



POSTER SESSION ABSTRACTS

Inspiration and Education

POSTER HALL OPEN

Thursday, April 21 9:00 am - 5:00 pm

Friday, April 22 9:00 am - 5:00 pm

Including Submissions for
Young Scientist Excellence Award
Innovator Award

CPSA Shanghai 2016
April 20 - 22, 2014
Renaissance Shanghai Pudong Hotel
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POSTER ABSTRACT

#102

Determination of Limaprost, an analogue of PGE1 in human plasma by QTRAP® 6500+ and SelexION®+ technology

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Introduction:

Limaprost, an analogue of prostaglandin E1 analogue, is a promising drug that has strong vasodilatory and antiplatelet activity for the treatment of various ischemic symptoms, such as ulcers, pain, and cold sensations associated with thromboangitis obliterans (TAO) and subjective symptoms associated with acquired lumbar spinal canal stenosis (LCS). Requirement of ultra-low limit of quantitation (sub- pg/mL level) and separation from endogenous interferences in human plasma becomes a big obstacle for its pharmacokinetic research. By now 2D-LC/MS/MS system^[1-2] is the principal solution to reduce or separate the endogenous interferences. However, it suffers from complexity of 2D-system optimization and method development, especially the pretty long analytical time (>50 min). We present here a more simplified 1D-LC/MS/MS assay based on a SCIEX QTRAP® 6500+ LC-MS/MS system equipped with a SelexION®+ differential mobility separation technology device aimed at better usability and higher efficiency in comparison of current 2D-LC/MS/MS.

References

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POSTER ABSTRACT

#103

A Generic Kit-Based Approach for Quantifying Monoclonal Antibody Drugs Through Direct Digestion of Discovery Study Samples

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INTRODUCTION

Over the past 5–10 years, there has been a significant shift towards a greater % of biologics in pharmaceutical pipelines. However, the industry finds itself in the middle of patent expiry for many of the critical monoclonal antibody and other protein-based drugs, with patent expiration dates ranging from 2012–2020. This has resulted in a focus on protein quantification in Bioanalytical labs, innovator pharma and CRO's as well as biomarker research labs. While immunoassay (IA) methods are sensitive and simple to execute, poor reagent reproducibility, lack of standardization, cross-reactivity, limited linear dynamic range, and other short-comings have led the drive to convert to LC-MS. These LC-MS workflows however, encompass a multitude of sub-segments, each having many steps. Those that are common to most workflows may include affinity purification, denaturation, reduction, alkylation, digestion, and SPE clean-up (each requiring optimization). Such traditional protein quantification protocols often require as much as a day and half for completion. Furthermore, the margin and possibility of error is significant within each individual step. There is a strong need for simpler, more standardized workflows which enable scientists to complete sample preparation and start an analytical run by mid-day. At the same time, ideally using generic, kitted methods, assay sensitivity must be high enough to accurately and precisely quantify low enough levels of the target protein to make critical decisions in discovery. The typical workflow complexity (see poster), often leads to errors and poor reproducibility or sensitivity. In this application note, we have used the ProteinWorks™ eXpress Direct Digest Kit to simplify and streamline the workflow process using the same universal protocol and reagents for all monoclonal antibody drugs tested. Infliximab, bevacizumab, trastuzumab, and adalimumab (Figures 2–5) in plasma were directly digested, and peptides extracted using SPE in under 4 hours total time. This enabled data to begin to be acquired the same day, with several 96-well plates being run by the following morning.



