. **Conclusion**: HZ08 may be the substrate of p-gp and acts as a multiple target modulator to invert the efflux function of p-gp.

Keywords: HZ08; p-glycoprotein; multi-drug resistance; drug interaction

S12.8

Inhibitory effect of baicalin on the plasma protein binding and CYP1A2 activity induce the pharmacokinetic changes of theophylline in rats

Na GAO, Yan FANG, Bing QI, Lin-jing JIA, Hai-ling QIAO*. *Department of Clinical Pharmacology, School of Medicine, Zhengzhou University, Zhengzhou 450052, China*
*To whom correspondence should be addressed.
E-mail: qiaohl@zzu.edu.cn

Aim: To elucidate the effect of baicalin on the pharmacokinetics of theophylline in rats, focusing on plasma protein binding displacement and inhibitory effect on CYP1A2 *in vivo* and *in vitro*. **Methods:** This study was a randomized, three-period crossover design. Theophylline (5 mg/kg, iv) was administered to rats immediately following the injection of baicalin (450 mg/kg, given 1 time or divided into 3 times). The effect of baicalin on CYP1A2 activity was determined by metabolism of phenacetin and plasma protein binding of theophylline was determined by ultrafiltration *in vitro*. **Results:** C_{max} of theophylline decreased by 30% and 31%, $T_{1/2}$ increased by 116% and 71%, V_d increased by 51% and 49% in different treatment group (baicalin 450 mg/kg given 1 time or divided into 3 times), respectively. No significant effects on the CL and AUC of theophylline were observed in the rats treated with baicalin given 1 time, but the CL significantly decreased and AUC increased in the group treated with baicalin divided into 3 times compared with control (P<0.05). Baicalin inhibited metabolism of phenacetin and exhibited a mixed-type inhibition in rat liver microsomes *in vitro*. Moreover baicalin was a competitive displacer of theophylline from plasma protein *in vitro*. **Conclusion:** The changes of theophylline in pharmacokinetics may be attributed to two mechanisms of baicalin, plasma binding displacement and metabolic inhibition.

Keywords: baicalin; theophylline; pharmacokinetics; CYP1A2; protein binding

S12.9

Validated spectrophotometric determination of diclofenac sodium

Jun Zheng GOH[1, *], Hoe Siong CHIONG[1], Muhammad Nazrul HAKIM[1, 2]. *[1]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; [2]Sports Academy, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
*Corresponding author.
E-mail: junzhenggoh@hotmail.com

Aim: A reliable assay for quantitative determination of pharmaceutical products is vital. Hence, present study was conducted to develop a rapid and reproducible spectrophotometric method to quantify a widely used non-steroidal anti-inflammatory agent in therapeutics, diclofenac sodium. **Methods:** A simple and economical spectrophotometric analytical procedure with estimation in UV-vis region was performed on diclofenac sodium using dimethyl sulfoxide as solvent. The parameters included time, temperature and types of solvent were studied for 20 μg/mL drug solution at 295 nm. All parameters and results of the analysis were statistically validated. **Results:** Under optimized parameters, Beer's law was obeyed in the range of 0.625–40 μg/mL at λ_{max} 295 nm. A linear working range of 5–35 μg/mL with regression coefficient of 0.9978 was obtained by using seven triplicate analyses of drug samples with seven different concentrations. The limit of detection and limit of quantitation was 1.194615 and 3.620 μg/mL, respectively. **Conclusion:** Present study successfully demonstrated an optimized and accurate procedure as per the guidelines by International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) for detection and evaluation of diclofenac sodium.

Keywords: NSAIDs; diclofenac sodium; UV-vis spectroscopy; spectrophotometry

S12.10

Nicotinamide phosphoribosyltransferase is required for the calorie restriction-induced improvements of metabolism

Yun-feng GUAN, Pei WANG, Jie SONG, Chao-yu MIAO. *Department of Pharmacology, Second Military Medical University, Shanghai 200433, China*

Aim: This study was designed to investigate whether nicotinamide phosphoribosyltransferase (NAMPT) plays an important role in the beneficial effects induced by calorie restriction (CR). **Methods:** FK866, a specific chemical inhibitor of NAMPT, was used. Sprague-Dawley rats were divided into 4 groups: *ad libitum* (AL), CR, AL+FK866 and CR+FK866 groups. AL and AL+FK866 animals were allowed unlimited access to standard chow, while the CR and CR+FK866 animals were restricted to 60% of the food intake consumed by AL and AL+FK866 animals. **Results:** CR upregulated NAMPT mRNA and protein levels in rat skeletal muscle and white adipose tissue. Inhibition of NAMPT did not affect the SIRT1 upregulation by CR but suppressed the CR-induced SIRT1 activity and deacetylation of Foxo-1. In addition, inhibition of NAMPT blocked the CR-induced improvement of whole-body insulin sensitivity, evidenced by insulin tolerance test and glucose tolerance test. At last, inhibition of NAMPT impaired CR-induced Akt signaling activation and eNOS phosphorylation. **Conclusion:** Our data demonstrate that the CR-induced beneficial effects in metabolism require NAMPT.

Keywords: nicotinamide phosphoribosyltransferase; insulin sensitivity; metabolism; calorie restriction; Akt
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S12.11

Determination of atomoxetine in human serum by HPLC-UV method using protein precipitation and on-line solid phase extraction

Jing HE, Wen-biao LI*, Can-jun RUAN. *Laboratory of Clinical Psychopharmacology, Beijing Key Lab of Mental Disorders, Beijing Anding Hospital, Capital Medical University, Beijing 100088, China*

Aim: To develop an HPLC-UV method for the determination of atomoxetine (ATM) in human serum with simple sample preparation and higher automation. **Methods:** After protein precipitation (PP) of the serum sample, 750 μL supernatant was injected into the HPLC system with two gradient pumps and a six port switching valve to do on-line solid-phase extraction by a reverse-phase C_{18} column (Waters XBridge, 4.6 mm×30 mm, 5 μm) and a gradient mobile phase consisted of acetonitrile and phosphate buffer. The eluent containing the analyte and internal

standard maprotiline (MPL) was transferred into an analytical column (XBridge C_{18}, 4.6 mm×150 mm 3.5 μm) by column switching to do further separation. The ultra-violet detection wave length was 210 nm. **Results:** Linear range of the method was 3.125–1600 ng/mL ATM in serum. The lowest limit of quantification was 6.25 ng/mL. This method was verified with within- and between-batch precisions of 0.6%–3.96% and 1.64%–4.15%, respectively. The within- and between-batch biases were 2.66%–13.95% and 4.39%–9.53%, respectively. The recovery rates of ATM and MPL through PP are 100%–106% and 98%, respectively. Commonly used psychotropic drugs and frequently co-administered drugs did not interfere with ATM and MPL. Comparing the external quantification method with internal, the results showed no difference. **Conclusion:** This is a highly specific, precise, and accurate method with simple sample preparation and robust method for the determination of atomoxetine in human serum and could be used in TDM or PK analysis.

