POSTER SESSION ABSTRACTS

Inspiration and Education

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POSTER ABSTRACT

Bioanlytical

#101

LC-MS/MS Method Validation for the Quantitative Determination of Drug A in the Cynomolgus Monkey Plasma

Jiaxu Zhou, Jingliang Gu, Xiuwen Liu

Department of DMPK, Joinn Laboratories (Suzhou)

This study validates a simple, specific high performance liquid chromatography tandem mass spectrometry method (LC-MS/MS) for the quantitative determination of drug A in the cynomolgus monkey plasma.

The method involves the addition of drug B as an internal standard (IS) and protein precipitation extraction. An API 4000 QTRAP triple quadruple mass spectrometer, operated in the positive electrospray ionization (ESI) ion mode, was used to monitor the precursor to production ion transitions of m/z 371.2¡æ353.2 and 343.2¡æ285.2 for drug A and drug B, respectively.

The method was specific for Drug A in cynomolgus monkey plasma and the concentration-response relationship was linear in the calibration range from 1.00 to 500 ng/ml. The square of correlation coefficients (r) of the calibration curves obtained from the method validation was 0.9940 or greater. The accuracy and precision of the method were determined at low, middle and high concentrations of quality control samples on three separate days. The within-day accuracy and precision ranged from 94.3% to 108% and 3.35% to 9.24%, respectively. The between-day accuracy and precision ranged from 100% to 104% and 7.38% to 8.33%, respectively. The accuracy and precision of the dilution QC samples at 10,000 ng/ml (100-fold) were 111% and 3.07%, respectively. The lower limit of quantification (LLOQ) was established at 1.00 ng/ml with the accuracy and precision of 100% and 9.83%, respectively. The mean recoveries of Drug A and Drug B from cynomolgus monkey plasma by protein precipitation extraction were 71.3% and 74.2%, respectively. There was no matrix effect observed within six individual plasma lots.

Drug A was stable in cynomolgus monkey plasma through up to three freeze-thaw cycles and was stable for at least 6 hours stored at room temperature. Drug A was stable in processed samples for at least 48 hours in the autosampler at approximately 4¨¬C. Drug A was also stable in the cynomolgus monkey plasma when stored at -70 ¡≤ 15¨¬C for at least 14 days. In addition, the stock solutions of Drug A and Drug B (IS) in DMSO were stable for at least 6 hours at room temperature and 14 days stored at -20 ≤ 5¨¬C.

In conclusion, the validation results of the method met the acceptance criteria. This method is applicable for the determination of drug A in cynomolgus monkey plasma for bioequivalence, bioavailability, pharmacokinetic or toxicology investigations..

References: 1.Guidance for Industry Bioanalytical Method Validation. FDA(May 2001)

POSTER ABSTRACT

Bioanlytical

#102

Liquid-liquid microextraction increases sensitivity of GC¨CMS-based assay for measurement of volatile borneol, isoborneol, and their metabolite camphor in rat plasma

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Both natural Borneolum (the products from resin of Dryobalanops aromatica or Cinnamonum camphora L. Presel) and Borneolum syntheticum (semisynthesized products from camphor and turpentine oil) are listed in the Chinese Pharmacopoeia under the item "Bingpian"; the former consists of (+)-borneol and the later contains (-)-borneol and (-)-isoborneol. Bingpian is an ingredient of the cardiovascular multi-herb medicine Fufang-danshen-fang. As part of our ongoing pharmacokinetic studies of Fufang-danshen-fang, we developed a bioanalytical assay for measurement of borneol, isoborneol, and their metabolite camphor in rat plasma samples. The newly developed assay involved using a liquid-liquid microextraction method, i.e., extracting 20 μL of plasma sample (spiked with 10 μL of the internal standard fenchol) with 30 μL of n-hexane. The resulting n-hexane extract (15 μL) was introduced into a GC-CMS system via a programmable temperature vaporizing injector operating ! in delayed splitless mode. The newly developed bioanalytical assay was sensitive, accurate, precise, and matrix effect-free and was applied successfully to rat pharmacokinetic studies of natural Borneolum (of D. aromatica), Borneolum syntheticum, and a kind of tablets of Fufang-danshen-fang. We found that the extraction efficiency for the analytes and the internal standard from plasma did not decease as the n-hexane volume decreased, which resulted in a significant increase of the compound concentrations in the post-extracting solvent. In addition, the liquid-liquid microextraction omitted the subsequent solvent evaporation and reduced the dilution due to use of the extracting solvent. The applicability of the liquid-liquid microextraction technique may be extended to measurement of other volatile and many nonvolatile medicinal compounds in biomatrices, which can be predicted according to the octanol/water distribution coefficient (LogD) and acid dissociation constant (pKa) of the analytes.

POSTER ABSTRACT
#103

Bioanlytical

A rapid and selective liquid chromatography/mass spectrometry method for simultaneously quantitative analysis of six neurotransmitters in mouse brain

Yuya Wang, Dan Pu, Even Wu and Kelly Dong

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The determination of brain neurotransmitters facilitates better understanding of complex neurobiology in the central nervous systems and monitors the progress stage of Parkinson's disease (PD) in animal models.

Dopamine (DA) and its three metabolites 3,4 dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-methoxytyramine (3MT), 5-hydroxytryptamine (5HT) and its metabolites 5-hydroxyindole-3-acetic acid (5HIAA) were analyzed simultaneously by a LC/MS/MS method using a combination of pH and organic gradient and ionization mode switching. Full chromatographic separation of these compounds has achieved within 7-minute total runtime to avoid cross interference. Acidification of brain homogenate had successfully maintained the stability of neurotransmitters during sample storage and processing. Due to endogenous background of these compounds, a surrogate biological matrix was selected based onto its representation to provide reliable quantification of study samples. This fully validated bioanalytical method has provided rapid and effective support in establishing MPTP and 6-OH dopamine in vivo models and guides drug discovery programs for Parkinson's disease.

POSTER ABSTRACT

Bioanlytical

#104

Development of a nucleic-acid-hybridization-based enzyme-linked bridging assay (ELBA) for quantification of oligonucleotides and its application in pharmacokinetic study

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With ongoing efforts to develop oligonucleotides (ONs)-based therapeutics, there is a need of a sensitive, high-throughput method for quantification of ONs-based drugs in biological matrices. To overcome the insufficient sensitivity and time-consuming sample extraction procedures involved in current conventional capillary gel electrophoresis (CGE) and high-performance liquid chromatography (HPLC) methods, a novel sensitive, rapid and high throughput nucleic-acid-hybridization-based enzyme-linked bridging assay (ELBA) was developed to quantify intact and metabolite ONs in biological matrices.

In principle, the ELBA was established on the basis of a two-step hybridization, first by base pairing of the intact analyte (N mer) to the immobile capture probe (N+9 mer), followed by hybridization with a detection probe (9 mer), which was then ligated to the analyte by T4 DNA ligase. In this format, any metabolite (N-1 or above 1), together with the capture probes (N+9 mer) and detection probes (9 mer), would form a double strand leaving a gap in one strand, which could be cleaved by a S1 nuclease, resulted in the loss of fluorescence signal derived from detection probe. The whole procedure ensures the selective collection of fluorescence solely by intact ODN-based drugs. Similarly, various metabolites could be detected by modification the length or the direction of capture-probe. Then, selective quantification of intact ONs or related metabolites could be realized by using the correct capture probes assisted with T4 and S1 enzymes.

In the present study, the ELBA was utilized to investigate the pharmacokinetics (PK) of AS1261, an antisense oligodeoxynucleotides targeting protein kinase C (PKC) alpha mRNA, in Rhesus monkeys following intravenously guttae (ivgtt) at the dose of 05, 1.5 and 5 mg/kg, respectively. And, the characteristics of ELBA on quantification of ONs was compared to that of CGE. Method validation for ELBA showed that the linearity range was 0.024 ~ 25 nM with the precision 19.70 % and the accuracy of -7.77 % ~ 6.46 %. In contrast, for CGE method, the linearity range was 25 ~ 25000 nM with the precision ¡Ü18.26 % and the accuracy of -16.85 % ~ 10.51 %. The sensitivity of ELBA was about 1000 times to that or CGE. Cross-test showed good consistency between the CGE and ELBA at different concentration level of AS1261 with various dilution factors (RE were 8.16 %, 3.75 %, and 1.68 % at 400000 nM, 80000nM, and 400 nM, respectively).

Results showed that, due to insufficient sensitivity, the PK profiles of AS1261 in monkeys at the dose of 0.5 and 1.5 mg/kg were failure to be well described by CGE method and at the dose of 5 mg/kg, the PK profile was concluded by 75 min post dosing. While by ELBA, the concentration-time curves of all three dose groups were fully described to 12~24 h post dosing, which revealed a comprehensive elimination phase of the PK profile.

In conclusion, here we successfully transferred a classical molecular biological technology to Bioanalysis fields, and developed a sensitive, rapid, and robust ELBA method for quantification of ONs-based therapeutics.

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POSTER ABSTRACT

Bioanalytical

#105

A unique LC/MS/MS method by combining MRM and Q3 ion modes for simultaneous determination of deuterium-containing drug and metabolites

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LC/MS/MS is a popular and powerful analytical tool for quantitative bioanalysis. It offers high sensitivity and selectivity by monitoring the MRM transitions (mass/charge based) characteristic of the drugs and metabolites. The development of an LC/MS/MS method for deuterium-containing drugs can be very challenging, as the deuterium atoms may come off during in vivo metabolism. MRM may not be an appropriate selection for deuterium-containing drug and the resulting metabolites due to isotopic interference among the drugs and metabolites containing varying number of deuterium atoms. The quantitative determination can be done via Q3 ion mode. Q3 ion mode is susceptible to high background and baseline interference due to lack of Q1 ion selectivity.

In this work, we present a unique LC/MS/MS method for simultaneous determination of a d5-labelled drug and its five metabolites (M1-M5). An analog internal standard was used. M5 metabolite does not contain deuterium as the deuterium group is cleaved during metabolism pathway. Q3 ion mode was used for quantitation of the deuterium containing drug and metabolites M1-M4 whereas MRM was utilized for M5 and the IS to eliminate interference and baseline noise. Phospholipids matrix effect was removed through on-line column switching.

POSTER ABSTRACT

Bioanalytical

#106

Simultaneous Determination of Estrone and Equilin in K2EDTA Human Plasma by LC-MS/MS

Ming Zuo, Qiaoling Yu, Wenzhe Lv, Tianyi Zhang

Frontage Laboratories (Shanghai)

Estrogens are endogenous compounds present at very low concentration levels in human subjects. LC/MS/MS is a very sensitive technique and is often employed in quantitative analysis of estrogens. In order to determine the presence of low level of Estrone and Equilin in human plasma from female subjects, an ultra sensitive method for accurate quantification of Estrone and Equilin K2EDTA human plasma by LC-ESI/MS/MS has been developed and validated in our lab.

In this work, we describe the development and validation of this method. Because these were endogenous compounds, charcol stripped human plasma was used to prepare the calibration standards. QCs were prepared in both unstripped plasma and charcol-stripped human plasma. d4-isotope labelled internal standards were used. The plasma samples were extracted by protein precipitation with acetonitrile.

To enhance ionization and sensitivity, Estrone and Equilin were derivatized with 2-fluoro-1-methylpyridine p-toluenesulfonate (FMPT) prior to LC/MS/MS injection.

The extract was injected on a Sciex API-5000 LC-MS/MS system using reverse phase chromatography with an electrospray source in positive ion mode. The total chromatographic runtime was 5 min per injection. The low quantification limits of Estrone and Equilin in plasma were achieved as low as 5 and 2 pg/mL, respectively.

A highly sensitive method for simultaneous accurate quantification of Estrone and Equilin in K2EDTA human plasma by LC-ESI/MS/MS has been developed and validated by chemical derivatization using 2-fluoro-1-methylpyridinium p-toluenesulfonate.

This method has been successfully used to analyze study samples from a bioequivalence study.

POSTER ABSTRACT

Pharmaceutical Analysis

#107

Analytical Technologies for Determination of Trace Level Genotoxic Impurities in Drug Substances

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Pharmaceutical industry and Regulatory authorities are very concern on the issues of genotoxic impurities (GTI) in drugs administered to human. FDA and CHMP Guidelines have established the staged genotoxic impurity limits as well as 1.5 g/day for long term (> 12 M) clinical trials and market products. Properly control genotoxic impurity under the safe level has become urgent and critical tasks to meet the regulatory requirements in order avoid the delay on clinic trials and market launching.

Determination of trace level of genotoxic impurities in active pharmaceutical ingredient (API) is a big challenging task for analytical method development. Complex diversity of molecular structures of genotoxic impurities results in great difference in their physical-chemical properties. Furthermore, reliable analytical results can usually be compromised by severely interference of the complicated sample matrix. The requirements for analytical methods used for determination of trace level of genotoxic impurities in sample matrix demand for high sensitivity, good selectivity, better compatibility with QC Lab of manufacture for easy operation, fastness, robustness, and smooth tech transfer.

This presentation will introduce method development strategy, GTI Chemical Structure & Property-Based Approach. GTIs are divided into several groups based on GTI physical-chemical properties. In order to achieve the best possible results and high QC standard method, specific analytical technology is applied to the method development according to GTI physical-chemical properties as well as sample matrix effect. For some extremely difficult GTIs on separation, detection for desired sensitivity, sample preparation, solution stability and matrix interference, careful selection and novel design of derivatization techniques are employed in order to overcome and eliminate these issues. The derivatization approaches which combined with suitable analytical tools have been proved to be very powerful technologies to solve trace level of GTIs in API. This presentation will demonstrate several examples of successful analytical methods using these technologies.