POSTER ABSTRACT

#104

Characterization and collision cross section determination of obesity related lipids within mouse models using travelling wave IMS-Qtof mass spectrometry

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Introduction

Obesity is a risk-factor associated with metabolic syndrome, causing excess body fat to be accumulated to the extent that it adversely affects health and life expectancy. This can lead to further health implications such as type 2 diabetes, heart and liver disease and potential links to various forms of cancer. It has been demonstrated that glucosylceramides play a crucial part in metabolic syndrome. The manipulation of the function of glucosylceramides with small molecule drug compounds has shown that symptoms can be negated. A previous multi-omic study showed differentiation between subjects treated with glucosylceramide synthase inhibitors. Lipid analyses have been conducted using a label-free LC-DIA-IM-MS approach, providing qualitative and quantitative information from a single experiment. This work is an extension, providing additional characterization of the liver cell lipid complement using ion-mobility and associated collision cross section databases, obtained with a novel geometry travelling wave IMS-QTOF MS platform, for obese mouse models which have undergone treatment to prevent or revert obesity. The curated datasets were then interrogated using pathway analysis tools, indicating that physiological processes such as hepatic system development, inflammatory response and carbohydrate metabolism are influenced following MZ-21 treatment.



POSTER ABSTRACT

#105

Singulus Pulpitum: Microfluidics Coupled With Mass Spectrometry For Multi-Omics And Targeted Assays In Translational Research

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Abstract

Translation medicine is an interdisciplinary science that aims at combining the information taken from bench to bedside. In this process molecules are isolated and identified in discovery and then utilized in the clinical setting as biomarkers of health and disease to better develop therapies. It has become recently apparent that proteomics, metabolomics, lipidomics, and glycomics data combined are necessary to address the challenge of translational research which places strain on available sample and instrument utilization [1-4]. Due to the complexity of deriving meaningful information from these studies, the development of new analytical technologies is critical [5-6]. Here we present the utilization of a microfluidic LC coupled with mass spectrometry for both discovery and targeted studies in translational research. And The robustness, reproducibility and ability to analyze multiple preparations of biofluids for multi-omics experiments indicates that the use of microfluidic LC/MS may play a future key role in the development of translational and personalized medicine.



POSTER ABSTRACT

#106

Tissue-engineered long term hepatocyte co-culture model and data independent mass spectrometry acquisition to identify multi generation metabolites

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Introduction

The determination of metabolic stability (Clint) and accurate prediction of major human metabolic profiles is critical to optimizing drug candidate selection. Traditional models such as subcellular fractions and suspended hepatocytes are short-lived and are often limited in their ability to resolve multi-generation metabolites or drug clearance, particularly for slowly metabolized compounds. Furthermore, many current LC/MS systems used for metabolite identification lack the sensitivity to generate quantitative bioanalytical measurements within the therapeutic dosing range. Thus, identification of metabolites and evaluation of drug clearance are traditionally collected from separate samples. In this study, we simultaneously identified and quantified metabolites along with drug clearance using 1 μ M concentration in a long-term hepatocyte co-culture model using Q-ToF and data independent acquisition strategy

Methods and Results

Tolbutamide was purchased commercially and 24-well human hepatocyte co-culture plates were manufactured at Hepregen Corporation. Compound (@1 μ M & 10 μ M) was incubated at 0, 4, 48, and 168 hours in human hepatocyte co-culture. After incubation, 400 μ L acetonitrile solution was added directly to the well and lysed cells and media (total cell fraction) were collected and transferred (with scraping/disruption of the cell samples) to a collection reservoir. Direct injections of sample at 10 μ L was used for LC/MS for 1 μ M tolbutamide. Mobile phase A was composed of water (0.1% formic acid), and mobile phase B was composed of acetonitrile (0.1% formic acid). The metabolite profiling method was developed on a TripleTOF[®] 6600 system coupled with a Shimadzu Nexera UHPLC. Human hepatocyte co-cultures incubations of tolbutamide were prepared at 1 μ M & 10 μ M concentrations over the time course of 7 days. The bioanalysis of samples was conducted on a high resolution accurate mass spectrometer to simultaneously a) to identify and confirm all possible phase I, II metabolites in single analysis b) resolve a strong correlation between the disappearance of parent compound and the appearance of metabolites. Data were acquired with high resolution TOF-MS survey scan and SWATH MS/MS, and processed using several algorithms such as principal component variable grouping (PCVG), multiple mass defect filtering (MMDF), isotope pattern filtering (IPF), common product ion and neutral loss and generic background subtraction to identify and elucidate chemical structures of the observed metabolites. The disappearance (%) of tolbutamide occurred at 87% by 2 days in culture and 50% by 7 days in culture. Hydroxytolbutamide (m/z 299.0702) and carboxytolbutamide (m/z 299.0809) were the predominant metabolites of tolbutamide (m/z 269.0965) upon 168h incubation in human hepatocyte co-culture. Comparisons of metabolite profiles post incubation of either 1 μ M & 10 μ M tolbutamide revealed the need looking up references to compare metabolites found in our study to clinical trial data