Keywords: HPLC-UV; human serum; protein precipitation; on-line solid phase extraction; atomoxetine

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S12.12

Pharmacokinetic and pharmacodynamic properties of batifiban coadministered with antithrombin agents in Chinese healthy volunteers

Xiao-meng HE[1], Ying ZHOU[1], Jie LI[1], San-lan WU[1], Meng-meng JIA[1], Ming-zhou LIU[1], Hui CHEN[1], Ke CHEN[2], Sheng-feng LI[3], Yao-hua WANG[3], Wei-yong LI[1, *]. *[1]Institute of Clinical Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China; [2]Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China; [3]Biothera Solutions Ltd, Guangzhou, China*

Aim: To observe whether antithrombin agents affects the pharmacokinetic and pharmacodynamic properties of batifiban in combination therapy and optimize clinical administration dosage of batifiban. **Methods:** An open-label, parallel study was conducted. Thirty healthy subjects were randomly divided into three groups, which were sequentially treated with batifiban alone, or oral coadministration of clopidogrel, aspirin and UFH, or batifiban coadministered with these antithrombin agents. Blood samples were collected at pre-specified times. The evaluation index included the inhibition of platelet aggregation and pharmacokinetic parameters. **Results:** The pharmacokinetic parameters of batifiban and batifiban coadministered with antithrombin agents showed no significant differences. The mean inhibition rate of platelet aggregation (%) suggested that neither batifiban alone nor antithrombin agents alone could provide such potent inhibition rate (>80%) to obtain the best clinical efficacy, but they have a synergistic effect on platelet inhibition. No serious adverse effects were observed. **Conclusion:** The results in these healthy subjects suggest that batifiban coadministrated with antithrombin agents could achieve optimum clinical treatment effect for patients with NSTE ACS, and also those scheduled for percutaneous coronary intervention.

Keywords: batifiban; glycoprotein IIb-IIIa inhibitors; antithrombin agents; pharmacokinetics; pharmacodynamics

S12.13

Isolation and identification of active metabolites of [Ile1,Thr2]-63-desulfatohirudin in rats

Su-yun JIANG[1, *], Jian JIAO[1], Ting-ting ZHANG[1], Xiao-yu LI[2],Guo-zhu HAN[3]. *[1]Dalian Institute for Food and Drug Control, Dalian 11621, China; [2]Dalian University of Technology, Dalian 116024, China; [3]Dalian Medical University, Dalian 116027, China*

*Corresponding author.

E-mail: syjiangsy@263.net

Aim: To isolate and identify the active metabolites of recombinant hirudin. **Methods:** Blood and urine samples of rats that had been injected intravenously with recombinant hirudin that harbors an N-terminal amino acids sequence of ITY, and radiolabeled with ^{125}I (^{125}I-recombinant hirudin), were collected, separated by gel filtration chromatography, and monitored by the gamma-counter. ELISA and the chromogenic substrate assay were employed to determine immunogenicity and anti-thrombin activity, respectively, in the elutions. Elutions that exhibit anti-thrombin activity were further purified by reverse phase high performance liquid chromatography (RP-HPLC). The molecular weight and the amino acid sequence of the purified recombinant hirudin metabolite were determined. **Results:** The recombinant hirudin is metabolized in the plasma, whose metabolites, presented in the urine, exhibited the anti-thrombin activity, but not hirudin immunogenicity. The anti-thrombin metabolite of recombinant hirudin is identified to be the N-terminal polypeptide fragment of recombinant hirudin, consists of the same first 52 amino acid residues as those in the recombinant hirudin, with a molecular weight of 5162 Da.

Keywords: recombinant hirudin; urinary metabolites; rats; pharmacokinetics

S12.14

Cross-species comparison of drug metabolism in liver microsomes using LC-HRMS based metabolomics

Hua LI[1], Xiao-mei ZHUANG[1], Yan TAN[1], Ke-rong ZHANG[2]. *[1]Institute of Pharmacology and Toxicology, AMMS, Beijing 100850, China; [2]AB SCIEX China, Shanghai, China*

Aim: Understanding species difference in drug metabolism is crucial for exploration of animal PK data to human. In this study, an LC-MS/MS based metabolomics approach was applied for cross-species comparison of drug metabolism in liver microsomes from 5 species, with isoimperatorin (ISOIM) and imperatorin (IM) as model drugs. **Methods:** ISOIM and IM (20 μmol/L) were incubated, in the presence of NADPH, with liver microsomes (1 mg/mL) from human, monkey, dog, rat, and mouse at 37 °C for 60 min. The blank and test samples were analyzed in parallel using LC-QTOFMS/MS. MS data were recorded using real time Mass Defect Filtering (MDF)-Information Dependent Acquisition (IDA) workflow for simultaneous metabolite identification and quantification. **Results:** A total of 26 metabolites for ISOIM and 17 metabolites for IM were found in liver microsomes. Oxidation was the major metabolic pathway for both ISOIM and IM. A number of mono-, di-, tri-oxidative metabolites were identified. ISOIM and IM showed different stability in liver microsomes. Metabolomic comparison revealed that the metabolite profiles of ISOIM in human, mouse and rat liver microsomes significantly differ from those in monkey and dog. In the case of IM, the profiles in human and dog remarkably differ from those in monkey, rat, and mouse. **Conclusion:** Significant species differences were observed in liver microsomal stabilities and metabolic profiles of ISOIM and IM.

Keywords: metabolism; species difference; isoimperatorin; imperatorin

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S12.15

Pharmacokinetic comparisons of Yu Ping Feng San with the different combinations of its constituent herbs

Tao LI, Yi-wei WANG, Yan-li WANG, Ri-xin LIANG, Dong ZHANG, Hui-hui ZHANG, Li CHEN, Zhong-ming ZHOU, Wei-peng YANG*. *Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China*

*To whom correspondence should be addressed.