POSTER ABSTRACT

Pharmaceutical Analysis

#108

High performance liquid chromatographic separation of thirteen drugs collected in Chinese Pharmacopoeia 2010 (Ch.P2010) on amylose ramification chiral stationary phase

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The enantiomers separation of thirteen pharmaceutical racemates collected in Ch.P2010 was performed on chiral stationary phase of amylose ramification by highperformance liquid chromatographic (HPLC) methods. In this study, thirteen types of racemates from Ch.P2010 have been selected which are commonly applied in clinics such as ß-receptor blocking drugs (propranolol hydrochloride (C8), atenolol (C9)), benzamide antipsychotic drugs (sulpiride (C10)), ß-agonist (clenbuterol hydrochloride (C11)), calcium antagonist (verapamil hydrochloride(C12)), antihistamine drugs(chlorphenamine maleate (C13)), proton pump inhibitor (omeprazole (C6)), dihydropyridine calcium channel blockers(nitrendipine (C3), nimodipine (C4), felodipine (C5)), antipyretic analgesics(Ibuprofen (C1),ketoprofen (C2)), and broad-spectrum antiparasitic agents(praziquantel (C7)). The chemical structures of these target racemates cover amines, alcohols, ethers, ketones, acylamide, halogenated aromatic hydrocarbons, polycyclic compounds, and heterocyclic compounds.The racemates were separated on AD column and AS column as stationary phase, and the mobile phase consisted of isopropanol and n-hexane. The detection wavelength and the flow rate were set at 254 nm and 0.7 mL/min, respectively. The enantiomersseparation of these thirteen racemates on chiralpakAD column and chiralpakAS column was studied, while the effects of proportion of organic additives and alcohol displacer, temperature on the separation were investigated. And the mechanism of part of racemates was discussed. The results indicated that eight types in thirteen chiral drugs could be separated on chiral stationary phase of amylose ramificationin normal phase chromatographic system. The chromatographic retention and resolution of enantiomers could be adjusted by factors including the changes of the concentration of alcohol displacer in mobile phase and organic alkaline modifier, column temperature. It was shown that the resolution was improved with reducing concentration of alcohol displacer. When the concentration of organic alkaline modifier was 0.2%, the resolution and the peak shape were fairly good. Most racemates mentioned above had the best resolution at column temperature of 25ºC. When racemates were separated, the best temperature should be kept unchanged so as to obtain stable separation results. KEY WORDS: HPLC; chiral stationary phase; optical enantiomers; amylose ramification

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POSTER ABSTRACT

Pharmaceutical Analysis

#109

Assessing Sites of Reactive Metabolite Formation via Combination of Ion Mobility and Molecular Modeling

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Reactive metabolite formation can represent a significant obstacle for drug discovery program especially in the cases where high clinical therapeutically doses are anticipated. To mitigate this risk, the tripeptide glutathione (œÃ-glutamyl-cysteinylglycine, GSH) is often used as a trapping agent to capture these chemical reactive and electrophilic intermediates. The current methodologies for GSH trapping rely on liquid chromatography/tandem mass spectrometry (LC/MS/MS) and sometimes accurate mass spectrometry. These approaches have been often restricted in providing definitive structural information on metabolism sites hence, offering only the limited guidance for lead optimization. In our present study, we propose a novel work flow to maximize the outcome from routine GSH trapping experiment and provide much desired structural information on metabolic soft spot. Seven model compounds with known glutathione conjugation liability were selected and analyzed by trave! lling-wave ion mobility mass spectrometry technology (IM) to acquire experimentally derived collision cross-section (CCS) values. Theoretical collision cross-section values were calculated by Projection Approximation (PA) algorithm embedded in DriftscopeTM after Molecular Operation Environment (MOE) software to generate lowest energy structures. The theoretical and experimentally derived collision cross-section values were compared in order to accurately assign possible metabolic spots. Advantages, limitations and future improvements of this novel approach are also discussed in the area of theoretical collision cross-section values calculation and its combination with the second-generation travelling-wave ion mobility separator to facilitate structure elucidation.

POSTER ABSTRACT

Instrumentation

#110

Simultaneous, Fast Analysis of Melamine and Analogues in Pharmaceutical Components Using Q Exactive - Benchtop Orbitrap LC-MS/MS

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Introduction:

Potential drug contamination by melamine and its analogues remain a major concern by FDA. We present a novel workflow for analysis of melamine and its analogues in at-risk pharmaceutical components using high resolution benchtop Orbitrap LC-MS/MS: Q Exactive. Simultaneous, fast screening and quantitation for melamine and its analogues in complex matrices were achieved by HRAM full scan, ms/ms in a data-dependent fashion with polarity switching.

Comparing with commonly used Triple Quadrupole MS method, this workflow allows for added post analysis flexibility, it avoids the upfront selection of specific compound masses pertaining to SRM methods. Confident identification is achieved by accurate mass measurement of both precursor and fragment ions, as well as the fine isotope pattern of 13C and 15N.

Method:

A calibration curve, containing melamine, ammeline, ammelide, and cyanuric acid, was prepared by serial dilution of a 1mg/ml stock solution to give a final concentration range from 5ppb to 50ppm. Sample preparation recovery was determined by spiking neat solutions into the commercial pharmaceutical excipients tested in this study. Q Exactive coupled to Accela UHPLC system (Thermo Scientific) was employed for the studies. LC base line separation of all four compounds was achieved within 7 minutes using Acclaim HILIC-10 column. Full scan MS at resolving power 70,000 FWHM and MS/MS at resolving power 35,000 FWHM were collected in a data-dependent fashion with polarity switching. The US FDA method for melamine and cyanuric acid was referenced with modifications.

Initial data showed that full scan analysis at 70,000 resolution (FWHM) provides spectra of high quality that have sub-2 ppm mass accuracy with external calibration and well-separated isotopic pattern of A+2 isotope ion on 13C and 15N. The high resolution accurate mass measurement, fine isotope pattern and signature product ions provide a multi-dimensional confirmation for melamine and its analogues analysis.

Product ions from MS/MS spectra were utilized for quantitation. The results have good linearity on five-point calibration curves over the range from 5ppb to 50ppm with excellent linear regression coefficients (r2>0.99) and above 85% recovery rate.

The preliminary data suggested that this robust UHPLC- HR MS/MS method provides simultaneous, simple, fast determination and quantitation of melamine and its analogues at a high confident level in a variety of pharmaceutical components.

We believe that this methodology of rapid screening using high resolution mass spectrometry, has broad implications for other arenas, including: OTC weight loss pills that contain harmful, undeclared API; performance enhancing drugs, for which there is no API present, the main ingredient being food flavoring; and some nutritional supplements, where the harmful impurity may be present at a dangerously high level.

POSTER ABSTRACT

Instrumentation

#111

Metabolic Stability Screening Workflow using a High Resolution Accurate Mass Benchtop Instrument and Novel Data Mining Software

Kate Comstock, Tim Stratton, Hai-Ling Sun, Daniel Soohoo, Kelly (Wei) Wang

1) Gilead Sciences, Foster City, CA; 2) Thermofisher Scientific, San Jose, CA

In vitro metabolic stability screening performs a very important role in the drug discovery stage for compound selection in pharmaceutical companies. The screening is primarily supported by LCMS, which involves the monitoring of the disappearance of parent compounds, using selected reaction monitoring (SRM) on triple-quadrupole instruments. If moderate to high turnover is observed, separate metabolite identification experiments are then conducted to characterize the biotransformation products. In this study, we present a novel workflow using a high resolution accurate-mass benchtop Mass spectrometer Q Exactive and data mining software MetQuest. This workflow combines relative metabolic stability and initial metabolite information from the same analysis. The high mass resolution with high scan speed data acquisition is compatible with UHPLC for high throughput screening.

Methods

Six model compounds were selected for this study. Compounds (3 µM) were incubated using human, dog and rat hepatic microsomes at nominal 0.5 mg protein/mL at 37°C for up to 60 minutes in the presence of NADPH. At 0, 5, 15, 30, 45 and 60 minutes, aliquots of the reaction mixture were transferred and mixed with quench solution. The samples were centrifuged, and supernatant was injected for analysis. The time-concentration profiles of parent compounds and identified metabolites were estimated from the acquired data by using an Q Exactive benchtop HR mass spectrometer in positive full scan/all ion fragmentation (AIF)/scans at 70,000 FWHM resolution, also full scan/top2 ms/ms at 70,000 FWHM /17.500 FWHM resolution respectively. Simultaneous acquisition and processing were performed using the relative Quan/Qual software package, MetQuest 1.1.

Results and Discussion

The metabolic stabilities of six diverse compounds- trifluoperazine, carbenoxolone, raloxifene, diclofenac, paclitaxel, and piroxicam were analyzed by UPLCMS on a benchtop Orbitrap MS with higher energy collision dissociation (HCD). Data were acquired as a full scan followed by an all-ion-fragmentation (AIF) scan, full scan/top2 data dependent ms/ms. The data acquisition was conducted by using MetQuest 1.1 metabolic screening software, which performed automatic relative Quan/Qual data analysis. Further metabolite structure determination was possible using the same data files, without reinjection, by using data mining software. Initial processing of the time-course samples was performed automatically, using a list of 50 mass changes representing commonly found biotransformations as well as screening for unexpected metabolites. To be considered an unexpected metabolite, the extracted ion chromatogram of an observed m/z value had to follow a peak shape, and not appear in the control sample. Expected metabolites were included only if the area, after comparison to the control, differed by more than 5X and whose exact mass value was within 5 ppm of the expected value.

This workflow, by employing ultra high resolution mass spectroscopy, automated software acquisition and processing for simultaneous relative quantitative and qualitative (Quan/Qual) analysis, reduces data turnaround time, increases compound throughput, and maximizes mass spectrometer usage.

POSTER ABSTRACT

Instrumentation

#112

A Dedicated, Ultra-low Flow Pump for Flow Injection Nanospray Useful for the Relative Quantification of Analytes

Gary A. Valaskovic, Ben Ngo

New Objective Inc., Woburn, MA USA

Ultra-low flow rate (< 20 nL/min) nanospray ionization has demonstrated reduced ion suppression, a trend toward equimolar response, and high ionization and utilization efficiency for small molecule analytes.(1) Experiments using ultra-low flow ESI are typically conducted using off-line (pumpless) nanospray, referred to as „static‰ nanospray. Static nanospray is operated in a regime in which the applied ESI voltage generates and controls the effective through-emitter flow rate of mobile phase. Static nanospray is difficult to control because the flow rate is a function of applied voltage, mobile phase composition, and emitter/source geometry. Furthermore, the real-time flow rate with static nanospray is usually unknown. In practice, the ESI voltage is tuned until the ion current (and hence flow rate) „looks‰ correct. Decoupling flow rate from the ESI voltage would significantly decrease the experimental uncertainty of ultra-low flow nanospray. The development of a dedicated ultra-low flow pump (1-100 nl/min) provides the analyst with the means to retain the analytical benefits of static nanospray in combination with the robustness and repeatability of pumped flow (dynamic) nanospray.

We have developed a robust, closed-loop control pneumatic pump capable of directly generating and delivering flow rates in the rage from 1-100 nl/min. Nitrogen pressure (30 psi), provided by tank or in-house gas, is fed into a digitally controlled liquid pressure cell. The outlet flow from the pressure cell is monitored by a real-time electronic flow rate sensor. A software package in communication with the pressure cell and flow sensor enables the calibration and control of the pump at flow rates with an uncertainty that is typically better than ± 3 nL/min. The pump software also controls a nanoinjection valve that enables the injection of samples into the flow stream upon exiting the pump. The level of flow control and accuracy is much better than that obtained using typical syringe pumps, or split-flow LC systems.

The pump has been combined with an on-line nanospray source fitted to a triple quadrupole mass spectrometer. Examples of using the pump to characterize mixtures of small molecule analytes will be presented. The pump enables independent control of nanospray flow rate and ESI voltage, giving the analyst full ability to optimize ionization signal, significantly reducing ion suppression.

POSTER ABSTRACT

Instrumentation

#113

The PicoChip: A Robust, Easy-to-use Solution for Nanospray Enabled LC-MS

Gary A. Valaskovic, Amanda Berg, Ben Ngo

New Objective Inc., Woburn, MA USA

Nanospray ionization, in combination with nanobore liquid chromatography tandem mass spectrometry (nLC-MS/MS), has been the method of choice for protein/peptide biomarker discovery in the life sciences. These qualititative methods have typically featured very small (75 to 100 µm) inside diameter (ID) columns running at flow rates typically between 300 to 1,000 nL/min. Columns are typically either self-made by the analyst or commercially available in the form of 380 um outside diameter (OD) fused-silica tubing. Given the small ID and OD of the tubing, and the low column volume, there are numerous challenges in the implementation of these tools for successful LC-MS analysis. The traditional laboratory solution has been a heavy investment in the education and skill set of the analyst. Key to success are critical skills in making "perfect" connections, tuning the nano-electrospray source, and deep experience in system troubleshooting. Injection-to-injection cycle times are typically long (> 30 minutes), so time lost to instrumental difficulties carries a high economic cost. Given the system and plumbing complexity, the ability to automate these processes has been limited. The growing need for analysis in the areas of protein quantification and biomarker validation places new demands on robustness, ease-of-use, and suitability for automation.

We have developed an integrated system for nanobore LC-MS that alleviates the need for specialized expertise in nano-scale separations. A novel design for a "PicoChip" based solution combines the functionality of the nanospray emitter, nanobore separation column, high-voltage contact, and autosam pler transfer line into a single consumable device. The comfortable design of the consumable package makes it exceptionally easy to handle and eliminates the risk of emitter or column breakage. A new high-voltage contact with every column change means consistent and stable spray ionization. Pre-assembly and testing of the assembled device in a production setting ensures results in the customer's lab.

The design of the PicoChip has a several-fold advantage for the end-user. PicoChip change-over can be accomplished by an in-experienced end user in a matter or minutes. The complexity of the interior design of the PicoChip means that the nanospray source itself is exceptionally simple and low-cost. The PicoChip design is readily adapted to front-end automation. A newly developed four channel source enables a facile nLC-MS/MS workflow and improves instrument duty cycle from 40% for a single channel system to greater than 95%. The enclosure of the nanobore column in the PicoChip housing also isolates the column from changes in ambient temperature and enables further temperature control such as column heating.

ABSTRACT
#114

Instrumentation

A collaborative LC/MS/MS solution for urine testing for detection and quantification of drugs-of-abuse and pain management

Martin Steel

McKinley Scientific, LLC, Sparta, NJ USA

McKinley Scientific is an organization that does not design or manufacture products, or own traditional IP. McKinley Scientific,s business is in equipment management and financing and being a conduit for our customers to more easily access technology. Our business model is to lease LC/MS/MS systems to allow users to refresh technology in a timely manner. We also marry other vendor technology with systems to support users in customizing configurations and performance. Instruments returned at lease-end are typically sold into R&D labs with a full compliment of support services provided by us or our partners.

Recognizing a need for LC/MS applications for drugs-of-abuse and pain management urine testing, it was clear that many of the businesses in this arena did not have the experience or resources to select an appropriate platform, develop and validate methods and effectively run high throughput testing services.

McKinley Scientific built a collaborative consortium to provide near turnkey testing capabilities to Clinical labs and Physician offices.

Due to the good availability of Thermo Quantum Ultra MS/MS, the Quantum platform was selected as the standard. Methods were developed by North Carolina based Opans, a contract analytical services lab, in conjunction with Georgia based Microliter Analytical Supplies, ITSP (Instrument Top Sample Prep), the manufacturer of a consumable device for solid phase extraction. The instruments are pre-configured and installed and serviced by North Carolina based Ion Technology Services. Onsite LC/MS training is provided by Opans and Validation coaching/consultation is provided by Molecular MS Diagnostics of Rhode Island who are clinically certified. McKinley Scientific is the program lead vendor, co-ordinating the services of the participating parties, and also provides financing options. Consequently our innovation is a targeted collaboration.