The preliminary results from this work showcase the ability to simultaneously resolve quantitative and qualitative data for drug metabolism of low turnover compounds in a human hepatocyte co-culture model using UHPLC-Q-TOF-MS with data independent acquisition to draw in vitro-in vivo correlation.

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POSTER ABSTRACT

#107

Quantitative determination of endogenous biomarker lysophosphatidic acids in mouse plasma by LC-MS/MS

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Lysophosphatidic acid (LPA) is an important class of endogenous biomarker for several potential drug targets because of its various signaling expression through G protein-coupled receptors. LPA level in human biofluids has been shown to correlate with various diseases, such as osteoarthritis, cancer and myeloma. The production of lysophosphatidic acid (LPA) is catalyzed by Autotaxin to remove choline group from lysophosphatidylcholines (LPC), one of well-characterized LPA biosynthesis pathway in vivo. Here we developed a highly robust and sensitive liquid chromatography–tandem mass spectrometry (LC-MS/MS) method to simultaneously measure the concentration of endogenous Lysophosphatidic acid (16:0, 18:0, 18:1, 20:4 LPA). LPA (17:0), undetectable in biological matrices, was used as internal standard in quantification. Protein precipitation by methanol extracted target analytes with high recovery. Chromatographic separation was performed on a short Waters Xbridge C8 column at the flow rate of 0.4 ml/min. Mobile phases consisted of water containing 0.1 mM ammonium acetate with 0.05% ammonium and MeOH containing 0.02% ethylenediamine (DEA) and 0.05% ammonium. The addition of ion pair reagent ethylenediamine (DEA) helps to generate sharper peaks and superior resolution. The slope of LPA calibration curves in charcoal-stripped plasma (CSP) parallels that in untreated plasma therefore calibrators were prepared in CSP over the range of 2-250 ng/mL. Matrix effect, recovery, selectivity, accuracy, precision and stability were evaluated in authentic matrix. Each injection takes about 3.5 min to allow the elution of abundant endogenous LPC that can interfere with accurate quantification of LPA. In addition, several factors, which could potentially influence the accuracy and precision of quantification, were evaluated during method development, such as adsorption, ion-source fragment, pH of extraction condition, LPC & LPA conversion. Abundant endogenous LPC, LPI and others lysophosphatidic species could degrade to respective LPA due to in-source fragment occurred on the weak bond connecting choline/inosine head group with LPA backbone. This would subsequently introduce significant bias/ interference during LC-MS/MS analysis when chromatographic baseline separation was not achieved. The artificial formation of LPA demonstrated that acidic pH and concomitant endogenous enzyme in plasma could accelerate LPC converting to LPA. Hence sample handling condition should be carefully controlled. The validated LC-MS/MS method was successfully applied to quantify LPA contents in mouse plasma in a pilot study.