E-mail: yangweipeng@icmm.ac.cn

Aim: Yu Ping Feng San (YPFS) is a well-known traditional Chinese medicine formula, containing Radix Saposhnikoviae (RS), Radix Astragali (RA) and Rhizoma Atractylodis Macrocephalae (RAM). Cycloastragenol and cimifugin are the key metabolites, which show good pharmacokinetic properties *in vivo* and simultaneously possess strong biological activities relevant to the effects of YPFS. The aim of this study is to compare the pharmacokinetics of cycloastragenol and cimifugin in rat plasma after oral administration of YPFS with different combinations of its constituent herbs. **Methods:** Male Wistar rats were randomly and equally divided into six treatment groups and orally administered with YPFS, RS & RAM, RS & RA, RA & RAM, RS, and RA, respectively. Plasma samples were collected and analyzed with a validated SPE-HPLC-MS method. The concentration-time data were then calculated by non-compartmental pharmacokinetic methods using WinNonlin software. **Results:** The main pharmacokinetic parameters of cycloastragenol for YPFS, RS & RA, RA & RAM, and RA group were: T_{max} (time to reach the maximum plasma concentration) 12.0, 8.0, 12.0, and 8.0 h; C_{max} (maximum concentrations in plasma) 15.08, 4.66, 5.46, and 11.97 ng/mL; AUC_{last} (area under the curve from the time of dosing to T_{last}, where T_{last} is the time of last measurable mean concentration) 186.4, 81.78, 86.51, and 93.4 h·ng/mL, respectively. The main pharmacokinetic parameters of cimifugin for YPFS, RS & RA, RS & RAM, and RS group were: T_{max} 2.0, 4.0, 0.5, and 0.5 h; C_{max} 1320.7, 1478.9, 1518.3, and 6992.8 ng/mL; AUC_{last} 10559.6, 11557.0, 12120.0, and 6992.8 h·ng/mL, respectively. **Conclusion:** Marked differences in the pharmacokinetic parameters of cycloastragenol and cimifugin were found in different combinations of YPFS, indicating potential herb-herb pharmacokinetic interactions among the ingredients in YPFS. This study could be helpful for clarifying the compatible mechanisms of constituent herbs in

YPFS.

Keywords: Yu Ping Feng San; pharmacokinetics; cycloastrageno; cimifugin; herbal combination

S12.16

Development of a new strategy for the analysis of chemical medications in serum with HPLC using protein precipitation, on-line SPE and column switching

Wen-biao LI*, Jing HE, Wei GUO, Zuo-li SUN, Can-jun RUAN. *Laboratory of Clinical Psychopharmacology, Beijing Key Lab of Mental Disorders, Beijing Anding Hospital, Capital Medical University, Beijing 100088, China*

*Corresponding author.

E-mail: wenstonli@yahoo.com.cn

Analysis of drug concentration in serum/plasma with high performance liquid chromatography (HPLC) is widely used in both overall drug development process and therapeutic drug monitoring (TDM). Commonly used HPLC is ordinarily a single channel system consists of one pump and one column with auto-sampler, detector and working station. Biological samples must be pretreated before injection into the system. Conventional sample preparation techniques include liquid-liquid extraction (LLE), solid-phase extraction (SPE) and protein precipitation (PP). The former two techniques are always labour intensive, low detection through-put and error prone processes with multi-step manual operations. PP is a simple and easy handling technique, but the supernatant is always impure with large volumn.

Dual chanel HPLC with two pumps, two columns and one switching valve has been developed in the last decades to do on-line SPE with direct injection of serum or plasma into the system. Three types of on-line SPE columns are commercially available: the different kinds of reverse-phase (RP) columns, restricted access material (RAM) columns and turbulent flow chromatography (TFC) columns. RP columns are easily to be contaminated and clogged, and need to be changed frequently. RAM and TFC columns function as a pre-column, in combination with an analytical column and column switching, to remove proteinaceous materials prior to analytical chromatographic separation. Many on-line SPE methods have been reported for the determination of different kinds of medications/metabolites in serum or plasma. But these methods have not been widely used, because of short on-line SPE column life-time, low drug recovery and high costs.

We have developed a new strategy by combining PP, on-line SPE and column switching together. The protein precipitation reagent we used has a very high protein removal efficiency (>99.4%), high drug extraction efficiency (>70%) and low organic solvent usage (less than the sample volumn). After centrifugation, the supernatant is directly injected into the HPLC system with large volumn (up to 1000 μL). The on-line SPE column is a short RP C_{18} column, and can durate for more than 3000 injections without significant column pressure rising. Only the eluent containing the analytes is swithched into the analytical column to do further separation and detection. Most of the impurities are washed directly to the waste. We have already successfully developed several new methods for the determination of atomoxetine, clozapine, *N*-desmethylclozapine, quetiapine, olanzapine, risperidone and 9-hydroxyrisperidone in human serum. All these HPLC methods are highly specific, precise and accurate with long duration of the on-line SPE column, simple sample preparation and high degree of automation.

Keywords: protein precipitation; on-line SPE; column switching; high performance liquid chromatography

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S12.17

Hepatotoxicity of pyrrolizidine alkaloid-containing natural products and development of the mechanism-based biomarker for clinical assessment

Ge LIN. *School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China*

It is now well recognized that the concept of no adverse effect and/or toxicity of traditional Chinese medicines (TCMs) is a misnomer. Similar to orthodoxdrugs, TCMs may also induce toxicity. In this presentation, using pyrrolizidine alkaloids (PAs)-containing natural products as an example, safety of TCMs will be illustrated. PAs are widely present in plant kingdom. PAs have been detected in herbal plants, teas, and dietary supplements, and also in foodstuffs, including grain crops, honeys and milks. Yearly numerous PAs poisoning cases occur worldwide. PAs are considered as a group of natural toxins with a high risk of acute poisonings, prolonged toxicities and potential tumorigenicity. PAs are pro-toxins and induce toxicity via metabolic activation to generate pyrrolic metabolites, which are chemically reactive and react rapidly with cellular macromolecules to form pyrrole-protein adducts leading to hepatotoxicity. Due to metabolites instability, to date there are no available method to analyze them and no clinical approach to diagnose PAs intoxication. Our research team has been working on PA-induced toxicity for many years. Recently, we developed pyrrole-protein adducts as the mechanism-based biomarker and determined the min blood samples of the patients who ingested PA-containing herbs in China or who exposed to PA-contaminated food stuffs in other country, demonstrating the successful development of a biomarker for the diagnosis and assessment of hepatotoxicity induced by PA-containing herbs and PA-contaminated foodstuffs.