ABSTRACT
#115

Instrumentation

An Automated On-Line Card Extraction LC/MS System for the Determination of Clozpaine and its Metabolites in Rat Blood

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Introduction: Dried blood spot (DBS) sampling provides several advantages when compared to conventional plasma sampling including use of small blood volumes, easy sample shipping and storage, and fewer biohazard concerns. When compared to traditional hole punching and off-line extraction methods, the on-line extraction LC/MS system enables automated flow through DBS analysis and reduces analysis time and manual preparation and increases sample throughput. This work describes the development and validation of an on-line card extraction DBS LC/MS method for the rapid quantitation of clozapine and its metabolites, norclozapine and clozapine-N-oxide, in rat blood. Excellent sensitivity, linearity, dynamic range, precision, accuracy, and reproducibility, were demonstrated. The quantitation performance of the method is comparable to that of an off-line sample extraction method. This approach could be readily applied to clozapine pharmacokinetics and metabolic profiling studies.

Method Development: The automated card extraction LC/MS system consists of a Prolab card extraction instrument for automated flow-through analysis of DBS cards, a binary pump for on-line sample extraction and cleaning, an isocratic pump for sample dilution, another binary pump for analytical LC separation, and an Agilent 6400 QQQ MS for analyte detection. The Prolab instrument is a CTC based autosampler which functions as master controller of the analytical setup to integrate with the pumps and the QQQ MS.

Method development was performed in two phases to establish the optimum parameters for the on-line extraction and the analytical LC/MS detection. Clozapine and its metabolites were extracted from the DBS cards, mixed with internal standard, trapped on Trap1 column, and separated from matrix components using water and acetonitrile. After eluting from Trap1, the analytes were diluted with water to enable their retention on Trap2 column prior to the analytical run. Analytical separation was performed on a C18 column using 0.1% formic acid in water and acetonitrile. MRMs of m/z 327.0>192.1, 313.0>192.2, and 343.0>192.2, were employed for quantitation of clozapine, norclozapine, and clozapine-N-oxide, respectively. In this method, the restart for the next DBS extraction is performed from the extraction pump program while the analytical LC gradient is still running. Thus, the extraction overlaps the analytical run and achieves a cycle time of less than 10 min.

Results and Discussions: The limit of detection (LOD) in rat blood is 0.1 ng/mL for clozapine, norclozapine, and clozapine-N-oxide, and the lower limit of quantitation (LLOQ) is 0.5 ng/mL for all three analytes. Excellent accuracy (95-105%) and reproducibility (RSD<8.9%) were obtained at the LLOQ level. The calibration curves for all three analytes showed excellent linearity (R2>0.999) with over four orders of dynamic range (0.5-10000 ng/mL). The method accuracy, reproducibility, and precision were evaluated at eleven standard concentrations. The accuracy of measurement was determined to be 85˜109% for all analytes. The triplicate reproducibility is excellent with RSD <6.2% for all analytes. The precision (RSD) of analyses over the calibration dynamic range is <8.9% for all analytes.

The quantitation performance of the on-line extraction method was compared to that of a hole punching manual extraction method and comparable results were observed. The off-line method delivered a linear dynamic range of 0.5-10000 ng/mL (R2>0.999) with an LLOQ level of 0.5 ng/mL. The accuracy, reproducibility and precision of the off-line method were determined to be 88-108%, <9.0% and <6.6%, respectively. The comparable quantitation performance demonstrated the validity of the automated card extraction LC/MS system for direct analysis of DBS cards for rapid pharmacokinetics studies and metabolite profiling of clozapine.

POSTER ABSTRACT
#116

Drug Delivery

Multidrug Resistance-Associated Protein 1 (MRP1) mediated Colchicine resistance: Effect of Saikosaponin D

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Background: Colchicine, as a substrate of MDR1 and multidrug resistance -associated protein 1 (MRP1) Simultaneously, which cellular accumulation will be decreased by MDR1 and/or MRP1. This study investigated effects of Saikosaponin D on MRP1-mediated resistance of colchicine in Human Embryonic Kidney (HEK293) cells. HEK293 cells were pretreated with Saikosaponins in the presence or absence of MDR1 inhibitor verapamil and/or MRP1 inhibitor MK571 for 48 hour before colchicine treatment. Colchicine levels were measured in the cell extracts by RP-HPLC. Results: The 50% inhibiting concentration (IC50) of Saikosaponin D to Human Embryonic Kidney (HEK293) cells is 20.37 μM. And 12.80 μM Saikosaponin D decreased the cellular colchicine concentration, besides, immunoblotting assay results indicated that Saikosaponin D enhanced MRP1 expression in HEK293 cells. Conclusion: The result demonstrates that Saikosaponin D has identical effect in MRP1 mediated colchicine resistance and may be a potential inducer of multidrug resistance in MRP1 overexpressing tumor cells.

POSTER ABSTRACT

Drug Delivery

#117

Effect of Saikosaponin A, D and Fatty Acid Extracted from Vinegar-baked Radix Bupleuri (VBRB) on Efflux Transporter of MRP1

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Background and purpose: Our previous research showed that Vinegar-baked Radix Bupleuri (VBRB) has liver-targeting enhancing effect on some anticancer agents not only increased drug accumulation in the liver but also decreased distribution in not-target sites, shows a synergy and attenuation effect. Further mechanism study showed that VBRB¡¡achieved this synergy and attenuation effect may be by affecting drug transporters. In order to demonstrate the hypothesis, in this paper we investigated effects of Saikosaponin A (SSA), Saikosaponin D (SSD) and fatty acid (FA) extracted from VBRB on efflux transporter multidrug resistance-associated protein 1 (MRP1) in Human Embryonic Kidney (HEK293) cells and the exact mechanism over it.

Method: The modified MTT assay was used for analyzing cell viability of SSA, SSD and FA to HEK293 cells. Cells were divided into 4 jumpbo control group, SSA group, SSD group and FA group. In the uptake efficacy research, colchicine was used as the substrate of MRP1. Since colchicine is a substrate of MDR1 as well, in order to get rid of the MDR1-mediated interference, each jumpbogroup was subdivided into 4 groups¡ªVerapamil(-)/MK571(-) group, Verapamil(+)/MK571(+) group , Verapamil(+)/MK571(-) group , Verapamil(-)/MK571(+) group. Verapamil and MK571 are inhibitors to MDR1 and MRP1, the concentrations of them are 20 and 50 0…8M, respectively. Cells were pretreated with SSA, SSD and FA in the presence or absence of verapamil and/or MK571 for 48 hours before 90 minutes colchicine (50 0…8M) treatment. Thereafter, the colchicine levels in the cell lysate were measured by RP-HPLC. And in the Western blotting assay study, cells were also divided into 4 jumpbogroups as above. After treated with SSA, SSD or FA for 48 hours, all protein extracted from cells were applied to MRP1 expression assay.

Results: The 50% inhibiting concentration (IC50) of SSA , SSD and FA to HEK293 cells are 45.38 , 15.91 and more than 100 0…8g 6Ó7ml-1 , respectively. SSA (20 0…8g 6Ó7ml-1) and SSD (10 0…8g 6Ó7ml-1) decreased the cellular colchicine concentration, while FA (100 0…8g 6Ó7ml-1) increased the accumulation. Besides, western blotting assay results indicated that SSA and SSD enhanced the MRP1 expression in HEK293 cells (1.55 and 1.21 fold of control group), however, FA weakened the MRP1 expression (0.50 fold of control group).

Conclusion: The results demonstrated that SSA and SSD have identical effect on MRP1-mediated drug transport and may be potential inducers of MRP1. While FA reverses efflux-transport of drug and seems a promising inhibitor of MRP1. The results of this paper indicated that affecting drug transporters may be the reason for liver targeting effect of VBRB.

POSTER ABSTRACT
#118

Other

The True or False Adverse Effects?

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Safety is always the number one factor for new drug candidates. We are facing much challenge to evaluate the true negative and false positive toxicity results since many factors would affect the assessment. The relationship between the toxic response and the exposure of a chemical is very important to reveal basis related to the mechanism of action. During the myotoxicity study in Cynomolgus monkeys, it was found that combined exposure level of drug concentration and sustained time played an important role for the occurrence of myotoxicity, rather than the simple Cmax or total AUC values. In the QT interval prolongation assessment using guinea pig, the hurry injection or the organic solvent would produce the false positive QT issue. Truth of safety assessment is essential to increase the survivability for candidates and reduce the risk in clinical trials. More detail and possible mechanism will be discussed.

POSTER ABSTRACT

Instrumentation

#119

Selectivity Enhancement in High Throughput Analysis of Testosterone using Differential Ion Mobility Coupled to LDTD MS/MS

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The Laser Diode Thermal Desorption (LDTD) ionization source has been coupled to a mass spectrometer equipped with the SelexION® differential ion mobility device, enabling a high throughput capacity for the analysis of testosterone in biological matrix.

The innovative LDTD source design allows a rapid laser thermal desorption of the sample at atmospheric pressure, followed by a gas phase APCI type ionization. The absence of solvent in the APCI provides high efficiency protonation with strong resistance to ionic suppression. Since no chromatographic separation is needed, a very high throughput capability of 7 seconds sample-to-sample analysis time is achieved, without carry-over.

The SelexION ion mobility device enables enhanced selectivity over traditional MS/MS analysis by providing an orthogonal means of separating isobaric species, using differential mobility spectrometry. This capability becomes especially important when liquid chromatography is not used to separate isobaric species prior to analysis by MS/MS. This makes the junction of SelexION with LDTD an interesting match; the combination of high-throughput capability of the LDTD with the selectivity of SelexION has an unparalleled potential.

Sample preparation consisted of a simple liquid-liquid extraction of human plasma using ethyl acetate, in a 1:4 v/v ratio. 2 µl of the upper layer was deposited directly into the 96 well plate and allowed to dry prior to analysis. In the work presented here, the ion mobility cell was interfaced directly to the front of a QTRAP® 5500 mass spectrometer, and was optimized for the detection of testosterone by tuning the Compensation Voltage (CoV) parameter to a value of 4 V.

When the analysis was performed with the ion mobility device turned off, the lower limit of quantitation for the LDTD-MS/MS determination of testosterone was limited by the blank interference arising from the presence of isobaric analytes. However, when the LDTD source and SelexION ion mobility device were used in tandem to analyze spiked serum testosterone calibrators, the interference in the blank was reduced from 65% to nearly zero, resulting in a 10x improvement in the lower limit of quantitation. The lower limit of quantitation was 0.1 ng/ml, and the assay displayed excellent linearity, with r2 = 0.99972, over 5 orders of magnitude. Accuracy and reproducibility were well within the acceptable values. The sample-to-sample run time was only 7 seconds. In comparison, the equivalent analysis using conventional LC-MS/MS would typically require approximately 3-5 minutes per sample.

POSTER ABSTRACT

Instrumentation

#120

Absolute quantitation of CYP3A4 and CYP3A5 proteins in human liver microsomes by LC/MS: Correlation of polymorphic expression of CYP3A5 with vincristine metabolism

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Cytochrome P450 (CYP) proteins are important drug-metabolizing enzymes responsible for the elimination of many drugs in humans. inhibition or induction of CYP enzyme activity by a co-administered drug may result in significant drug-drug interactions. In addition, if a drug is mainly cleared by a polymorphic CYP enzyme such as CYP2D6 or CYP2C9, it may lead to significant individual pharmacokinetic variability in humans. Therefore, it is important to understand roles of CYP enzymes in major drug clearance pathways of a drug candidate in drug development. CYP3A5, a polymorphric CYP enzyme, is expressed in 75% of African-Americans, while only 10 - 20% of Caucasians express high levels of the CYP form. CYP3A4 and CYP3A5 often share the same substrates. However, determination of relative contributions of and CYP3A4 and CYP3A5 to a metabolic pathway is very difficult due to the lack of specific chemical inhibitors of CYP3A4 or CYP3A5. The main objective of the current study was to quantitatively determine absolute CYP3A4 and CYP3A5 protein contents in a panel of human liver microsomes (HLMs) that were genotyped for CYP3A5. A panel of 24 HLMs with genotypes of CYP3A5*1*1 (n = 7), CYP3A5*1*3 (n = 8) and CYP3A5*3*3(n=9) were analyzed. CYP3A4 and CYP3A5 contents of individual HLMs were determined by measuring digested peptides specific to either CYP3A4 or CYP3A5 using triple quadrupole selected reaction monitoring. Synthetic stable isotope labeled peptides were used as internal standards. Results showed that the contents of CYP3A4 were 94, 71, and 85 pmol/mg protein for genotype CYP3A5 *1*1, *1*3 and *3*3 respectively, similar across the genotypes of CYP3A5. In contrast, the contents of CYP3A5 were 37, 21 and 1.1 pmol/mg protein for CYP3A5*1*1, *1*3 and *3*3 respectively, which varied significantly among different genotypes. The CYP3A5 contents were strongly correlated with the formation rates of a primary metabolite (M1) of vicristine (r2 = 0.82) in the panel of HLMs. It was reported that the formation of M1 was mainly mediated by CYP3A5. In summary, absolute protein contents of CYP3A4 and CYP3A5 in the panel of HLMs genotyped for CYP3A5 were determined by LC/MS. The CYP3A5 contents in HLM were associated with metabolism rates of vicristine, a known CYP3A5 substrate. The HLM panel will be served as a useful tool for the reaction phenotyping of CYP3A4 and CYP3A5 and assessment of contribution of CYP3A5 to metabolic variability of drug candidates in vitro.

POSTER ABSTRACT

Instrumentation

#121

Fast and Clean Sample Extraction Procedures

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Today, there are three commonly used sample extraction methods in pharmaceutical industries and CRO labs. The first choice is protein precipitation, which is fast, easy and applied to all kinds of chemicals. However, it provides limited clean up and generally has strongest matrix effect. When using UHPLC-MS/MS to analyze protein precipitate samples, UHPLC columns are blocked quickly.

Liquid-liquid extraction and its high throughput version, supported liquid-liquid extraction (SLLE), are the second choice. SLLE is a relative cleaner method, while still easy to develop. However, it,s limited to hydrophobic compounds, and it still extracts phospholipids, which could cause matrix effect.

The third choice is a solid phase extraction method. It is the cleanest method and applied to a broader range of chemicals, but most of time, chemists consider it hard to develop, and extraction procedure is too complicated.