POSTER ABSTRACT

#108

Quantification of Acetaminophen Protein Adducts as a Biomarker in Human Plasma by using LC-MS/MS

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Abstract:

Acetaminophen (APAP) is a popular OTC drug commonly used as analgesic and antipyretic agent. APAP is considered safe at the therapeutic doses but overdoses of its acetaminophen-containing products can lead to hepatotoxicity and progressed to acute liver failure. Acetaminophen-induced hepatotoxicity has been attributed to covalent binding of reactive metabolite *N*-acetyl-*p*-benzoquinone imine to cysteine groups on proteins as an acetaminophen-cysteine conjugate (APAP-Cys).

Early studies of APAP protein adducts relied on radiometric and immunochemical methods for the analysis of APAP-Cys. Later, HPLC-ECD method was developed. The matrix was first dialyzed to remove non-protein-bound APAP-Cys and the dialyzed samples were subjected to digestion with protease and then prepared for HPLC-ECD analysis. Recently, LC-MS/MS methods using dialysis or gel filtration were reported to quantify APAP-Cys in human serum. However, the methods used relatively large volume of serum sample and obtained a 10nM LLOQ. In present study, a more sensitive and rapid LC-MS/MS method on API6500 were developed and applied in a clinical study to monitor APAP protein adduct as biomarker.

Novel Aspect:

It is the first reported sensitive and specific LC-MS/MS method to achieve 1ng/mL LLOQ using 0.1 mL human plasma for the determination of APAP-Cys.

Preliminary Results:

During method development, we utilized dialysis devices in 96-well plate format to significantly reduce the cost and achieve higher sample preparation throughput. In addition, positive control sample using microsomal incubation was used to evaluate digestion efficiency.

Figure 1. Representative LC-MS/MS chromatogram of APAP-Cys (positive control sample) in human liver microsome (See Poster)



POSTER ABSTRACT

#109

Hepatocyte-Based in Vitro Model for Evaluation of Drug-Induced Cholestasis and Hepatotoxicity

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Purpose: to explore the toxicity and intracellular of bile acids and the link between drug-induced cholestasis and hepatic transport of bile acids (BA) and bilirubin.

Methods: First, toxicity and intracellular accumulation of various bile acids including DCA, CDCA, UDCA and GCDCA were tested in both day-1 and day-3 sandwich cultured rat hepatocytes (SCRH) model. And then, with SCRH model and biomarkers detection including ALT, AST, ALP, LDH and urea generation, fifteen compounds including compounds known associating with liver injury (such as Cyclosporin A, Labetolol, Diclofenac, Sorafenib, Chlorpromazine, Verapamil, Benzbromarone, Acetaminophen) were tested in the following assay: (i) drug-induced hepatotoxicity with bile acids at 10-fold concentration of rat serum level or 40-fold concentration of human serum level; (ii) Inhibition of test compound on Ntcp-mediated hepatic uptake of d5-taurocholate; (iii) Inhibition of test compound on Bsep-mediated biliary efflux of d5-taurocholate; (iv) drug-induced hepatotoxicity with 100 μ M bilirubin in presence or absence of bile acids at 10-fold concentration of rat serum level. Briefly, on day-0, seeded fresh prepared rat hepatocytes and formed 2-layer configuration with ice cold BD Matrigel™ after 4hr incubation. The hepatotoxicity study was performed on day-1 and day-3, when cells were treated with test compounds alone or co-incubated with bile acids mixture or bilirubin for 24 hours. After 24h-treatment pipetted the supernatant media to determine the leakage of AST, ALT, LDH and ALP. And then urea generation was determined to calculate drug-induced cholestasis index (DICI). The inhibition studies of test compounds on hepatic uptake and biliary efflux of d5-taurocholate were performed on day-1 and day-4, respectively. On day-4 formed with bile canalculus, the accumulation of d5-taurocholate in hepatocyte and bile canalculi was quantified under the incubation with regular HBSS, while the accumulation only in hepatocyte was quantified in the presence of 5mM EGTA to chelate Ca^{2+}/Mg^{2+} and disrupt bile canalculi tight junction. The concentration of d5-taurocholate in both studies was determined by LC/MS/MS.