Keywords: mechanism-based biomarker; pyrrolizidine alkaloids; hepatotoxicity; toxicity of natural products

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S12.18

Combined contributions of impaired hepatic CYP2C11 and intestinal breast cancer resistance protein activities and expression to increased exposure of oral glibenclamide in streptozotocin-induced diabetes mellitus

Hai-yan LIU[1], Xiao-dong LIU[2], Li LIU[2], Sen YU[1], Jia LI[2], Dan MEI[2], Ru DUAN[2]. *[1]Jiangsu Chia Tai Tianqing Pharmaceutical Co Ltd, Nanjing 210023, China; [2]Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, China*

Aim: To evaluate the contribution of the impaired cytochrome P450 and breast cancer resistance protein (BCRP) activity and expression to drug pharmacokinetics under diabetic states. **Methods:** Diabetic was induced in rats by intraperitoneal administration of streptozocin. Glibenclamide (GLB), a substrate of BCRP, served as a model drug. The pharmacokinetics of orally administered GLB (10 mg/kg) were studied. **Results:** Diabetes mellitus significantly increased exposure (area under the curve and peak concentration) to GLB after oral administration. Data from hepatic microsomes suggested impairment of GLB metabolism in diabetic rats. GLB metabolism in hepatic microsomes was significantly inhibited by a selective inhibitor (sulfaphenazole) of CYP2C11 and anti-CYP2C11 antibody. Western blotting further indicated the contribution of impaired CYP2C11 expression to the impairment of GLB metabolism. Excretion data showed that approximately 72% of the orally administered dose was excreted via feces of normal rats, which indicated an important role for intestinal BCRP. Diabetes significantly decreased recovery from feces, which was only 40% of the orally administered dose. Results from *in situ*, single-pass, intestine perfusion experiments revealed that diabetes significantly increased the apparent permeability and decreased efflux of GLB through the intestine; this suggests impairment of intestinal BCRP function, which may play a role in the increased exposure to orally administered GLB in diabetic rats. Insulin treatment partly or completely reversed the changes in diabetic rats. **Conclusion:** The impaired hepatic CYP2C11 and intestinal BCRP expression and activity induced by diabetes contributed to the increased exposure of orally administered GLB.

Keywords: diabetes mellitus; pharmacokinetics; glibenclamide; CYP2C11; breast cancer resistance protein

S12.19

Investigation on stereoselective metabolism of phencynonate *in vitro* by the chiral HPLC method

Ying LIU[1, 2], Yu-ying KOU[1], Pin-xiang XU[1], He LIU[3], Ke-liang LIU[3], Ming XUE[1, *]. *[1]Department of Pharmacology, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China; [2]Department of Pharmacy, Yan Jing Medical College, Capital Medical University, Beijing 101300, China; [3]Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China*

Aim: To investigate the stereoselective metabolism of phencynonate. **Methods:** Phencynonate enantiomers were isocratically separated and determined by high performance liquid chromatography (HPLC) on a LiChroCART 250-4 (250 mm×4 mm, id 5 μm, Merck) chiral column. **Results:** The mechanism of stereoselective metabolic metabolism of phencynonate was investigated by incubation of the drug with the rat liver microsomes, protein binding rate with rat plasma and membrane permeability across Caco-2 cell monolayer. The results revealed that there were significant differences in the metabolic stabilities of phencynonate enantiomers

in rat liver microsomes. The absorption and plasma protein binding had some effects on the stereoselective pharmacokinetics of phencynonate. **Conclusion:** The stereoselective metabolism of phencynonate isomers was obviously observed *in vitro*.

Keywords: phencynonate; chiral drugs; drug metabolism; cytochrome P450; protein binding; Caco-2; stereoselectivity

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S12.20

A single dose pharmacokinetic study of Vitamin K1-fat emulsion injection in Beagle dog

Na-na PING, Yan-ni MI, Xue XIAO, Yin-jing ZHU, Sen LI, Yong-xiao CAO. *Department of Pharmacology, Xi-an Jiaotong University College of Medicine, Xi-an 710061, China*

Aim: To study the pharmacokinetic of vitamin K1-fat emulsion injection and compared with vitamin K1 injection. **Methods:** Beagle dogs were administered intravenously vitamin K1-fat emulsion injection or vitamin K1 injection, using HPLC method to detection of the concentration of vitamin K1 in the plasma and BAS 3.0 to calculate the pharmacokinetic parameters. **Results:** Vitamin K1 concentration and peak area regression equation was A=13.6C+278.5 (r=1.00), the linear range from 3 to 30 000 ng/mL, the minimum detectable concentration was 0.56 ng/mL. The concentrations of vitamin K1 0.1, 1, and 10 μg/mL absolute recoveries were 91.6%, 90.0%, and 79.6%; relative recovery to 114.2%, 116.1%, and 114.4%; intra- and inter-day precision relative standard deviations were 1.2%, 2.5%, and 1.5% and 3.5%, 2.5%, and 2.9%. After intravenous injection of saline or fat emulsion injection was not measured vitamin K1 in plasma. Dogs intravenous vitamin K1-fat emulsion injection of 1.0 mg/kg, C_{max} 10.09 g/mL, $AUC_{0-\infty}$ 4511 g/L·h, $T_{1/2(\alpha)}$ 2.0 min, $T_{1/2z}$ 2.54 h. Vitamin K1-fat emulsion injection 0.25 mg/kg group C_{max} was 2.364 g/mL, $AUC_{0-\infty}$ 1019 g/L·h, $T_{1/2(\alpha)}$ 2.5 min, $T_{1/2z}$ 2.26±1.44 h. Vitamin K1 injection of 0.25 mg/kg group was C_{max} 2.725±0.625 μg/mL, $AUC_{0-\infty}$ 1781±615 g/L·h, $T_{1/2(\alpha)}$ 14.2±4.1 min, $T_{1/2z}$ 3.70±1.96 h. **Conclusion:** The vitamin K1-fat emulsion injection intravenous majority fit a two compartment model, the distribution of fast and elimination of relatively slowly. The distribution of the fat emulsion injection of vitamin K1 was faster than that of vitamin K1 injection, and there was no significant difference in elimination half-life.

Keywords: vitamin K1-fat emulsion injection; vitamin K1 injection; pharmacokinetics

S12.21

Pharmacokinetics determination of Ozagrel polymorphs

Zhi-zhen QIN[1], Qian-xi CHEN[2], Yang LV[2], Guan-hua DU[1,*]. *[1]Beijing Key Laboratory of Drug Target and Screening Research, Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100050, China; [2]Polymorphs Drug Research Beijing City Key Laboratory, Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100050, China*

Aim: To enhance the quality and efficiency of Ozagrel by investigating the differences among the Ozagrel polymorphs in bioavailability. **Methods:** Solid Ozagrel in different forms were orally administered to rats, and an HPLC method was established to determine plasma level of metabolite-Ozagrel and the bioavailability was analyzed. **Results:** The indirect pharmacokinetic parameters of Ozagrel, as the metabolites of the form I and II of Ozagrel, were as follows: C_{max} were (36.21±17.48) and (31.70±18.03) mg/L, respectively; AUC_{0UC} were (64.44±15.49) and (79.64±21.73) mg·L^{-1}·h, respectively; $T_{1/2}$ were 3.32 h and 11.89 h, respectively; T_{max} were 0.92 h and 1.19 h, respectively. **Conclusion:** There was no statistically significant difference in pharmacokinetic parameters among form I and II polymorphs of Ozagrel while the $T_{1/2}$ of form II is longer than form I, which suggested that form II polymorphs could be used as long-acting drug.