In our lab, we focused on developing a SPE method working for a wider range of chemicals, and the cost of SPE cartridges (or plates) and solvents are compatible with SLLE and protein crash plates. In this way, chemists will not be worried about method developments, while still using a clean extraction method for LC-MS/MS analysis.

We developed a single extraction method to extract both hydrophobic (hydrocortisone and carbamazepine) and hydrophilic (acetaminophen and caffeine) compounds in plasma samples. Plasma sample can be loaded directly to Sagacity HL PDVB extraction cartridges (or plates), followed by simple wash and elute steps with different percentage of methanol. Complete recoveries (96-102%) are obtained for all those compounds with good reproducibility, and matrix effects are between 83%-103%.

Similar extraction method can also be used for Celerity Deluxe 2 mg/well 96-well extraction plate. One of the advantages of this procedure is that it eliminates evaporation step and uses very little extraction solvents. In general, it only needs 100 ˆ 200 µL of wash solvents, and 100 µL elution solvent. The recovery and reproducibility are as good as those of regular size extraction plates.

POSTER ABSTRACT

ADME

#122

A new strategy for discovery and identification of metabolites using high-performance liquid chromatography coupled with a hybrid linear ion-trap and fourier transform ion cyclotron resonance mass spectrometry after oral administration of five epimedium flavonoids monomers and the herbal extract of epimedium to rats

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A new strategy using high-performance liquid chromatography coupled with a hybrid linear ion-trap and fourier transform ion cyclotron resonance mass spectrometry (HPLC-LTQ-FTICR-MS) and mass frontier software was established to discovery and identification of metabolites in rat after oral administration of five epimedium flavonoids monomers and the herbal extract of epimedium, respectively. The rat bile, feces and urine samples were collected and purified by methanol precipitation, and then injected onto an Agilent Zorbax-SB C18 column (250mm 4.6 mm, 5 μm) with a gradient elution system composed of 0.3 % acetic acid and acetonitrile at a flow rate of 1.0 mL/min. Through dynamic exclusion and data dependent scan, HRMS and multiple-stage mass spectrometric data of compounds in biological samples could automatically be acquired even when dealing with rapidly changing concentrations observed across a typical HPLC peak. For rapidly exploring metabolites from complicate!

d biological matrixes, we developed a novel mass spectrum tree similarity filter (MTSF) technique by in-depth excavation mass frontier software potential function. It was a simple four-step approach to discovery and identification of metabolites. First, HRMS and multiple-stage mass spectrometric data of all detect compounds were changed to mass spectrum tree data by mass frontier software. Second, predictable metabolites which molecular weights can be easily predicted, were detected by the high resolution extracted-ion chromatography. Then mass spectrum tree data of parent compounds and predictable metabolites were imported into a new established library. Third, the probability matching scores were statistical calculated by comparing mass spectrum tree data of all detected compounds in biological samples with that of the compounds in the library. The highest matching score is 1000, while the no matching score is 0. According to our experience, the metabolites with the scores greater than 200 were confidential, Score over 200 as a limit can effectively filter endogenous interference compounds and fish possible metabolites. Finally, possible metabolites were identified by further using the high accurate molecular mass combined with multiple-stage mass spectrometric data and fragmentation rules. This new stategy was tried to discovery and identify the metabolites of five flavoniod monomers and, a total of 12 metabolites of icarrin 20 metabolites of epimedin A, 15 metabolites of epimedin B, 15 metabolites of epimedin C and 15 metabolites of baohuoside were identified in rats, respectively. Then the mass spectrum tree data of above metabolites were imported to the library and expanded database. The new strategy was put into practice to screen and confirm the metabolites of herbal extract of epimedium in rats, and 108 metabolites were discovered and identified. As the results, the metabolic profile of epimedium was obtained while the metabolic pathway was proposed. However, in our previous study, we just identified 9 major metabolites of epimedium using common metabolites mining approaches. In a word, the results demonstrated that this strategy should be useful to discovery and identification of metabolites in complex biological matrixes, especially low levels of unpredictable metabolites.

POSTER ABSTRACT

ADME

#123

Involvement of CAR and PXR in the transcriptional regulation of CYP2B6 Gene Expression by Traditional Chinese Medicines

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Abstract: Chinese herbal medicines are widely used for clinical treatment in China and other Asian countries. To date, drug-drug interactions involving Chinese herbal compounds have been reported. Induction of hepatic drug-metabolizing enzymes (DMEs) can affect drug efficacy and cause toxicity, however, so far, limited information is available regarding the molecular mechanism how herbal medicines induce human CYP2B6 which metabolizes many of the clinically used therapeutics and activates several pro-carcinogens or toxicants. Accumulated evidence suggests that the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) play important roles in trans-activation of CYP2B6. Here, the effect of several Chinese herbal compounds on the dose dependency and receptor specificity of PXR and/or CAR mediated CYP2B6 induction was examined by CYP2B6 luciferase reporter gene assays. The HepG2 cell line was transiently transfected with human CYP2B6 luciferase promoter report! er plasmids along with hPXR or hCAR expression vectors. Furthermore, the gene expression of CYP2B6 in human intestinal LS174T cell lines treated with different herbal compounds was determined by using real-time PCR. By combining cell-based reporter assays and quantitative analysis of gene expression in cell line, the CYP2B6 inducers were classified as hPXR or hPXR/hCAR dual activators. We observed that an array compounds extracted from traditional Chinese medicines can up-regulate CYP2B6 gene expression by activation of hPXR in a concentration-related manner, and several herbal compounds acted as the ligands of both hPXR and hCAR to induce CYP2B6 gene expression. In conclusion, our results of hPXR/hCAR-mediated induction of CYP2B6 by Chinese herbal medicines have important implications in herb-drug interactions.

POSTER ABSTRACT

ADME

#124

Absorption and Pharmacokinetic Study of Radix Rehmanniae in Rats

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Abstract: Radix Rehmanniae was a commonly used herbal medicine. In this paper, absorption and pharmacokinetic study of Radix Rehmanniae was conducted in rats after oral administration of Radix Rehmanniae water extract. Physicochemical properties of main components in Radix Rehmanniae, such as molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, logP, number of rotatable bonds, topological polar surface area, were calculated by Molinspiration software. Combined with content in the extract and bioavailability of some components, absorption of main components was predicted by Lipinski's rule of five and Veber's rule. Iridoid mono-glycosides, such as catalpol and ajugol, were the main components absorbed, with bioavailability 10% to 20%. Biological samples, such as plasma, urine and bile, were analyzed by LC-MSn. Catalpol and ajugol were identified as the main absorbed components, excreted to urine in their prototype. They were not detected from bile samples post administration. There was no other component and metabolite in those samples. MS2 fragmentation of those absorbed components was evaluated under full product ion scan mode. Ammonia adduct ions of those components were selected as parent ions, and fragment ions produced by cleavage of glycosidic bonds, or with further dehydration, were selected as product ions. The pharmacokinetic study of those absorbed components was conducted after oral administration of 6 g/kg Radix Rehmanniae water extract to rats. Pharmacokinetic parameters were calculated with Non-compartmental model by WinNonlin software. Both catalpol and ajugol were absorbed and excreted rapidly with Tmax at 1 h and MRT0-¡fi at 1.7 h and 2.0 h. There was gender difference in plasma exposure of catalpol and ajugol from Radix Rehmanniae. Both Cmax and AUC of catalpol and ajugol in female rats were higher than those in male rats.

Catalpol and ajugol were proved to be the main components absorbed from Radix Rehmanniae, which was consistent with the in silico prediction in this thesis. Plasma exposure of them in female rats was higher than that in male rats, with gender difference.

KEY WORDS: Radix Rehmanniae, catalpol, ajugol, liquid chromatography coupled with mass spectrometry, absorption, pharmacokinetics, in silico prediction

POSTER ABSTRACT

ADME

#125

In vivo study on metabolic transformation of bioactive xanthones from Halenia elliptica D. Don by high performance liquid chromatography coupled to ion trap time-of-flight mass spectrometry

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Halenia elliptica D. Don (Gentianaceae) is commonly used as a Tibetan medicinal herb in the treatment of hepatitis and cholecystitis. The preliminary results indicated that xanthones from this Tibetan medicine caused vasodilation in rat coronary artery pre- contracted, exhibiting either endothelium-dependent or endothelium-independent mechanisms in rat coronary artery. In this study, major components, such as 1-hydroxy-2, 3, 5- trimethoxy-xanthone (HM-1), 1-hydroxy-2, 3, 4, 7-tetramethoxy-xanthone (HM-2) and 1- hydroxy-2, 3, 4, 5-tetramethoxy-xanthone (HM-3), which are abundantly present in the original herbs, have been investigated on their metabolic transformation in vivo. A method of high performance liquid chromatography coupled to ion trap time-of-flight mass spectrometry (LC-ESI-IT-TOF) was applied for on-line analysis and identification of metabolites in plasma, bile, urine and feces of SD rat.

In the present study, twenty-five metabolites were detected after intravenous administration and their structure could be determined on the basis of difference of fragmentation behaviors with increase of stages or relative abundances and previous results from study of HM-1, HM-2 and HM-3 in vitro. Consequently, metabolic pathways of HM-1, HM-2 and HM-3 in vivo were proposed. The results indicated in vivo metabolic transformation of HM-1, HM-2 and HM-3, phase II reaction glucuronidation and sulfation were mainly occurred, and only six hydroxylated products of phase I reaction were detected among twenty-five metabolites in total. HM-5 and HM-3A, which, according to the in vitro study previously, were the major metabolites of HM-1 and HM-3 with same structure of 5- OCH3, were found to have the sulphate conjugations. Compared with HM-1 and HM-3, the amounts of metabolites of HM-2 with the structure of 7-OCH3 were detected the most. HM-1, HM-2 and HM-3 existed as in the original form in blood, eliminated through bile and excreted through urine and feces. This study provided scientific basis for comprehensive and systematic early development and research of biologically-active xanthone derivatives of Halenia elliptica D. Don. KEY WORDS: Halenia elliptica D. Don; xanthones; metabolites identification; in vivo; LCMS- IT-TOF

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POSTER ABSTRACT

ADME

#126

Establishment of Cell Model with Stable Expression of hPepT1

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The human intestinal di/tri-peptide transporter, hPepT1, has extremely broad substrate specificity, including di-and tripeptides arising from breakdown of food protein, peptide- like drug molecules such as β-lactam antibiotics, bestatin, ACE inhibitors. Because of its unusually broad substrate selectivity, large transport capacity, and significance in prodrug design, intensive investigation correlate with hPepT1 has been done. With the hope of supplying an useful tool to test PepT1-dependent transport, our study aimed to establish a cell model with stable-expressed human oligopeptide transporter 1(hPepT1) in MDCK.

The recombinant plasmid pcDNA3.1(+)-hPepT1 was constructed and transfected into MDCK cells, after G418 screening for 10 to 14 days, monoclones were obtained by limiting dilution. The transport activity of monoclones were preliminarily testified by fluorescent substrate β-Ala-Lys(AMCA), and Glysar was applied subsequently to further testify their transport activities, while the reverse transcription-polymerase chain reaction (RT-PCR) was employed to validate the expression of hPepT1 at transcription level. Finally, two monoclones named as D19 and A11 possessed highly uptake of AMCA than that of mock cells(cells transfected with empty pcDNA3.1(+)) were obtained, the uptake of Glysar in D19 and A11 were found to be increased up to 24, 11-fold when compared to that of mock cells, respectively, and the mRNA level of hPepT1 in D19 and A11 were significantly higher than that in mock cells, the uptake of Glysar in D19 were markedly inhibited by known hPepT1 inhibitors, such as cefalexin, valacyclovir. In conclusion, a MDCK/hPepT1 cell model was established, which can be applied to test PepT1-dependent transport, such as screening substrates and inhibitors of PepT1,in prodrug design targeting PepT1, as well as studying the drug-drug interaction mediated by hPepT1.

POSTER ABSTRACT

ADME

#127

Assessment of Drug-induced Liver Injury at Early Discovery Stage using Sandwich-cultured Hepatocytes Model

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Drug induced liver injury (DILI) is a critical safety issue for pharmaceutical industry. Efficient and effective prediction of DILI at early stage of drug discovery may facilitate drug screening process greatly.

A rat sandwich-cultured hepatocytes (rSCH) model was successfully setup to evaluate potential DILI with the help of metabonomics. rSCH maintained hepatic polarity ,morphology , liver specific metabolic activities and developed functional bile canalicular networks over time. Three traditional hepatotoxic compounds were tested in rSCH model. Conventional cytotoxic endpoints such as MTT and ATP measurement could identify liver injury induced by valproate and chlorpromazine but not alpha-naphthyl isothiocyanate (ANIT). However, ANIT reduced biliary excretion index (BEI) dose dependently in rSCH which indicated ANIT may damage rat hepatocytes biliary excretion ability directly. As a widely acknowledged sensitive method to predict hepatotoxicity, metabonomics assay was carried out to check ANIT toxicity to rat hepatocytes. The metabonomic profile was consistent with BEI observation. The results suggested ANIT may directly damage biliary excretion function in rSCH without significa! nt cell death. rSCH plus metabonmic assay could be developed into a promising alternative platform, which may predict DILI at very early discovery stage for hepatotoxicity screening.

POSTER ABSTRACT

ADME

#128

Metabolism of BYZX in Human Liver Microsome: Identification of Its Metabolites and Its Metabolic Pathways

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BYZX, [(E)-2-(4-((diethylamino) methyl) benzylidene)-5,6-dimethoxy-2,3-dihydroinden-one, is one of the series of novel acetyl cholinesterase inhibitors, synthesized as a new chemical entity for the treatment of Alzheimer's disease (AD) symptoms. BYZX could be rapidly transformed into some specific products in rat and dog and these products were also found in human liver microsome (HLM). In this study, the metabolism of BYZX was performed by using HLM and recombinant human cytochrome P450s (CYP). Three major products named M1, M2, and M3 were found in HLM incubation. The Km values of the formation of M2 and M3 were determined in HLM to be 19.59 ¡À 1.27 μM and 102.8 ¡À 15.27 μM, respectively. To identify the chemical structures of these metabolites, M1, which was also found to be the main metabolite in rat, was isolated and was verified as a N-des-ethylated and C-C double bond-hydrogenated product by LC-MS/MS and NMR (including 1H-NMR, 13C-NMR, H-H COSY, HMQC and HMBC), and M2 and M3 were identified as a hydrogenated metabolite and a des-ethylated metabolite, respectively, compared their mass spectrometric data with that of M1. Studies utilizing chemical inhibitors and recombinant CYPs were further carried out. The results indicated that the metabolic pathway of des-ethylation was primarily catalyzed by CYP3A4, and CYP2C8 might probably participate in this process according to the result giving by the recombinant CYPs metabolism study. Otherwise, the hydrogenation of BYZX found at C-C double bond site instead of carbonyl group site had been detected in all of the HLM and recombinant CYPs incubation samples and this reaction seemed not to be inhibited by any chemical inhibitors of CYPs, which indicated that the NADPH-CYP450 reductase (OR) existing in all samples might be one of the enzymes related to this hydrogenation pathway. It was further testified by using recombinant OR and Cytochrome b5.