Result: The results demonstrated that increasing intracellular accumulation of bile acids would cause cholestasis and hepatotoxicity. And the inhibition effect of compounds on hepatic transport of bile acids could aggravate the hepatotoxicity, such as drug-induced cholestasis. And sandwich cultured rat hepatocytes was a useful tool to evaluate drug-induced hepatotoxicity in vitro.

Conclusion: The sandwich cultured rat hepatocytes are an excellent model to characterize drug induced hepatotoxicity especially transporter-based cholestasis



POSTER ABSTRACT

#110

Optimization of detection sensitivity for derivatized and underivatized neurotransmitters on different triple quadrupole mass spectrometer platform

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Abstract:

Norepinephrine (NE), serotonin (5-HT) and dopamine (DA) play important roles as neurotransmitters and are classical biomarkers for diseases such as hypertension and neuroblastoma. Therefore high-sensitivity measurement of catecholamines by LCMS is very much warranted. One of many approaches for improving the sensitivity of catecholamine detection by LCMS is derivatization of catecholamines to attach a hydrophobic moiety so that they retain better on reverse-phase columns. While this approach is effective from a chromatographic point of view, the extent of this effect on the overall sensitivity of detection depends on ionization efficiency and ion transmission in mass spectrometry, which vary amongst instrument type due to different architecture. We compared herein the sensitivity, background noise level and repeatability of the neurotransmitters measurement between Shimadzu and AB Sciex instruments.

Standard solutions of norepinephrine (NE), serotonin (5-HT) and dopamine (DA) were prepared at 0.002-1, 0.002-1 and 0.02-10 ng/mL, respectively. Internal standard solutions were prepared by mixing 2 ng/mL d6-NE, 2 ng/mL d4-5-HT and 10 ng/mL d4-DA in ethanol. As a quality control sample, artificial CSF composed of NaCl, KCl, CaCl₂, MgCl₂ and sodium phosphate buffer were spiked with standard solutions. Prior to measurement, 10 µL of standard solutions or model samples were mixed with equal volume of internal standard, and then derivatized by adding 50 µL of derivatization reagent. The reaction product was directly subjected to LCMS analysis. Alternatively, the samples were directly subjected to analysis without derivatization and quantified by external calibration.

Novel Aspect:

The sensitivity of neurotransmitters was directly compared using different high-end mass spectrometers under identical LC condition.

Preliminary Results:

With AB Sciex Triple Quad 5500, the lower limit of quantification (LLOQ) of derivatized NE, 5-HT and DA were determined to be 0.005, 0.005 and 0.05 ng/mL, respectively. The peak area ratio reproduced well with quality control sample at LLOQ, showing that sample preparation and derivatization resulted in no significant loss of analyte. However, LOD (without derivatization) was limited to 0.2 ng/mL NE, 0.02 ng/mL 5-HT and 0.1 ng/mL DA in artificial CSF.

On the other hand, the LLOQ of intact catecholamines on Shimadzu LCMS-8060 was determined to be 0.005 ng/mL for NE, 5-HT, and 0.01ng/mL for DA, without derivatization. The LLOQ of derivatized NE, 5-HT and DA were determined to be 0.01, 0.002 and 0.02 ng/mL, respectively. We are currently standardizing the sample and chromatographic conditions for direct comparison between the two instruments.



POSTER ABSTRACT

#111

Improved predictability of MDCKII-MDR1-BCRP assay by lower substrate concentration to 0.1 μ M: potentially overcoming saturation of transport activity