Keywords: Ozagrel; polymorphs; pharmacokinetics; HPLC

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S12.22

Absorption of SH-I-5 after oral administration in rats

Jun-ke SONG, Jia-lin SUN, Guan-hua DU*. *Beijing Key Laboratory of Drug Target Identification and Drug Screening, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China*

Aim: To develop and validate a good HPLC-MS method for the determination of SH-I-5 in rat plasma samples for further pharmacokinetic study. **Methods:** Six male Sprague-Dawley rats weighing 235±5 g was orally administered (*po*) SH-I-5. An Agilent Eclipse Plus C18 column (2.1 mm×100 mm, 3.5 μm) was used by the Agilent 1200HPLC system for chromatographic separation. The mobile phase consisted of acetonitrile – water (54:46, *v/v*) with 0.46% formic acid using an isocratic elution. Mass spectrometric detection was performed with Agilent 6110 single quadrupole MS equipped with an ESI source. The MS detector was operated in selective ion monitoring (SIM) mode using the quantification ions $[M+H]^+$ at m/z 460.10 for SH-I-5 and m/z 322.80 for IS (Clopidogrel Hydrogen Sulfate). The fragmentor voltage was 120 V for both SH-I-5 and internal standard. **Results:** The specificity, accuracy, precision and recovery of this method was good, and the linear relationship ranged from 5.68–1428.57 ng/mL. The regression equation was R=0.0017C–0.0024 (R^2=0.9997). Major pharmacokinetic parameters were as follows: $AUC_{(0-t)}$ 2073.047±627.172 μg/L·h, $T_{1/2}$ 1.298±0.595 h, T_{max} 5.333±1.033 h, C_{max} 303.216±56.377 μg/L. **Conclusion:** A rapid HPLC-MS method was developed and validated which was successfully applied to the pharmacokinetic study of SH-I-5 in rats.

Keywords: SH-I-5; pharmacokinetics; absorption; HPLC-MS; rats

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S12.23

Absorption, distribution and excretion of salvianolic acid A after oral administration in rats

Jia-lin SUN, Li ZHANG, Jun-ke SONG, Chao HUANG, Guan-hua DU*. *Beijing Key Laboratory of Drug Target Identification and Drug Screening, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China*

Aim: To study the pharmacokinetic characteristics of salvianolic acid A (Sal A) after a single oral administration in rats, including the absorption, distribution, and excretion. **Methods:** Rats were allocated into the following treatment groups: in the absorption experiment, rats (male/female, 1:1) were given a single oral dose of 5, 10, and 20 mg/kg and received a single dose of 50 μg/kg iv injection; rats (male/female, 1:1) in the distribution and excretion studies were given a single oral dose of 5 and 20 mg/kg, respectively. Plasma was sampled before and after administration at designated time points. Animals were sacrificed at 10 min, 1 h, and 2 h post-dosing and multiple tissues were harvested. Bile, urine, and feces samples were collected over designated time intervals. Caco-2 cell model was applied in the absorption mechanism study of Sal A. **Results:** After oral administration, Sal A reached the peak concentration in plasma within 1 h followed by a quick elimination. The absolute bioavailability was calculated to range from 0.39% to 0.52%, which indicated poor absorption. Besides, Sal A was shown to have poor permeability (less than 1×10^{-6} cm/s) across the Caco-2 cell monolayer, and the ratio of P_{B-A} to P_{A-B} was greater than 1.5 at different concentrations. Sal A could distribute widely in different tissues with different concentrations. Excretion results showed that total excretion of unchanged Sal A accounted for 7.81‰ of the administered dosage. **Conclusion:** The pharmacokinetic properties of Sal A in rats after oral administration were characterized as a rapid oral absorption, a poor absolute bioavailability, a wide tissue distribution, and a quick but low elimination.

Keywords: salvianolic acid A; pharmacokinetics; absorption; distribution; excretion

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S12.24

Effects of celastrol, derived from *Trypterygium wilfordii* Hook F, on metabolism of model CYP1A2, 2C11, 2E1 and 3A2 probe substrates in rat liver *in vitro*

Xin WANG*, Min SUN, Yu TANG, Tong-gui DING, Ming-yao LIU. *Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai 200241, China*

*Corresponding author.

E-mail: xwang@bio.ecnu.edu.cn

Aim: The present study was to investigate the effects of celastrol, derived from *Trypterygium wilfordii* Hook F ("Thunder of God Vine"), a traditional Chinese medicine plant, on the metabolism of model probe substrates of CYP isoforms, CYP1A2, CYP2C, CYP2E1, and CYP3A, which are important in the metabolism of

a variety of xenobiotics. **Methods:** The effects of celastrol on CYP1A2 (phenacetin *O*-deethylase), CYP2C11 (tolbutamide 4-hydroxylase), CYP2E1 (chlorzoxazone 6-hydroxylase) and CYP3A2 (testosterone 6β-hydroxylase) activities were investigated using rat liver microsomes *in vitro*. HPLC-DAD was used to measure the model substrates and metabolites. **Results:** Celastrol weakly inhibited the metabolism of model CYP1A2, 2C11, 2E1, and 3A2 probe substrates in rat liver. Inhibition of rat CYP isoforms (IC_{50}) by celastrol in potency order was CYP2C11 (10.8 μmol/L) > CYP3A2 (23.2 μmol/L) > CYP1A2 (52.8 μmol/L) > CYP2E1 (74.2 μmol/L). Enzyme kinetic studies showed that the celastrol was the competitive inhibitor of these CYP isoforms. **Conclusion:** Celastrol inhibits CYP1A2, 2C11, 2E1, and 3A2 activities in rat liver microsomes *in vitro*. Further systematic studies in human *in vitro* and *in vivo* are needed to identify the interactions of celastrol with cytochrome P450s.