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ADME

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Cloning and expression of the rat long-chain 2-hydroxy-acid oxidase

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(S)-2-hydroxy-acid oxidase is an FMN-dependent enzyme which can catalyze the oxidation of 2-hydroxy acids to 2-keto acids with the reduction of oxygen to hydrogen peroxide. This enzyme is a peroxisomal enzyme and exists as two major isozymes, isozyme A and B. The B form of rats is also called long-chain 2-hydroxy-acid oxidase (LCHAO) and its physiological substrate is unclear. In this work, rat LCHAO isozyme â1 and â2 (an insertion of three amino acids, -VRK-, to the mature â1 isozyme) have been successfully cloned and expressed with expression vector pET-28(a) in the E.coil strain Rosetta(DE3). It was confirmed by SDS-PAGE and western blot results at protein level. After purified by Ni-NTA purification system, the two recombinant enzymes can show high activity toward L-mandelic acid and 2-hydroxyoctanoate through measuring the change of electron acceptor DCIP in A605. For L-mandelic acid, the Km value and Vmax of isozyme A were 206.85±32.03 μM and 1434.50±28.9!

And to isozyme B, the Km value and Vmax were 229.95±99.91 μM and 1696.50±13.44. For 2-hydroxyoctanoate, the Km value and Vmax of isozyme A were 32.70±0.89 ìM and 839.25±20.44. And to isozyme B, the Km value and Vmax were 30.47±0.40 μM and 1192.50±120.92 . These two isozymes can be used to screen the substrates, inducers and inhibitors of LCHAO. It would be useful to identify chemical agents that can modulate the activity of 2-hydroxy-acid oxidase as therapy drugs of Metabolic Syndrome and to study the biological role of 2-hydroxy-acid oxidase in lipid biochemistry in mammals.

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ADME

Mechanistic study on the metabolism and transport of scutellarin: selective OATP2B1-mediated hepatic uptake is responsible for its unusual pharmacokinetic characteristics

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Unusual oral pharmacokinetics of scutellarin (scutellarein-7-O-glucuronide, S-7-G) has been observed in humans: S-7-G was hardly detected, while its isomeric metabolite (S-6-G) dominated in the circulation. Previous rat study revealed that S-7-G is absorbed mainly as its aglycone, which undergoes intestinal glucuronidation and forms S-7-G and S-6-G with high preference for S-7-G, but S-7-G/S-6-G ratio dramatically declines due to higher hepatic clearance of S-7-G. This study was aimed to uncover the mechanisms underlying the formation and elimination of S-7-G and S-6-G in humans and to further explain the human pharmacokinetics. Opposite to the pharmacokinetic observation, the formation of S-7-G through glucuronidation of aglycone was superior to that of S-6-G in human intestinal and hepatic microsomes (mainly mediated by UGT1A9), while the further glucuronidation into S-6,7-diG, their major metabolic pathway in humans, prefers S-6-G to S-7-G (mediated by UGT1A1, 1! A8, and 1A10). Efflux transporter study showed BCRP and MRP2 played important roles in the intestinal and biliary efflux of S-6-G and S-7-G, but none exhibited significant substrate selectivity. Uptake transporter study indicated that OATP2B1 was responsible for the hepatic uptake of S-6-G and S-7-G, which showed significant preference to S-7-G, especially at low concentration levels (Km values were 1.77 and 43.0 μM for S-7-G and S-6-G respectively). Furthermore, a co-incubational system would further enlarge the difference between them. In conclusion, the formation and elimination of S-6-G and S-7-G in humans involves various UGTs and transporters, and the selective OATP2B1-mediated hepatic uptake greatly contributes to the much lower exposure of S-7-G than S-6-G.

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ADME

#131

Pharmacokinetic preliminary evaluation on a novel CNS drug IMMLG5645-2 and its derivative compounds

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IMMLG5645-2 is a novel central nervous system (CNS) candidate, and it is developed by the Institute of Materia Medica. Its six derivative compounds were screened by pharmacokinetic method to choose a preferable one, which could pass blood-brain-barrier for further development. In this study, a LC-DAD-MS method was established for determining the concentration of IMMLG5645-2 and its six derivative compounds in rat plasma and cortex. An Alltima C18 column (4.6 mm ◊ 150 mm, 5 μm) was used; the mobile phase was consisted of a mixture of acetonitrile and 0.02 M ammonium formate (10:90) with a gradient elution (the condition is as follows, 0 to15 min, acetonitrile of 10% to 80%), and the flow rate was 0.8 mL/min; the detective wavelength and column temperature were set at 332 nm and 35oC, respectively; electrospray ionization (ESI) source was operated under a positive ion mode with selected ion monitoring mode (SIM); the selected ion for IMMLG5645-2 was m/z 304.9. The standard calibration curves for IMMLG5645-2 and its six derivative compounds (including hydrochloride (C1), oxalate (C2), tosilate (C3), maleate (C4), malonate (C5), citrate (C6)) were linearly proportional to the concentration from 0.1 μg/mL to 30 μg/mL in plasma and from 1 ng/mL to 2000 ng/mL in cortex, respectively. All the detection limits of IMMLG5645-2 and its derivative compounds were 0.05 ng. This method was applied to evaluate pharmacokinetic features of IMMLG5645-2 and its six derivative compounds. Rats were i.v administered with doses of drug (2◊10-5 mol/kg). Plasma and cortex samples were subjected to protein precipitation with acetonitrile and were determined by LC-DAD-MS. Concentration-time curves of IMMLG5645-2 and its six derivative compounds were acquired using GraphPad Prism 5.0 software. Statistical analysis of the data was carried out by one-way ANOVA, and the p values less than 0.05 were considered statistically significant. IMMLG5645-2 group was set as the control group. In plasma samples, there were no significant differences (P>0.05) in two groups of oxalate (C2) and malonate (C4), but significant differences (P<0.05) could be observed in other groups compared with control group. Moreover, in cortex samples, there were no significant differences (P>0.05) in all derivative compounds groups compared with control group. This method is simple and rapid, and could be applied to the pharmacokinetic screening study of IMMLG5645-2 and its derivative compounds.

KEY WORDS: IMMLG5645-2; LC-DAD-MS; pharmacokinetics

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POSTER ABSTRACT

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#132

Effects of Ketoconazole and Rifampicin on Morinidazole Pharmacokinetics of in Healthy Subjects

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Aim: Morinidazole is a new third-general 5-nitroimidazole antimicrobial agent that is metabolized primarily by N+-glucuronidation mediated by UGT1A9 and undergoes oxidative metabolism by CYP3A4. In addition, the pharmacokinetics of metronidazole and ornidazole were reported to be influenced by CYP3A4 inhibitor and inducer. The aim of this study is to characterize the impact of ketoconazole and rifampicin on morinidazole pharmacokinetics.

Methods: Two studies were performed in healthy volunteers. One study examined the effect of ketoconazole 200 mg once daily for 7 days on a single 500-mg dose of morinidazole by intravenous infusion administered over 40 min in 12 healthy subjects. The other study examined the effect of rifampicin 600 mg once daily over 6 days on a single 500-mg dose of morinidazole in 12 healthy subjects. Morinidazole and its main metabolites in human plasma up to 36 h were simultaneously determined by a validatd LC-MS/MS method.

Results: When co-administration with ketoconazole, there was no statistically significant difference observed in the pharmacokinetic parameters AUC, Cmax and T1/2 of morinidazole. While the AUC of N+-glucuronides (M8-1 and M8-2) were decreased by 20% and 15%, respectively. Rifampicin altered morinidazole AUC and Cmax with geometric mean (95% CI) decreases of 77% (66, 91) and 72% (68, 76), respectively. Conclusion: Systemic exposure to morinidazole was not altered by ketoconazole, indicating that morinidazole was safe and well tolerated when given alone and in combination with potent inhibition of CYP3A4. The AUC of morinidazole was slightly decreased by rifampicin, and more pharmacodynamic data will be needed to determine whether dosage adjustment is required.

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#133

Hepatic SLC and ABC Transporters Mediating Biliary Excretion of Ginsenoside Rg1 and Their Impact on Rat Systemic Exposure to the Saponin

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Background and purpose: Ginsenosides Rg1 and Rb1 are major pharmacologically active triterpene saponins of Panax notoginseng roots (Sanqi), a Chinese cardiovascular herb. Our earlier studies indicated that rapid and extensive biliary excretion of ginsenoside Rg1 was associated with its differences in systemic exposure from ginsenoside Rb1. The aims of the current studies were to elucidate the molecular mechanism of biliary excretion of ginsenoside Rg1 and to assess the effects of hepatic transporters on rat systemic exposure to the saponin.

Experimental approach: Transport experiments and kinetic assessment were performed using rat or human transporters-expressing HEK293 cells and membrane vesicles, the transport activities of which were validated using the respective known substrates. The transport rate was calculated using LC-MS/MS-measured intra-cellular or intra-vesicular concentrations of ginsenoside Rg1 or relative ATPase activity. Ginsenoside Rb1 was used as a control compound. In vitro and in vivo activities of rat Oatp1b2 were inhibited with rifampin. In addition to normal Sprague-Dawley rats, Mrp2-dysfunctional Eisai hyperbilirubinemic rats were also used to study the effect of Mrp2 on the systemic exposure to ginsenoside Rg1.

Key results: Rat Oatp1b2 and human OATP1B3 exerted potent in vitro activities towards the transport of ginsenoside Rg1, with Km and Vmax values of 31 and 15 μM, respectively, which could be inhibited competitively by rifampin (the Ki values 0.7 and 3.1 µM, respectively). Meanwhile, rat Mrp2 and human MRP2 also had potent in vitro activities towards the transport of ginsenoside Rg1 with Km and Vmax values of 20 and 94 μM, respectively. In contrast, ginsenoside Rb1 was not the substrate of these transporters except for rat Mrp2 exhibiting a low activity (Km value of 206 μM). Concomitant i.v. administration of rifampin in Sprague-Dawley rats resulted in a 2.5-fold increase in the mean AUC0¡ú4h of ginsenoside Rg1, while the associated mean biliary excretion clearance (CLB) was decreased to 11% of that of the control rats. In addition, the Mrp2-dysfunctional Eisai hyperbilirubinemic rats also exhibited a 4.5-fold increased mean AUCUof ginsenoside Rg1 as compared with the data in the normal rats, whereas the associated CLB was diminished to only 9% of that of the control rats.

Conclusion and implications: Both Oatp1b2 and Mrp2 are implicated in the rapid biliary excretion of ginsenoside Rg1 in rats, which can affect the systemic exposure level of the saponin. OATP1B3 and MRP2 may be important for biliary excretion of ginsenoside Rg1 in humans. The reason for the differences in systemic exposure level and pharmacokinetic behaviors between the two ginsenosides is that ginsenoside Rb1 is not the substrate of these hepatic transporters except for rat Mrp2 revealing a low activity.

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#134

Short Oral Absorption model: One Stone to Shoot Multiple Birds

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One major challenge in CNS drug discovery is to identify compounds with decent systemic and brain exposure which are commonly evaluated by in vivo tools such as rodent brain penetration, and rodent PK after IV and oral administrations. To be compliant with 3R (reduction, refinement and replacement) principles, we have adapted a short oral absorption (SOA) model in rats or mice (See figure below). After single oral dose administration, pre-systemic, systemic, liver, brain and CSF samples were obtained from each animal and analyzed by LC/MS/MS. Total brain-to-blood ratio, oral exposure, rough estimation of hepatic extraction and an insight of oral bioavailability can be obtained from only 6-8 animals. Moreover, free drug exposure in brain can be estimated by measuring its concentration in CSF, a surrogate of free drug brain exposure. Model validation with selected 4 tool dr

POSTER ABSTRACT

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#135

Pharmacokinetic Study of Rhubarb Anthraquinones in Normal and Ulcerative Colitic Rats

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Rhubarb (Rhei Rhizoma et Radix) is an important medicinal herb used in clinical practice of Chinese medicine for the treatment of digestive diseases such as constipation, gastritis, enteritis, gastric ulcer and hepatitis. Recent studies also support its health benefits in alleviation of ulcerative colitis (UC), an inflammatory destructive disease of the large intestine. This study aimed to compare systemic exposures of Rhubarb anthraquinones, the main bioactive components of this herb, between UC and normal rats and to identify the factors causing the pharmacokinetic difference. Male Sprague-Dawley rats were treated with dextran sulfate sodium (UC group) or drinking water (normal group). On day 21, both groups received a single oral dose of Rhubarb extract and blood samples with drawn for quantification of Rhubarb anthraquinone aglycones directly or the total of each anthraquinone (aglycone + conjugates) after acidic hydrolysis pretreatment. Rhubarb anthraquinones predominantly existed as conjugates in rat plasma from both groups. Compared to those in normal rats, systemic exposure of each anthraquinone (aglycone+conjugates) decreased while the proportion of each aglycone to the total (aglycone+conjugates) increased in UC rats. Decreased metabolic activity of gut bacteria from UC rats on hydrolysis of anthraquinone aglycosides to form respective aglycone was demonstrated in vitro. Correspondingly, bacterial compositional analysis using 16S rRNA-based real-time quantitative PCR revealed decreases of Clostridium leptum and the probiotic genera Bifidobacterium and Lactobacillus as well as an increase of Bacteroides fragilis in gut bacteria from UC group. The beta-glucosidase and beta-glucuronidase activities of UC group were also significantly decreased. Moreover, the intestinal microsomes from UC rats catalyzed glucuronidation of anthraquinones with higher activities, while the activities of hepatic microsomes showed no difference between normal and UC groups. In conclusion, there were acompositi! onal alteration and metabolic functional decrease of gut bacteria as well as an increase of intestinal glucuronidation activity in DSS-induced colitic rats. These factors may account for the pharmacokinetic difference of Rhubarb anthraquinones observed between UC and normal rats.