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Abstract

P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), both ATP-dependent efflux transporters, are expressed at the apical membrane of blood-brain barrier (BBB) which play an important role in limiting entry of drugs to CNS. Double-transduced MDCKII cells with human P-gp and BCRP has been widely employed as an *in-vitro* tool to study efflux potential of drugs in CNS drug discovery. In one of AstraZeneca CNS projects, we observed that *in-vitro* P-gp&BCRP cells failed to efficiently screen out low brain penetrable compounds in rat CNS model at substrate concentration of 1 μ M, which is the most commonly adapted concentration. For this assay, one of the reasons is that transport activity of P-gp and BCRP may be saturated at 1 μ M test compound concentration leading to incapable of identify efflux potential¹. Here we presented transport experiment by lowering the test compound concentration to 0.1 μ M to avoid the possible saturation of efflux protein. The modified assay was validated by using CNS drugs. The model was then successfully applied for the project with good *in vitro-in vivo* correlation between P-gp and BCRP mediated transport activity and *in vivo* brain penetration in rats. The present findings are consistent with literature reports, indicating that lower concentration was more sensitive to identify drug efflux potential. The results provide an efficient way in selecting brain penetrable candidate for human.



POSTER ABSTRACT

#112

LC-MS/MS method for the determination of bile acid from serum sample using Cleanert MAS-MAW

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Cleanert MAS-MAW was used to purify the serum sample which with a mix mechanism of reverse phase and anion exchange interaction. It has proven to be an efficient and simple way to extract bile acid from serum sample. The mix-mode material eliminated matrix effect of serum such as proteins and phospholipids and obtained sufficient recovery with acceptable precision.

Sample preparation

100 μ L of serum sample diluted with 100 μ L of 1% formic acid aqueous solution was loaded into Cleanert MAS-MAW which was pre-conditioned by 2 mL of acetonitrile and 2 mL of 3% formic acid aqueous solution sequentially. The cartridge was washed with 2 mL of methanol/ water (50/50, v/v), then discarded the eluate. The target compounds were eluted with 2 mL of methanol and 2 mL of triethylamine/ water/ methanol (2/10/88, v/v/v) sequentially. The eluates were collected together and concentrated at 40 $^{\circ}$ C under a gentle stream of nitrogen to dryness. The residues were reconstituted with 100 μ L of methanol/ water (7/3, v/v) and filtrated by PTFE filtration with the pore size of 0.22 μ m, and then analysis by LC-MS/MS.

Results and discussion

Cleanert MAS-MAW is a mixed-mode mechanism cartridge with reverse phase and anion exchange material. Reverse phase interaction exist between polystyrene/ divinylbenzene materials and bile acids with the functional groups of carboxylic acid and hydroxyl. Anion exchange interaction was supplied by amino group which extracted the bile acids with sulphonate functional group.

Formic acid was used to adjust pH of loading sample, which could maintain a part of bile acids as molecular state. The step was important to get admired recovery data of bile acids with the functional groups of carboxylic acid and hydroxyl.

Most of the protein and phospholipids were washed out by 50% methanol in water without loss of bile acids. The elute process was divided into two steps. Reverse phase interaction was disrupted by 100% methanol in the first step which obtained bile acids with the functional groups of carboxylic acid and hydroxyl. In the second step, mixture of triethylamine and high percentage of methanol eluted the bile acids with functional group of sulphonate. Triethylamine as organic base was used in ion exchange SPE mode while ammonium hydroxide incompetent to restraint or ionize the target compound and absorbent material. In this application, triethylamine was used as ionization suppression to control the state of absorbent material.

The average recoveries of bile acid from the sample pretreatment method with two concentration levels at 50ng/mL and 500ng/mL are range from 82.7% to 130.8%, LOD data of bile acid are ranged from 0.33 to 2.39 ng/mL.

Conclusions

The mixed-mode SPE plate could be used for eliminating matrix effect of phospholipids and proteins prior to the analysis of bile acid in serum by LC-MS/MS. A sufficient recovery and great precision were obtained. The method can be applied for the assay of bile acid in patient's serum samples.