Keywords: celastrol; cytochrome P450 (CYP); competitive inhibitor; liver microsomes
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S12.25

Ethnic difference in drug response among Asians

Hiroshi WATANABE[1], Shinichi KAWAI[2], Masahiro TOHKIN[3]. *[1]Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Japan; [2]Division of Rheumatology, Department of Internal Medicine, Toho University School of Medicine, Japan; [3]Department of Medicinal Safety Science, Nagoya City University Graduate School of Pharmaceutical Sciences, Japan*

Drug response may vary between Asian and Caucasian. Ethnic or racial differences in pharmacokinetics (PK) and pharmacodynamics (PD) which have been attributed to distinctions in genetic, physiological, and pathological factors between ethnic groups should be an important consideration when we design and conduct a global clinical study. These PK/PD differences are influenced by several extrinsic factors such as diet, socioeconomic background, and culture. However, it is still open to question whether we have ethnic differences in drug response among Asians. Moxifloxacin, simvastatin, and meloxicam have been shown to have PK differences among Asians and Caucasian. To examine factors involved in PK differences of the 3 drugs among populations, prospective global PK studies (Principle Investigator: Prof Kawai, Toho Univ, Japan) under the same protocol with strictly adjusted trial conditions to remove possible extrinsic bias were conducted in 4 countries; Japan, China, Korea, and USA. In contrast with previous information, no significant PK difference was observed in moxifloxacin, simvastatin, and meloxicam among these groups. These results strongly suggest the importance of the influence of extrinsic bias including diet in drug response. For example, green tea is the most common beverage in Japan and has been shown to interact with drugs by changing the pharmacokinetics and pharmacodynamics of orally administered drugs. In this symposium, we would like to briefly introduce the results from the global PK studies and the data of green tea-drug interaction as a potential extrinsic factor that may influence ethnic difference in drug response.

S12.26

Species and gender differences affect the metabolism of Baicalein via glucuronidation

Yuan WU, Rui-hua DONG, Ze-yuan LIU. *Department of Clinical Pharmacology, Affiliated Hospital, Academy of Military Medical Sciences, Beijing 100071, China*

Aim: Baicalein, a flavone present in *Scutellaria baicalensis* Georgi, has been demonstrated to possess a variety of biological activities, including treatment of inflammation, cardiovascular disease, and microbial infections. Accumulating evidence has demonstrated the antitumor activity of this flavone in a variety of human cancer cell lines. Though it has so many pharmacological effects, its species diversity remains unclear. The aim of this study was to define the mechanisms responsible for poor bioavailability of Baicalein by its metabolism in liver *in vitro*. **Methods:** Liver microsomes of Rhesus monkeys, SD rats, Beagle dogs, pigs, and humans were employed to phase II metabolic incubation system. Three glucuronidation metabolites of Baicalein were determined in different species liver microcosms by using HPLC. **Results:** Our data revealed that the K_m values of Baicalein-7-glucuronidation in human liver microsomes (K_m=1.74) was about 25-fold lower than in female SD rats (K_m=43.8). The metabolite Baicalein-6-glucuronidation was catalyzed by liver microsomes, which is an active metabolite with 16-fold higher affinity to the SD rats (K_m=0.56) than Rhesus monkeys (K_m=9.39). The other metabolite, Baicalein-6-*O*-glucuronidation-7-*O*-glucuronidation, in native hepatic microsomes from female SD rats (K_m=156) liver displayed higher K_m values than that in pigs (K_m=6.48). Besides, our data also shows that there is a great difference in gender in some species. The species and gender metabolic differences we observed between animals and human liver provide key information for delineating Baicalein pharmacokinetics needed for human health risk assessment. **Conclusion:** In conclusion, species and gender affected Baicalein metabolism to a different degree, and experimental animals are expected to be useful predicting Baicalein glucuronidation in humans.

Keywords: Baicalein; metabolism; glucuronidation; UDP-glucuronosyl transferase; enzyme kinetics

S12.27

Effect of different compatibility of ingredients from Qingkailing Injeciton on their pharmacokinetics and pharmacodynamics in rat brain

Jie-kun XU[1], Wei-ku ZHANG[2], Hui-hua QU[1], Yan ZHAO[1], Qing-guo WANG[1]. *[1]Beijing University of Chinese Medicine, Beijing 100029, China; [2]Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing 100029, China*

Aim: To study the effect of different compatibility of ingredients (baicalin, geniposide, cholic acid and deoxycholic acid) from Qingkailing Injection on the metabolic process and the therapy of ischemic stroke of the major effective constituents. **Methods:** The neurological function grade, the volume and edema degree of cerebral infarction in 12 h after ischemic reperfusion were observed in rat MCAO model, respectively. In order to observe the effect of different compatibility of ingredients from Qingkailing Injection on metabolic process of geniposide in brain and its therapy of ischemic stroke, cerebrospinal fluid (CSF) was collected by microdialysis and the changes of ingredient content was measured by UPLC-MS/MS. **Results:** The model for cerebral ischemia in rats was made successfully. The collection of cerebrospinal fluid was uniform and stable by microdialysis sampling. The prescription contains all the four constituents reduced more markedly the volume and edema degree of cerebral infarction the most than those of lack of any of the four constituents in the prescription. Furthermore, the addition of baicalin, cholic acid and deoxycholic acid could significantly influence the metabolic characteristics of geniposide, increase its effective concentration and prolong half life in rat brain. **Conclusion:** The different compatibility of the four constituents in Qingkailing Injection could affect significantly the metabolic characteristics in rat brain of the other constituents, and might be one of the mechanisms which affect pharmacological effect.

Keywords: Qingkailing Injection; compatibility; ischemic stroke; pharmacokinetics and pharmacodynamics

S12.28

Effects of *CYP3A4*1G*, *CYP3AP1*3* and gender on pharmacokinetics of simvastatin and simvastatin acid in healthy Chinese volunteers

Wei-hong YANG[1], Hong-ping XU[2], Deng-zhi ZHAO[1], Xiao-fei WANG[1], Sheng-jun ZHANG[3], Li-rong ZHANG[1, *]. *[1]School of Basic Medical Sciences, Zhengzhou University, Zhengzhou 450001, China; [2]Zhengzhou Railway Vocational-Technical College, Zhengzhou 450052, China; [3]The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China*
*Correspondening author.
E-mail: zhanglirongzzu@126.com

Aim: To study effects of *CYP3A4*1G* (G>A), *CYP3AP1*3* (G>A) and gender on pharmacokinetics of simvastatin and simvastatin acid. **Methods:** The polymorphisms of *CYP3A4*1G* and *CYP3AP1*3* were determined by polymerase chain reaction-restriction fragment length polymorphism. Serial blood samples were obtained up to 24 h after a single dose of 40 mg simvastatin intake in healthy Chinese volunteers. Plasma concentrations of simvastatin and simvastatin acid were detected by HPLC/MS/MS. **Results:** Weight-adjusted apparent volume of distribution (NV_d/F) of simvastin in *CYP3A4*1G* AA genotype was higher than that in GG genotype. However, dose-normalized by the body weight C_{max} (NC_{max}) in AA genotype was lower than that in GG genotype. NV_d/F of *CYP3AP1*3* AA genotype was higher than that of GA genotype, while NC_{max} of AA genotype was lower than that of GA genotype. NC_{max} of simvastatin acid was higher in females than that in males. **Conclusion:** *CYP3A4*1G* or *CYP3AP1*3* has some effects on NV_d/F and NC_{max} of simvastatin, and the NC_{max} of simvastatin acid is higher in female than that in male.