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ADME

#136

Metabolism of and its Impact on Rat Systemic Exposure to Tanshinol, a Ca2+-Channel Blocker from Salvia miltiorrhiza (Danshen)

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Background and purpose: Tanshinol, a main constituent of Salvia miltiorrhiza roots (Danshen), is a Ca2+-channel blocker and has antioxidant activity. This phenolic acid also has favorable pharmacokinetic properties. Our earlier study indicated that, besides renal excretion, metabolism was another major elimination route for tanshinol. The aim of this study was to investigate systematically the tanshinol metabolism in rats. Experimental approach: A Waters Synapt G2 mass spectrometer interfaced via an ESI probe with a UPLC module was used to measure tanshinol metabolites in plasma, urine, bile%2

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#137

Alterations of Composition and Metabolic Function of the Predominant Fecal Microbiota from Ulcerative Colitic Rats

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Clinical observations and experimental data obtained from animals indicate an association of imbalanced intestinal bacteria with the initiation and amplification of ulcerative colitis(UC). The objectives of this study were to characterize dynamic compositional alterations of the predominant intestinal bacteria, as well as to monitor the change of metabolic activity of fecal microbiota, during UC progression in a rat model. UC was induced in Male Sprague-Dawley rats by administration of dextran sulfate sodium(DSS) in drinking water. Rats receiving drinking water served as controls. Fecal samples were collected at day 0 and every 3 days till day 21 for measurement of metabolic activity with probe substrates and fecal microbiota profiling using 16S rRNA based real-time quantitative PCR (RT-PCR) analysis with genus-speciﬁc primers for six dominant genera and a universal primer for the total bacteria. In bacterial samples from normal rats, the proportions of 6 genera were relatively stable throughout the experimental period. In contrast, after initiating DSS-stimulation for 7-10 days, fecal microbiota from UC rats exhibited higher contents of Bifidobacterium, Clostridium coccoides and Bacteroides fragilis, which decreased at the end of DSS treatment, while the relative amounts of Lactobacillus and Clostridium leptumwere the same as or lower than those in normal group. Moreover, there was an increase in beta-glucuronidase activity and a decrease in beta-glucosidase activity during UC progression, while at the end of DSS-intervention both activities were significantly lower than those from control group. These findings agreed well with previously reported fecal microbiota alterations in human UC and support the DSS-induced rat as a suitable model for fecal microbiota-oriented drug discovery from and evaluation of Chinese medicine.

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POSTER ABSTRACT

ADME

#138

Structure-metabolism relationships of angular-type pyranocoumarins from Peucedani Radix

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As the major bioactive components of Peucedani Radix (Chinese name: Qian-hu), angular-type pyranocoumarins (APs) have attracted extensive research interests as sources for discovering drug candidates for anti-cancer and cardioprotective therapies. The present study aimed to identify the structure-metabolism relationships (SMRs) of APs in rat liver microsomes (RLMs) and plasma. A total of twenty-one APs that belong to 6 core structures and include 7 pairs of enantiomers were investigated. In general, APs underwent oxidation, hydrolysis and acyl migration in RLMs and hydrolysis and acyl migration in plasma. In RLMs fortified with NADPH, hydrolytic removal of C-3′ and/or C-4′ acyl substituents was revealed as the major reaction for all APs, oxidation only occurred at large (isovaleryl, angeloyl or senecioyl) side chain, not at smaller (acetyl or isobutyryl) substituent, yet acyl migration was only observed for cis-khellactone derivatives that have ester bonds at both C-3′ and C-4′ positions in the same configuration. When in absence of NADPH, carboxylesterase(s)-mediated hydrolysis was the major reaction type and occurred to APs having a combination of angeloyl and acetyl/carbonyl or acetyl and isobutyryl substituents with at least one R-configuration at C-3′ and/or C-4′ in liver microsomes. Very interestingly, those APs with a carbonyl or cis-khellactone derivatives with R-angeloyl group at C-4′ could be hydrolyzed by liver microsomal carboxylesterase(s) but were resistant to rat plasma carboxylesterase(s). When hydrolysis occurred, the skeleton structure would be generated as one of main products and kept the absolute configuration of respective APs. Stereoselective metabolism was observed for each pair of enantiomers in all types of reactions in RLMs and plasma.

Carboxylesterase(s)-catalyzed hydrolysis exhibited a preference for enantiomers with R configuration at C-3′ position. These findings demonstrated regio- and stereo-selective metabolisms of angular-type pyranocoumarins and the SMRs may guide structural modification of this type of compounds during drug discovery.

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ADME

#139

Triptans are substrates of OATP1A2 and can be transported by this transporter across MDCKII monolayer

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Membrane transporters at the blood-brain barrier (BBB) have profound effect in drug transport to brain. OATP1A2 has the highest expression in the human brain (Kullak-Ublick et al., 1995) and is exclusively localized on the luminal membrane of BBB (Bronger et al., 2005). However, the role of OATP1A2 for CNS drug discovery is unknown. We have established a robust BacMam2-OATP1A2 transduced HEK293 system. Among the 37 CNS marketed drugs tested, triptans, 5-HT1B/1D receptor agonists for the treatment of migraine attacks, were identified as OATP1A2 substrates. Kinetics (Km and Vmax) were determined for seven marketed triptans. OATP1A2 is localized to the apical side of BBB which do not necessarily imply that OATP1A2 must be involved in drug uptake into the brain. We have transduced BacMam2-OATP1A2 into MDCKII cells and demonstrated that OATP1A2 was specifically expressed on the apical membrane of MDCKII monolayer and was capable to facilitate transport of triptans across the MDCKII monolayer from apical to basolateral side. Involvement of OATP1A2 for brain penetration of triptans in human requires further investigation.

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POSTER ABSTRACT

ADME

#140

Absorption and Disposition of Sodium Picosulfate in Humans: A Pharmacokinetic Explanation for the High Exposure of Its Conjugated Metabolites

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Sodium picosulfate (PICO), a disulfate conjugate, is a member of the polyphenolic group of stimulant laxatives. Its aglycone BHPM is the active laxative form, released in the colon via enzymatic hydrolysis of PICO. The objectives of this study were to characterize the pharmacokinetic profiles of PICO and its major metabolites, and explain the high exposure of the conjugated metabolites. After an oral administration of 5.0 mg PICO to 12 healthy subjects, monoglucuronide conjugate (M1) of BHPM was the major circulating chemical species, followed in decreasing abundance by parent drug and monosulfate (M2) of BHPM. The AUC0-36 h values for PICO, M1 and M2 were 130, 2512 and 14.2 ng/ml/h, respectively. The time of peak concentration for M1 (13.6 ± 7.4 h) was much longer than that of the parent drug (3.8 ± 1.4 h). The concentration-time curves of M2 showed double peaks, which occurred at the Tmax of PICO and M1, respectively. The AUC0-t ratio of BHPM, M1 and M2 in rats receiving 2.77 mg/kg BHPM hepatoportally was 1: 23: 1.5, whereas the value was 1: 407: 1.3 in oral group. The result revealed that intestinal metabolism may be a great contributor to the first-pass glucuronidation and liver metabolism for the first-pass sulfation. After PICO and BHPM administered orally to rats in equimolar quantities, the AUC0-t of M2 in PICO group was 1.5-fold greater than that in BHPM group, and the AUC0-t value of M1 were similar between the two groups, suggesting that M2 was derived partially from the conjugation of the aglycone. In incubations of PICO with the artificial gastric juice, a small amount of M2 was detected, implying that M2 was also formed by acid hydrolysis of PICO. In conclusion, after oral administration, PICO undergoes extensive first-pass metabolism. In the stomach, PICO was partially hydrolyzed to M2, and then the metabolite as well as a small amount of PICO could be directly absorbed unchanged into the bloodstream. The rest PICO entered the colon, where it was hydrolyzed to aglycone BHPM. While passing through the intestinal wall, the vast majority of BHPM absorbed was glucuronidated into M1. The fraction of BHPM escaping intestinal metabolism was conjugated to M1 and M2 in the liver before entering the systemic blood circulation.

POSTER ABSTRACT

ADME

#141

In Vitro Assessment of the Role of P-glycoprotein on the Permeability of Periplocin: A Cardiac Glycoside from Cortex periplocae

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P-glycoprotein (P-gp) is an ATP-dependent transmembrane drug efflux pump which belongs to members of ABC transporters. Undesirable and unintentional drug interactions with P-gp efflux transporters have led to reduced oral bioavailability or drug-induced toxicity of many orally administered drug compounds. This has been a potential setback for drug discovery projects because of the reduced oral bioavailability due to poor drug absorption, rapid excretion through bile and drug-drug interaction. Periplocin is a major bioactive compound isolated from the dried root back of Periploca sepium, indicated for rheumatic arthritis, and weakness of the loins and knees, cardiac palpitation, shortness of breath and edema of the lower extremities. Periplocin has been shown to have low bioavailability. This has been attributed to acid hydrolysis and intestinal bacterial degradation to form the respective cardiac glycosides, periplogenin and periplocymarin. However, compounds with low bioavailability may also be due to permeability problems and interactions with P-gp efflux transporter. Hence, this study was carried out to investigate the permeability, and the possible contribution of P-gp to the low bioavailability of Periplocin, using Madin-Darby Canine Kidney cells (MDCK) cells transfected with or without human multidrug resistance gene, MDR1.

Functional P-gp in the transfected cell line, MDCK-MDRI was assessed by inhibition of uptake or efflux of Rhodamine 123 (R123) in the presence of cyclosporine A or verapamil. The capacity of the cell system to distinguish an investigational drug as a potential P-gp substrate was also assessed. Bi-directional transport assay on periplocin was performed in MDCK-MDRI cells, using MDCK cells as a negative control and R123 as a probe substrate, in the presence or absence of cyclosporine A, a potent P-gp inhibitor. R123 uptake-inhibition assays by periplocin compared to cyclosporine A and verapamil was determined using MDCK and MDCK-MDR1 cell. The presence of functional P-gp was confirmed with significant efflux of R123 in MDCK-MDR1 cells, relative to MDCK cells. There were correspondingly significant increases in accumulation of R123 in the presence of cyclosporine A or verapamil, in both MDCK-MDR1 and MDCK cells. However, the increments were higher in the MDCK-MDR1 cells relative to the MDCK cells. The cell system was sufficiently capable in determining whether or not an investigational drug was a substrate of P-gp with a net efflux ratio > 2 for R123. The apparent permeability of periplocin (75μM, 100μM) from the apical to basolateral in MDCK cells were within 1.0x106 cm/s to 10.0x106 cm/s. The net efflux ratios after transport of R123 (5μM) and periplocin (75μM, 100μM) were > 2. These were significantly reduced to a net efflux approximately one in the presence of cyclosporine A. Additionally, periplocin at 100μM and 200μM significantly increased R123 accumulation to levels comparable to verapamil but not Cyclosporine A. Thus, periplocin is a moderate permeable compound and P-gp contributes to its low bioavailability.

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ADME

Human Metabolism of the Antiviral Drug Arbidol: An in Vivo and in Vitro Study

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Arbidol is a potent broad-spectrum antiviral with demonstrated activity against a number of enveloped and non-enveloped viruses. Our previous study indicated that glucuronide arbidol and glucuronide sulfinylarbidol were the major metabolites in human urine. In the present study, metabolites in plasma and urine obtained from healthy volunteers receiving a single oral dose of 200 mg-arbidol were identified and characterized by ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF MS). The results showed that arbidol was extensively metabolized after oral absorbtion, and the structures of five primary oxidative metabolites were confirmed by comparison with synthetic reference standards. Sulfinylarbidol (M6) was proved to be the most abundant circulating component, whereas urine specimens contained mainly the glucuronide conjugates of arbidol (M19) and sulfinylarbidol (M21). The principal biotransformation pathways included sul! foxidation, dimethylamine N-demethylation, glucuronidation, and sulfate conjugation. Moreover, some other new metabolic pathways including 4′-hydroxylation, oxidative S-dealkyaltion, methylindole N-demethylation, and di-N-demethylation were also identified. When arbidol was incubated with human liver microsomes (HLMs), sulfinylarbidol was detected as the majoe metabolites. The chemical inhibition study indicated that both P450s and FMOs have a role in the sulfoxidation of arbidol. Furthermore, using 15 human P450 and FMO isoforms, it was found that several isoforms contributed to the sulfoxidation of arbidol, including CYP1A2, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, FMO1, FMO3, and FMO5. According to the calculated percentage total normalized rate (% TNR), CYP3A4 and FMO3 were identified as the primary isoforms responsible for the production of sulfinylarbidol. Based on our results, an extended biotransformation profile is proposed for arbidol.

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Qualitative and Quantitative Analysis of Metabolites of a Bioactive Component Using Q-TOF LC/MS; A Novel Approach to Studying ADME of Chinese Herbal Medicine in Humans

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Metabolism and pharmacokinetics data of bioactive components from traditional Chinese medicine (TCM) provide critical information for better understanding of pharmacological mechanisms of TCM. The most commonly used approach to study absorption, distribution, metabolism and excretion (ADME) of drugs in animals and humans is administration of a radiolabeled testing compound, followed by metabolite detection and quantitative analysis using radiochromatographic detection and metabolite structural characterization using LC/MS. However, human radiolabeled ADME experiments are rarely performed in China due to regulatory and cultural differences. In this presentation, we describe a novel analytical strategy for qualitative and quantitative analysis of a non-radiolabeled bioactive component and its metabolites in humans using a bench-top Q-TOF LC/MS to provide key human ADME information. Rotundine (L-tetrahydropalmatine), a tetrahydroprotoberberine isoquinoline alkaloid isolated from Corydalis Yanhusuo W.T.Wang, was employed as amodel compound. The analytical strategy is consisted of three steps. (1) Rotundine metabolites in liver microsomes were profiled and identified using Agilent 1290 U-HPLC coupled to 6540 Q-TOF and MassHunter Metabolite ID and Qualitative Analysis software. Concentrations of the drug and metabolites were then determined based on their UV responses and/or MS responses using rotundine and a few synthetic metabolites as standards. (2) Human urines, plasma and feces were collected after oral administration followed by metabolite profiling and identification using the same HR-MS methodology. (3) Rotundine and metabolites in human samples were further quantitatively analyzed using LC/UV/MS and synthetic metabolites and in vitro metabolites as standards. Result showed that more than a dozen of phase I metabolites were formed in rat liver microsomal incubations with rotundine (50 μM), including products of demethylation, mono-hydroxylation, dehydrogenation, and combinations of these biotransformation reactions. Major metabolites in vitro were four desmethyl-rotundine. In a follow up pilot experiment, a healthy male Chinese volunteer received two oral doses of rotundine with a total of 180 mg in the time interval of 6 h. Urine samples collected pre-dosing, 0-3h and 3-8h after the second dose were analyzed. More than 20 metabolites were detected and characterized in urine based on accurate full-scan MS and MS/MS spectral data. A majority of the in vitro and in vivo rotundine metabolites are not reported in the literature. Quantitative analysis showed that two desmethyl sulfate conjugates were major metabolites in urine, while other metabolites and rotundine were minor drug-related components in urine. The results provided key information on renal elimination of rotundine and its metabolites in humans. At current time, identification and quantitative estimation of metabolites in human plasma and fecal samples are on-going. In summary, this study explored a novel analytical strategy as an alternative to radioactivity analysis in studying human ADME of non-radiolabeled TCM bioactive components. In addition, the effectiveness of using the Agilent bench-top Q-TOF as a single LC/MS platform for qualitative and quantitative analysis was demonstrated. Based on our best knowledge, this was the first time that major circulating metabolites and metabolic clearance pathways of rotundine in humans were identified.

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ADME

#144

Detection and characterization of unknown ticlopidine conjugates in rat bile using Orbitrap LC/MS: Applications of various data acquisition and processing tools

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Neutral loss and precursor ion (PI) scans on a triple quadrupole mass spectrometer are routinely used for detection of unknown drug conjugates (phase II metabolites) based on their predicted fragmentation patterns. For example, neutral loss scan of 176 Da is capable of detecting most glucuronides. However, to detect different types of conjugates, multiple LC/MS injections with different neutral loss and PI scans are required. Recently, high resolution mass spectrometry and post-acquisition data processes using mass defect filter (MDF), extracted ion chromatogram (EIC) analysis, isotope pattern filter (IPF) are extensively employed for fast and comprehensive detection of oxidative metabolites. The main objective of the study was to apply multiple data acquisition and data mining tools to the detection and characterization of different types of unknown conjugates of ticlopidine in rat bile. Ticlopidine, an antiplatelet drug, undergoes extensive oxidative metabolism and bioactivation in liver microsomes; however, in vivo metabolism of ticlopidine remains to be thoroughly investigated. Ticlopidine was administrated to Wister rats (20 mg/kg). Bile samples (0-8 hr) were collected, processed and injected into an LC/Oribitrap system. Accurate full-scan MS and MS/MS data sets were recorded using isotope dependent or targeted MS/MS acquisition, on-line H/D exchanging and high collision energy dissociation (HCD). Data processes were carried out using EIC, MDF and IPF. In addition, an isotope pattern simulation technique was utilized to confirm molecular formulae of unknown metabolites. A total ion chromatogram of a bile sample displayed a few major conjugated metabolites and many endogenous components. Profiles from EIC and IPF processes clearly exhibited multiple conjugates with no or minimal false positives. However, ticlopedine conjugates that are not predictable or lost Cl atom were not detected by EIC and IPF, respectively. MDF was able to detect the ticlopedine conjugates that were missed by IPF or EIC, but it led to more false positives. As a result, more than 20 significant conjugated metabolites of ticlopedine were found in rat bile, including glucuronindes, GSH adducts, and their further derivatives, such as Cys-gly, Cys, and N-acetyl-Cys conjugates. Structural assessments of these metabolites were accomplished based on accurate MS and MS/MS spectra and H/D exchanging data. A majority of the ticlopedine conjugates characterized in the current investigation have not been reported in the literature. The study demonstrated that high-resolution mass spectrometry together with data processing using MDF, IPF and EIC as well as data acquisition using H/D exchanging and HCD is a powerful approach for the detection and identification of unknown conjugated metabolites in complicated biological matrixes. In addition, it provided insight into metabolism and bioactivation of ticlopedine in rats.

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ADME

#145

Evaluation of Efflux Transporter Mediated In Vitro Biliary Excretion of Thienophine and Its Metabolite in Sandwich-cultured Rat Hepatocytes

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Thienorphine (TNP) is a new partial opioid agonist discovered and developed in the authorâ ™s institute. It is currently under clinical evaluation as a promising drug candidate for the treatment of opioid dependence. Pre-clinical pharmacokinetic studies revealed that the glucuronide conjugate of TNP (TNP-Glu) was the major metabolite in the circulation with the peak level about one order of magnitude higher than the parent drug in rat and dog. In an excretion study with 3H-TNP, a significant amount of radioactivity (26% in 24h) was recovered from the bile of BDC rats after an oral dose. Further study with non-labeled TNP showed that nearly one fourth of the oral TNP dose (23%) was excreted as its glucuronide from bile within 24h. Enterohepatic circulation of TNP and TNP-Glu was confirmed using a paired rat model and in situ perfused rat intestinal preparations. The previous results indicated that TNP and its glucuronide conjugate underwent a significant biliary clearance, and the enterohepatic circulation had a considerable influence on the terminal elimination of TNP. In the present study, TNP was identified as a substrate of P-gp and its glucuronide conjugate was a MRP2 substrate, using ABC transporter membrane and APTase assay. The role of efflux transporters on their bile excretion was further evaluated with a hepatocytes based biliary clearance model (B-clear model). After 5 days of culture in standard Ca2+ Hanks buffer, sandwich-cultured rat hepatocytes (SCRH) formed functional bile canalicular networks. TNP (20 Î¼M) was incubated with the SCRH for 60 min in the presence and absence of Ca2+. The cell accumulations of TNP and TNP-Glu under Ca2+ and Ca2+-free conditions were measured with a LC-MS/MS method. The biliary excretion index (BEI) values for TNP and TNP-Glu were calculated to be 19.7Â±1.5% and 55.0Â±5.8%, respectively. The intrinsic biliary clearance (CLbile, int) of TNP was 33.1Â±2.10 mLâ ¢minâ˜1â ¢kgâ˜1. To assess the effects of known transporter inhibitors on the biliary efflux of TNP and TNP-Glu, the selected inhibitors were pre-incubated separately with SCRH for 30min, and then co-incubated with TNP for additional 60 min. P-gp inhibitors tariquidar (0.5-5 Î¼M) and verapamil (10-100 Î¼M) could dose dependently inhibit the biliary excretion of TNP. Tariquidar (5 Î¼M) or verapamil (100 Î¼M) could decrease the TNP BEI to 34.5% or 42.4% of the control (Pï¼œ0.001). Similarly, the MRP2 inhibitors, probenecid and methotrexate also showed dose dependent inhibitory effects on the biliary efflux of TNP-Glu. Probenecid and methotrexate at 100 Î¼M could reduce the BEI of TNP-Glu to 79.0% and 70.0% of the control (Pï¼œ0.05). These results indicated the major roles of efflux transporters in the biliary excretion of TNP and TNP-Glu. The combination use with P-gp or MRP2 inhibitors may significantly effect the biliary excretion of TNP and its metabolite. The clinical implication of the potential drug-drug interaction between TNP and transporter inhibitors needs to be addressed in clinical studies.

POSTER ABSTRACT

ADME

#146

The in vitro ADME platform to support the new drug discovery and development

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The failure of the clinical trails and the withdrawn of the market drugs have pointed to the drug interaction issues. Recent FDA and ICH guidelines have revealed much more preclinical approaches should be conducted to avoid such crash. An ADME in vitro screening platform has been established, focusing on the inhibition and induction of major drug metabolizing enzymes. These include the phase I enzymes of Cytochromes P450 and phase II enzymes of UGTs using individual expression or liver microsomal systems as well as the P-gp transporter using Caco-2 or MDR1-MDCK systems. Additionally, the hPXR transfected fluorescent report-gene system is established to the estimate the induction potential for cyp3A4. Those positive control compounds have been tested to ensure the platform is up running, producing results with agreement of the literatures. The platform has been applied for screening many new drug candidates to increase the candidate survivability.

POSTER ABSTRACT
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ADME

The metabolism of buflomedil hydrochloride in rats

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Buflomedil hydrochloride is a peripheral vasodilatator, which is widely employed in the treatment of peripheral vascular diseases. However it has been forbidden temporarily by EMA in 2011 because of its life-threatening intoxications, whileas the metabolism of buflomedil in mammal which may play an important role in its toxicities seems to be scarcely investigated. To provide the interpretation of toxicities, we explored the metabolism of buflomedil in rats by qualitative metabolite profiling here. The urine, plasma, feces and bile samples of rats were collected after oral administration of buflomedil hydrochloride at a dosage of 30 mg/kg. The analytes were extracted through solid-phase extraction cartridge and chromatographed on an Agilent Extend C18 column(150¡Á4.6 mm, 5 œÌm) using methanol-water£¨containing formic acid 70 mmol/L, 27:73, v/v) as mobile phase with an isocratic flow rate of 0.5 ml/min. Liquid chromatography combined with mass spectrometry was used !

for structural investigation and identification of metabolites. There were 23 metabolites including 8 phase I and 15 phase ¢ò metabolites identified in vivo, among which 21 metabolites elucidated in our work still have not been reported until now. The main metabolism routes of buflomedil in rats were O-demethylation, oxidation, mono-hydroxylation, glucuronate conjugation and sulfate conjugation. The proposed metabolic pathways of buflomedil in rats will provide comprehensive data to increase our understanding of the metabolism of buflomedil. The results may give scientific explanation for toxicities of buflomedil hydrochloride for clinical practice.

POSTER ABSTRACT

ADME

#148

Disposition and Metabolism of [14C]Asunaprevir, a Potent HCV NS3 Inhibitor, After Oral Administration to Humans and Animals

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Asunaprevir (BMS-650032) is a potent and selective inhibitor of hepatitis C virus (HCV) NS3 protease that is currently under development for the treatment of HCV. Preclinical and clinical studies demonstrated that the compound was highly effective and well tolerated in humans. Following a single oral dose of [14C]asunaprevir to mice, rats, dogs, monkeys and humans, the majority of the radioactivity was excreted through feces. In BDC rats and monkeys, a significant portion of radioactivity was excreted in bile. Asunaprevir underwent extensive metabolism prior to excretion. The prominent biotransformatiom pathways include hydroxylation, ether cleavage and hydrolysis. In plasma from animals and humans, unchanged asunaprevir was the most abundant drug-related component. All the metabolites that humans were exposed to were also present in toxicological species. Using recombinant enzymes and selective enzyme inhibitors, it was demonstrated that the metabolism of asunaprevir in humans was mainly catalyzed by 3A4.

POSTER ABSTRACT

ADME

#149

Assessment of Biliary Clearance in Early Drug Discovery Using Sandwich-Cultured Hepatocyte Model

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It is challenging to predict biliary clearance (CLb) for new chemical entities (NCEs) in early drug discovery. Although sandwich-cultured hepatocyte (SCH) model has offered a valuable tool for characterizing hepatobiliary disposition and drug¨Cdrug interaction potential of NCEs, no comprehensive study was reported to project in vivo biliary clearance (in vivo CLb,observed) potential using in vitro SCH model during the drug discovery stage. In this study, the CLb of 110 discovery compounds was evaluated using rat SCH model. Parallel artificial membrane permeability assay, Caco-2, and rat liver microsomes were employed in parallel to explore the interplay of biliary excretion with cellular permeability and liver metabolism. Selected compounds were further tested in bile-duct-cannulated rats, confirming the value of the SCH model for ranking and predicting in vivo CLb,observed during drug discovery. For compounds with extremely low passive permeability and metabolism, rat SCH may underestimate in vivo CLb,observed. The combination of passive permeability, metabolic intrinsic clearance, and the SCH model could serve as an initial screening platform for biliary excretion potential as well as a means for improving compound liabilities and properties. A preliminary evaluation strategy was proposed to highlight biliary excretion risk evaluation during the drug discovery process.

POSTER ABSTRACT

ADME

#150

Biliary excretion of curcumin is mediated by multidrug resistance-associated protein 2

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Curcumin has a wide spectrum of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Recently, its potential as effective chemoprevention against cholangiocarcinoma, a highly malignant tumor of the bile duct with limited therapeutic options, was reported. The purpose of the present study was to investigate the contribution of multidrug resistance-associated protein 2 (Mrp2) to the biliary excretion of curcumin using Sprague-Dawley rats (SDR) and Eisai hyperbilirubinemic rats (EHBR). After intravenous administration of curcumin with a loading dose of 4.5 mg/kg, followed by a constant infusion of 18 mg/kg/h to the SDR and EHBR, the pharmacokinetic parameters of curcumin were estimated. In EHBR, the total area under the bile concentration˜time curve from 0 to 80 min following curcumin administration was dramatically decreased (0.094%) compared to that in SDR. In addition, the plasma-to-bile and liver-to-bile clearances were both significantly decreased compared to SDR. These results provide the first evidence that Mrp2 mediates the biliary excretion of curcumin and thus may be a major factor in the control of exposure of curcumin to the bile duct. This study may be helpful to the potential use of curcumin as a treatment for bile duct cancer, and to understanding the genetic polymorphism of Mrp2 for clinical trials of curcumin.

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ADME

A high-throughput bioanalytical platform, Xevo TQ-S for application in in vitro early ADME screening

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Liquid chromatography/tandem mass spectrometry (LC/MS/MS) is the bioanalytical method of choice to support plate-based, in vitro early ADME (Absorption, Distribution, Metabolism and Excretion) screening such as metabolic stability, cytochrome P450 (CYP) inhibition/induction, protein/tissue binding and so on. Although different purposes could be achieved to screen candidate compounds based on the results, the process of these experiments could be separated into two groups: one for detecting changes of specific metabolites from probe substrates to calculate IC50, such as CYP inhibition/induction and the other group included analysis of concentrations from candidate compounds. For the first group, LC/MS/MS methods developed for metabolites of probe substrates could be utilized for all screening, but results couldn't provide direct correlation with compounds, which had some potential risks for mistakes clients always concerned. The RADAR mode acquisition, a Xevo TQ-S function, enables the collection of Q1 full scan in the samples while simultaneously collecting MRM data for the quantitation of analytes, the quality of which weren't compromised. This efficient method has been developed in our lab and applied into 3A4 (midazolam, nifedipine and atorvastatin) screening without repeated injection to confirm candidate compounds. As for the other group of bioanalysis, in general, it is time-consuming and pains-taking to manually or semi-automated optimize MS/MS methods and build acquisition and quantitation methods to acquire and process data for hundreds and thousands candidate compounds mainly due to the lack of a high-quality and automated platform to reduce tedious and repeated work. Quanoptimize embedded in MasslynxTM software was a highly automated bioanalytical platform. MRM methods could be generated by software and directly applied into optimization of UPLC conditions. After data acquisition, global quantitation methods could be utilized to process the data. It was not necessary to build the quantitation method to each compound. A set of 20 compounds with diversified structures were used to demonstrate the speed, quality and reproducibility of MS/MS method optimization, sample analysis and data processing using this automated platform. Metabolic stability results for the validation compounds in microsomes from two species (rat and mouse) showed good consistency with each batch, and also between batches conducted on different days. Therefore, in drug discovery program, the Xevo TQ-S facilities could meet every requirement from clients to CRO companies for in vitro studies to provide optimal, cost-effective and time-saving screening method for different experiments.