Keywords: *CYP3A4*1G*; simvastatin; simvastatin acid; pharmacokinetics; *CYP3AP1*3*

S12.29

Transportation of neuroprotective flavonoids, based on cell toxicity: a comparison using the blood-brain barrier cell and Caco-2 cell models

Yu-ya YANG, Lu BAI, Chen-yang GUO, Bin-bin XIA, Xiao-rong LI, Pin-xiang XU, Ming

XUE*. *Department of Pharmacology, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China*

Aim: Comparative evaluations of the transmembrane transport of eight cerebro-vascular protection flavonoids including puerarin, rutin, hesperidin, quercetin, genistein, kaempferol, apigenin, and isoliquiritigenin, based on the cellular toxicity and lipophilicity were investigated using rat blood–brain barrier cell and Caco-2 cell monolayer model. **Methods:** The apparent permeability coefficients (P_{app}) of the tested flavonoids were calculated from the unilateral transport assays in Transwell system with simultaneous determination using a HPLC method. The toxicity of the flavonoids to rat brain microvessel endothelial cell was determined by the MTT assay. **Results:** The toxicity orders were genistein, isoliquiritigenin and apigenin, kaempferol, hesperidin and quercetin, puerarin and rutin. The tested flavonoids exhibited time-dependent P_{app} values in these models. The efflux mechanisms related with P-glycoprotein exist with some flavonoids and verapamil could enhance the permeation of rutin and quercetin. The position of the glycoside and hydroxyl group in flavonoids also affected the P_{app} value. The oil-water partition coefficient and toxicity of the flavonoids modified by the number and position of the glycoside and hydroxyl group were the key determinant for the transmembrane transport. **Conclusion:** These findings provided important information for establishing the transport relationship and predicting the oral bioavailability of these active flavonoids.

Keywords: flavonoids; blood–brain barrier; Caco-2 cell; transport; toxicity

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S12.30

Studies on the impact of Lignum Dalbergiae Odoriferae on the pharmacokinetic profile of Radix Salviae Miltiorrhiae in GDD decoction

Zhi-hong YANG, Xiao-bo SUN*. *Chinese Academy of Medical Sciences and Peking Union Medical College, Institute of Medicinal Plant Development, Beijing 100193, China*

Aim: Guanxin-danshen decoction (GDD, including Radix Salviae Miltiorrhiae, Radix Notoginsen, and Lignum Dalbergiae Odoriferae), the classic TCM prescription, has been with systematic pharmacodynamic studies. It will be further studied on the pharmacokinetic profile. Via studying the four effective components (cryptotanshinone and salvianolic acid B of Radix Salviae Miltiorrhiae, naringenin and volatile oil of Lignum Dalbergiae Odoriferae), we intend to elucidate the reasonable and scientific TCM compatibility theories from the aspect of pharmacokinetic characteristics and absorption mechanism in GDD. **Methods:** To study the effect of naringenin and volatile oil on the pharmacokinetic profile of cryptotanshinone and salvianolic acid B *in vivo* and *in vitro*, a rapid and sensitive LC–MS/MS method for the determination of the lipophilic and hydrophilic active components from Radix Salviae Miltiorrhizae in rat plasma, tissues and Caco-2, MDCK cells is developed and validated. To determine the pharmacokinetic parameters of cryptotanshinone and salvianolic acid B, the concentration–time data are analyzed by non-compartmental method using the DAS Software. **Results:** In the presence of naringenin and volatile oil, the pharmacokinetic parameters of cryptotanshinone and salvianolic acid B vary significantly. The data of $t_{1/2}$, MRT, $AUC_{(0-t)}$, C_{max}, V_d, and CL are changed from 56.90 min, 73.13 min, 159.31 mg·min/L, 2.96 mg/L, 9.84 L/kg and 0.12 L/min/kg to 47.57 min, 66.45 min, 191.29 mg·min/L, 3.90 mg/L, 6.99 L/kg and 0.10 L/min/kg, respectively. **Conclusion:** Naringenin and volatile oil may increase the amount of cryptotanshinone and salvianolic acid B in rat plasma, prolong the absorption process, promote the two active components into the target tissues, and increase their bioavailability. Furthermore, the promoting absorption mechanism of naringenin and volatile oil may be in a different manner.

S12.31

In vitro metabolism of methyl salicylate-2-*O*-β-*D*-lactoside

Dan ZHANG,Tian-tai ZHANG*, Guan-hua DU*. *Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing 100050, China*

The study aimed to evaluatethe metabolic characteristics of methyl salicylate-2-*O*-β-*D*-lactoside (DL0309) in rat intestinal flora and extrahepatic tissues. Identify the major metabolite and drug metabolizing enzymes involved in the metabolism of DL0309 in rat liver microsomes by selective inhibitors *in vitro*. DL0309 was incubated with rat intestinal flora and S9. The concentration of DL0309 was determined by HPLC-UV method. The metabolite of DL0309 in rat microsomes and intestinal flora was isolated and identified by UPLC-Q/TOF-MS. DL0309 was metabolized 50% within 4 h in rat intestinal flora and metabolized faster in intestine and liver. CYP2C19 and CYP3A4 was the major drug metabolizing enzymes in the metabolism of DL0309. The major metabolite of DL0309 in rat liver microsomes and intestinal flora were lactose, methyl salicylate and salicylic acid.

Keywords: methyl salicylate-2-*O*-β-*D*-lactoside; metabolism; liver microsomes

S12.32

Effects of tetrahydroxystilbene glucoside on mouse liver cytochrome P450

Feng ZHANG, Hang LIU, Yan-ying WANG, Qin WU, Yuan-fu LU, Jing-shan SHI. *Department of Pharmacology and Key Lab of Basic Pharmacology of Guizhou, Zunyi Medical College, Zunyi 563099, China*

Aim: Tetrahydroxystilbene glucoside (TSG) has been found to possess anti-oxidant, anti-aging and anti-inflammatory properties. In this study, the effects of TSG on cytochrome P450 (CYP) in mouse livers were investigated. **Methods:** Forty-five male mice were randomly divided into the blank, the low dose and the high dose of TSG groups. Three, five, and seven days after intragastrical administration of TSG, mice were sacrificed and the mRNA expressions of CYP isoenzymes in mouse livers were measured by real time RT-PCR, respectively. **Results:** (1) The high dose of TSG significantly inhibited CYP1A2 and CYP3A4 mRNA expression 3, 5, and 7 d after treatment, whereas the low dose of TSG decreased CYP1A2 mRNA expression 3 d after treatment. (2) The high dose of TSG time-dependently increased CYP2E1 mRNA expression 3, 5, and 7 d after treatment. (3) The high dose of TSG significantly inhibited CYP4A14 mRNA expression 7 d after treatment. (4) TSG had no significant effects on CYP2B10, CYP3A11, and CYP3A25 mRNA expressions. **Conclusion:** TSG has significant effects on CYP1A2, CYP2E1, CYP3A4, and CYP4A14 mRNA expressions but no significant effects on CYP2B10, CYP3A11, and CYP3A25 mRNA expressions.

Keywords: tetrahydroxystilbene glucoside; mice; cytochrome P450; real time RT-PCR

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S12.33

CYP2D6*10 genetic polymorphisms and Bisoprolol's pharmacokinetics

Zhi-guo ZHANG, Jing WANG, Hong-ying LI, Shu-bo SUN. *The First Affiliated Hospital of Jiamusi University, Jiamusi 154003, China*

Aim: To assess the effect of CYP2D6*10 different genotypes on Bisoprolol's pharmacokinetics in the healthy volunteers. **Methods:** CYP2D6*10 genotypes were determined by real-time PCR. Twenty-four healthy subjects were divided into three different genotype groups of CC, CT, and TT in the study. All the subjects were administered with Bisoprolol Fumarate tablets at a single dosage of 5 mg. Blood samples were collected at different time points and plasma concentrations of the drugs were measured by high-performance liquid chromatography (HPLC). **Results:** The main parameters of $T_{1/2}$, C_{max} and AUC_{0-t} among the volunteers with three genotypes of CC, CT, and TT were 8.25±1.80 h, 7.70±1.18 h, 8.19±1.86 h, and 41.69±11.22 μg/L, 37.69±6.53 μg/L, 43.14±5.85 μg/L and 394.38±104.70 $\mu g \cdot h \cdot L^{-1}$, 380.04±84.04 $\mu g \cdot h \cdot L^{-1}$, 414.08±104.40 $\mu g \cdot h \cdot L^{-1}$. There were no significant differences among the three genotypes by T test (P>0.05). **Conclusion:** The individual differences of Bisoprolol's pharmacokinetics are not related to CYP2D6*10 genotypes.

Keywords: bisoprolol; pharmacokinetic; CYP2D6*10; genetic polymorphism

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Transport study of polymeric micelles across epithelial cell monolayer

Shan-shan ZHAO[1], Jing LI[1], Yan-qiu ZHOU[2], Wen-wei SHAN[3], Jie GUAN[2], Li-li ZHANG[1], Ping ZHAO[1], Wen-xin XUE[1]. *[1]Pharmacy Department; [2]Central Lab; and [3] Personnel Section, China Meitan General Hospital, Beijing 100028, China*

Aim: To investigate the transport properties of polymeric micelles across Madin Darby Canine Kidney (MDCK) cells. **Methods:** Coumarin 6 loaded PEG-PLA (PEG_{3000}-PLA_{3000}) micelles were prepared by solvent evaporation method, and the HPLC determination method of coumarin 6 was constructed. Concentration, temperature effect on transport of micelles across MDCK cell monolayer were studied, the concentration of Ca^{2+} and EGTA were also studied. **Results:** Coumarin 6 loaded micelles have little toxic effect on MDCK cells. The transport of micelles was affected by concentration and temperature, which means that this process is an active and energy-dependent process. The concentration of Ca^{2+} and EGTA could

affect the transport of the micelles, which means micelles could transport across the cell monolayer via paracellular pathway. The amount of transported coumarin 6 micelles was rather limited compared with coumarin 6 micelles added to the apical side. **Conclusion:** Micelles transport across MDCK cell monolayer via both paracellular and transcellular pathway at the same time.

Keywords: polymeric micelles; coumarin 6; epithelial cells; transport

S12.35

Identification of *DDAH1* transcript variants and correlations with endothelial ADMA metabolic activity in human

Ji-peng ZHOU[1, 2, #], Tao SUN[2, #], Lei CHEN[2], Da-bin KUANG[1], Mu-peng LI[1, 2], Jie TANG[1], Xiao-ping CHEN[1, 2, *]. *[1]Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Central South University, Changsha 410078, China; [2] Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, Changsha 410008, China*

[#]These two authors contributed equally to this work.

*Correspondening author.

E-mail: chenxp74@hotmail.com

Aim: Dimethylarginine dimethylaminohydrolases 1 (DDAH1) is the major enzyme responsible for inactivation of endogenous nitric oxide (NO) synthases inhibitor asymmetric dimethylarginine (ADMA). Besides the *DDAH1* transcript variants 1 and 2 (*V1* and *V2*), we identified a novel human *DDAH1* transcript variant designated as *DDAH1-V3* (*V3*) here. The aim of this study is to determine the correlation between expression levels of *DDAH1* transcript variants and their role in ADMA metabolism in human. **Methods:** mRNA expression of *DDAH1* transcript variants in primary cultured human umbilical vein endothelial cells (HUVECs) and peripheral blood mononuclear cells (PBMCs) of patients with acute ischemic stroke (AIS) and acute myocardial infarction (AMI) and intracellular ADMA metabolic activity in HUVECs was determined. **Results:** *DDAH1-V3* mRNA expression correlated significantly with those of *V2* and *V1* in HUVECs ($P<0.05$, respectively). In PBMCs from patients with AIS and AMI, significant correlations between mRNA expression of *DDAH1* transcript variants were also observed. Only the expression level of *V1* mRNA correlated significantly with ADMA metabolizing activity in HUVECs ($R=0.805$, $P=0.002$). **Conclusion:** There are close correlations among mRNA expression of *DDAH1* transcript variants, while only the *DDAH1-V1* is responsible for ADMA metabolism, and transcript specific primers are suggested to determine *DDAH1* mRNA expression level.

Keywords: asymmetric dimethylarginine; dimethylarginine dimethylaminohydrolases 1 (DDAH1); human umbilical vein endothelial cells (HUVECs); peripheral blood mononuclear cells (PBMCs); transcript variants

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