POSTER ABSTRACT

ADME

#152

Identification of Single Nucleotide Polymorphism by HPLC and LC-MS/MS: Quantification of Residual Deoxynucleotides Post PCR Reactions

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Single nucleotide polymorphism (SNP) plays a critical role in biological activities that may elicit individual differences in efficacy, ADME and toxicity of drugs. To facilitate the identification of SNPs for various target genes, we developed an analytical platform for SNP genotyping by using HPLC-UV and LC-MS/MS methods for the separation and quantification of residual deoxynucleotide triphosphates (dATP, dCTP, dGTP, and dTTP) after the completion of PCR reactions. Using a Cosmosil C18 column (4.6 ¡Á 250mm, 5 œèm) with 20 mM phosphate buffer (pH 8.0) as the mobile phase, a baseline separation of the four dNTPs was achieved by the conventional HPLC-UV method and the peak resolution was found to be sensitive to the pH value of the mobile phase. HPLC-UV afforded a detection limit of 0.313 œèM with a linear range from 0.625-10 œèM. Intra-day and inter-day precision for the four dNTPs were <5% and <10%, respectively. Stability at room temperature was 0.9-1.4% for the 12-hour period. The current HPLC method was able to detect CC and CT genotypes upon the completion of designated PCR reactions. In addition, LC-MS/MS method was explored to determine dNTPs using negative ionization with MRM mode. Chromatography was performed using a Venusil C18 (2.1¡Á50mm, 3œèm) column and a linear gradient with mobile phases consisting of 2 mM N, N-dimethylhexylamine in water (A) and methanol (B). The LC-MS/MS method demonstrated a detection limit of 10 nM with a linear range from 30-1000 nM. In conclusion, both HPLC-UV and LC-MS/MS methods can be used for the analysis of deoxynucleotides, particularly for SNP genotyping in support of PCR reactions. While the HPLC-UV method is suitable for the analysis of deoxynucleotides present at high concentrations, LC-MS/MS is advantageous in the identification and quantitation of less abundant dNTPs.

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ADME

Principal Scientist

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Cerebrospinal fluid (CSF) is the single most important sampling matrix for assessing CNS drug exposures and biomarkers levels in drug research and clinical development including monitoring disease progressions. CSF drainage plays a crucial role for clearing drugs, neurological wastes (and biomarkers) from the CNS system. The purpose of this study was to assess the kinetic correlation of CSF flow rate with CNS drug exposures upon systemic administration of monoclonal antibodies. CNS uptakes of two monoclonal antibodies, A and B, were assessed in rats. Upon systemic administration, CSF concentration increased over time and then become parallel with serum concentration time profiles. In summary, for drugs with long systemic t∏, the concentration ˆ time curves of CSF and plasma converge and become parallel at steady-state. The time needed to reach steady state between serum and CSF is about 4 ˆ 5 rounds of CSF turnover. Thus, for long t∏ drugs, a few sampling time points, after steady-state is achieved, are sufficient to estimate CSF to plasma ratios. For drugs with short systemic t∏, shorter than the CSF turnover rate, the two curves are likely not parallel. A measurement of total AUC ratio may be necessary to assess CSF to plasma ratios.

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ADME

#154

Pharmacokinetic and Pharmacodynamic Drug-Drug Interactions and Serotonin Toxicity

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Indolealkylamines (IAAs) are a class of chemical derivatives of monoamine neurotransmitter 5-hydroxytrypatmine (serotonin or 5-HT). Acting on serotonergic system, overdose or combined use of IAA drugs may induce severe or even fatal serotonin toxicity/syndrome due to extensive drug-drug interactions, which is reasoned to occur at both pharmacokinetic and pharmacodynamic levels. In this thesis, we employed 5-methyoxy-N,N-dimethyltryptamine (5-MeO-DMT) and harmaline as model IAA drugs to define IAA drug interactions and serotonin toxicity. Concurrent harmaline significantly enhanced and prolonged the systemic and brain exposure to 5-MeO-DMT and its active metabolite bufotenine. Co-administration of harmaline potentiated 5-MeO-DMT-induced serotonin toxicity, as manifested by enhanced hyperthermia, hyperlocomotor activity and neuromuscular dysfunction as well as a sharp increase of acute toxicity. Of particular note, a more severe hyperthermia is shown in mice treated with 5 mg/kg of harmaline plus 2 mg/kg of 5-MeO-DMT than that treated with 10 mg/kg of 5-MeO-DMT alone, which is in contrast to a higher exposure to 5-MeO-DMT in mice treated with 10 mg/kg of 5-MeO-DMT. These findings support that harmaline interacts with 5-MeO-DMT not only at the pharmacokinetic level but also at the dynamic level, and concurrent use of MAOI will increase the hazards of 5-MeO-DMT intoxication.

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POSTER ABSTRACT

ADME

#155

Application of SWATH for detection of glutathione-trapped reactive metabolites and in vivo metabolite profile in biological samples

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In the drug discovery and development process, liquid chromatography/mass spectrometry (LC/MS) has been played a very important role in drug metabolite detection and structure elucidation. Various LC/MS techniques and approaches has been developed for sensitive and selective detection of metabolites and rapid in vitro screening. Here, in vitro GSH conjugate incubates and in vivo human bile sample for metabolite identification was characterized by using two different acquisition schemes: (1) information-dependent acquisition (IDA) with a TOF full MS scan as survey scan and intensity triggered product-ion scan as dependent scan; and (2) ¡°Sequential windowed acquisition of all theoretical fragment-ion mass spectra ¡°(SWATH) in which sequential precursor ion windows (typically 25 Da) are used to collect the same spectrum precursor and fragment ions using a collision energy range (20-50 eV). By comparing the acquisition of MS/MS spectrum as well as MS/MS spectrum quality, SWATH was found to be superior to IDA. When combine SWATH with UHPLC, in vivo bile sample was successfully profiled in 20 minutes, it significantly shortened the run time of metabolite profile for biological samples.

POSTER ABSTRACT

ADME

#156

Using diluted plasma for plasma protein binding in rapid equilibrium dialysis (RED)

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The measurement of the free drug concentration in plasma protein binding (PPB) assays can be very difficult while dealing with very high bound compounds. Here we describes the results of determining the free fractions of compounds using diluted plasma in Rapid in Equilibrium Dialysis (RED) device. The results of plasma protein binding from 10 compounds demonstrated plasma protein binding data from diluted plasma is very close to the values obtained from undiluted plasma when we tested at 3, 5, and 10 fold dilutions.

POSTER ABSTRACT

ADME

#157

Investigating Diclofenac and its Glucuronide Metabolite as Drug Transporter Substrates In Vitro

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Diclofenac, a nonsteroidal anti-inflammatory drug, is extensively metabolized to a glucuronide that is excreted into bile and urine. The aim of this study was to examine the role of hepatic uptake and efflux transporters in the hepatobiliary excretion of diclofenac and its glucuronide. Before the transport studies were conducted, the pH-dependent (5.5-7.4) chemical stability of diclofenac glucuronide was assessed by LC/MS/MS. The transporter phenotyping studies on diclofenac and diclofenac glucuronide were performed using stably transfected HEK cells that singularly expressed human OATP1B1, OATP1B3, or OATP2B1. For efflux transporters, studies were performed using the membrane vesicles prepared from Sf9 cells expressing human BCRP or MRP2. Diclofenac glucuronide was chemically stable at pH 5.5 or 6.0, but began to degrade to diclofenac at higher pH values (6.5-7.4). Thus, all the transport studies with diclofenac glucuronide had to be performed at either pH 5.5 or 6.0. Although the uptake of diclofenac into OATP1B1, 1B3 or 2B1/HEK cells was the same as mock/HEK cells, the cellular uptake of diclofenac glucuronide was increased in OATP1B1 and 2B1/HEK cells (vs. mock cells). In addition, the differential uptake was inhibited by known OATP inhibitors (BSP and taurocholate). When BCRP membrane vesicles were employed, both diclofenac and diclofenac glucuronide uptake was higher in the presence of ATP (vs. AMP). Diclofenac glucuronide uptake into MRP2 vesicles was also ATP-dependent, whereas diclofenac was not. Diclofenac is a substrate of BCRP, but not OATPs and MRP2. Diclofenac glucuronide is a substrate of OATP1B1, 2B1, BCRP and MRP2. These results suggest that after diffusion into the liver, diclofenac may be either metabolized to diclofenac glucuronide or effluxed into bile via BCRP. On the other hand, the clearance and disposition of diclofenac glucuronide, once formed, is governed by the interplay between OATP1B1/2B1, BCRP and MRP2. The data described herein provide additional information regarding the transporter- mediated disposition of diclofenac, and its glucuronide.

POSTER ABSTRACT

ADME

#158

Cytotoxicity of ginkgolic acid based on matabolized by CYP3A4

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Ginkgolic acid (GA) is a mixture of structurally related n-alkyl phenolic acid compounds and exists in leaves, nuts especially in external seed coat of ginkgo biloba L. The alkyl side chain in molecular structures varies from 13 to 17 carbons in length with 0ˆ2 double bonds.Ginkgo acid has a wide range of pharmacological activities including antitumour, antibacterial and antidepressant activities,etc..It was also reported that ginkgo acid could cause contact cytotoxic,mutagenic, carcinogenic and genotoxic. Ginkgolic acid (17:1, GA) ,6-[(10Z)-Heptadecenyl]salicylic acid, is the second major component ,which accounts for about 40% of the total ginkgolic acids. To determine whether the phase I metabolism and P-gp could contribute to their cytotoxicity, we investigated the cytotoxicity of ginkgolic acid (17:1), using in vitro bioassay systems such as primary rat hepatocytes, HepG2 cells, Madin-Darby canine kidney (MDCK) cells, MDR1-transfected MDCK cells,and MDR1-CYP3A4 cotransfected MDCK cells. It has been demostrated that CYP 3A and CYP1A are main metabolizing enzymes for GA(17:1).The addition of GA(17:1) to HepG2 cell culture resulted in dose-dependent cytotoxicity, and the GA(17:1) IC50 value in HepG2 cells was 59.3±6.21 ìM. After the pretreatment with 25ìM â-naphthoflavone (an inducer of CYP1A2) or10ìM rifampin (an inducer of CYP2C9, CYP3A4). Both of them increased sensitivity of HepG2 cells to GA(17:1) cytotoxicity, the IC50 values were 40.5 ± 4.78ìmol/L, 46.7 ± 5.43 ìmol/L respectively. The dose-dependent cytotoxicity was also found in primary rat hepatocytes after incubation with various concentrations of GA(17:1). IC50 values were 76.3±9.37 ìmol/L. Co-incubated with á-naphthoflavone (an inhibitor of CYP1A1/2) or ketoconazole (an inhibitor of CYP3A2) both of them were observed to decrease GA(17:1) toxicity. While co-incubated with â-naphthoflavone and rifampin the IC50 values 53.4 ± 6.12ìmol/L , 48.7 ± 5.63ìmol/L respectively. These findings suggest that HepG2 cells were more sensitive to the cytotoxicity of ginkgolic acid than primary rat hepatocytes, and CYP1A and CYP3A could metabolize ginkgolic acid to more toxic metabolites, the CYP1A was possibly more important than the CYP3A in this process.

MDCK-MDR1 MDCK-MDR1-CYP3A4 cells were used to evaluate the effect of P-gp mediated efflux of GA(17:1), After 12 h exposure, GA(17:1) showed a dose-dependent effect against the viability of all kinds of cells, the IC50 values were 37.9 ±7.47 μmol/L, 48.9 ±9.61 μmol/L, 31.8 ±5.34 μmol/L respectively. The results suggested that P-gp may affected GA(17:1) entry into kidney cells, corresponding to the previous results, the CYP3A4 also increased the toxicity in the MDCK cells.

Keywords: ginkgolic acid, MDCK-MDR1 cells, MDCK-MDR1-CYP3A4, CYPs, Metabolic cytotoxicity

POSTER ABSTRACT

ADME

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Metabolic Profiles of Isocorydine - An Important Aporphine Alkaloid

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Isocorydine is an important aporphine alkaloid, which is widely present in herbs. Isocorydine shows various pharmacological activities such as antiplasmodial, antiarrhythmic, vasodilative effect and nonspecific spasmolytic effect on diverse visceral smooth muscles. However, there are very limited data regarding hepatic metabolism of isocorydine. Therefore, we investigated the metabolism profiles of isocorydine in various vitro models including rat liver microsomes (RLMs), human liver microsomes (HLMs), recombinant human CYPs (rhCYPs) and HepG2 cells. In RLMs, the isocorydine metabolism was inhibited by 88% by ketoconazole, while the metabolic rate of isocorydine incubated with RLMs pretreated with dexamethasone was notably higher (6.4¨Cfold) than the control (non-induced RLM), indicating that CYP3A2 can be involved in isocorydine oxidative metabolism. Classical CYPs¡¯ substrates were used to investigate the inhibition effect of isocorydine on the CYPs, with the results that isocorydine almost has no inhibition effect on these main CYP isoforms (Ki values: 151.0 ¡À 4.2 œèM on CYP1A2, 190.3 ¡À 57.0 œèM on CYP2C6, 264.0 ¡À 12.7 œèM on CYP2C11, 182.7 ¡À 13.3 œèM on CYP2D1, 107.4 ¡À 13.8 œèM on CYP2E1, 400.2 ¡À 14.7 œèM on CYP3A2). The metabolites of isocorydine were preliminarily characterized by UPLC-MS/MS including one mono-demethylation and one mono-hydroxylation metabolites. Metabolic profiles of isocorydine in human were a little different from the circumstances in rats. In HLMs, the metabolic pathways of isocorydine were studied using specific chemical inhibitors. It was found that CYP3A4 and CYP2C8 were two CYP enzyme isoforms that mediated the metabolism of isocorydine in human, and these results were confirmed by rhCYP3A4 and rhCYP2C8. It was found that isocorydine did not inhibit CYP2C19, CYP2D6, CYP2E1 and CYP3A4 as the IC50 values of isocorydine on these CYPs isoforms were all > 400 œèM. However, the IC50 values of isocorydine on CYP1A2, CYP2C9 and CYP2C8 were 132.7 ¡À 1.1 œèM, 146.8 ¡À 1.1 œèM and 53.8 ¡À 1.1 œèM, respectively, implying that isocorydine may have weak inhibition effect on CYP2C8. In addition, the induction effect of isocorydine on some metabolic enzyme isoforms regulated by two nuclear receptors (NRs) human pregnane X receptor (hPXR) and human constitutive androstane receptor (hCAR) was investigated by dual-luciferase reporter assay in HepG2 cells. Results revealed that isocorydine almost has no gene induction effect on CYP2B6, CYP3A4 and UGT1A1. These finds should be useful for the clinical application and further studies of isocorydine.

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