POSTER ABSTRACT

#113

N-ethylmaleimide (NEM) as the stabilizer for the small molecule-drug conjugates with disulfide bond

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DMPK, ICC, Asia & Emerging Markets iMed, AstraZeneca

Small molecule-drug conjugates (SMDCs) directed against tumor markers such as FR^[1-3], PSMA^[4], CAIX^[5], somatostatin receptor^[6] represent an efficient chemical strategy for the delivery chemotherapeutic agents to tumors to generate anti-tumor activity without causing much of the associated off-target toxicities. SMDCs are built with three modules: a high affinity targeting ligand, a suitable linker and a cytotoxic payload (Fig. 1)^[1,2]. Disulfide bond is an attractive linker widely used in SMDCs product, which can be efficiently cleaved to release active drug from ligand by antioxidants (eg. Glutathione, GSH) inside endosome after the SMDC is taken up by the diseased cells^[7,8]. The challenge for DMPK scientist is to precisely quantify SMDC level in biological samples like plasma which contains GSH and other thiol containing molecules and can degrade SMDC. Here, we have described the utility of N-Ethylmaleimide (NEM) as the stabilizer of disulfide bond SMDCs in plasma. 2 mM of NEM in PBS can significantly reduce the generation of payload from SMDC. The performance of NEM on stabilizing SMDC in plasma was tested with different concentrations and different temperature. NEM at 10mM is capable of blocking GSH and thus stabilizing the disulfide bond SMDC in plasma. The method was applied successfully in the study of mouse pharmacokinetics (PK) of SMDC: plasma samples after collection were immediately pretreated by 10mM of NEM. In conclusion, our study provided a way of determining precisely disulfide bond SMDC level in plasma and help project team to understand *in-vivo* PK of SMDC.

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POSTER ABSTRACT

#114

Comprehensive workflow for sequence variant analysis using a new bioinformatics platform and a UHPLC coupled to a tribrid mass spectrometer.

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Misincorporation of an unintended amino acid will generate a sequence variant of the target protein. During the development of recombinant protein therapeutics, sequence variant proteins are a concern for the biopharmaceutical industry because they can potentially induce immune response. Sequence variant proteins, present at very low level, are classified as product-related impurities. Here we present a workflow tailored sequence variant analysis. To test the workflow, synthetic peptides representing sequence variant peptides were spiked into the NIST IgG tryptic digest at different levels. Triplicate LC-MS/MS runs were acquired in data dependent acquisition mode using a tribrid mass spectrometer coupled to an UHPLC for each condition. The raw files were processed with BioPharma Finder 1.0 software and only the native sequence of the IgG was used as an input.

In DDA mode, spiked peptides were selected and high quality MS/MS were acquired even when the sequence variant peptides were spiked in at less than 0.5% of the native peptide. The biopharma software successfully identified and quantified spiked sequence variant peptides and also reported coefficient of variation (%CV) below 10% for triplicate runs. Previously identified sequence variant peptides of the NIST digest IgG were also identified with the present workflow. Sequence variant analysis is a challenging analytical experiment and the preliminary data showed that the LC-MS hardware as well as the software are well suited for this type of assay.



POSTER ABSTRACT

#115

HPLC Separation Polar Compounds with a Focus on the Role Stationary Phase

ES Industries

The chromatographic separation and the analysis of polar compounds using HPLC has always been a challenging. It would seem that HPLC analysis would be well suited for the determination of the polar compounds however routine reverse phase HPLC analysis has yielded poor quality results for these types of compounds. The analysis of polar compounds via routine HPLC analysis has been deficient in several main aspects including poor retention, unacceptable low k' values and poor peak shapes. One of few HPLC techniques capable of analyzing polar compounds has been traditional normal phase chromatography (polar stationary phase with a non-polar mobile phase). Unfortunately, normal phase chromatography suffers from several major deficiencies including poor retention time reproducibility, poor column to column reproducibility and very low or no solubility of the target analytes. A number of HPLC stationary phases have introduced to improve the analysis polar compounds. These HPLC stationary phases include high aqueous stable phases, HILIC (hydrophilic interaction chromatography) phases and polar embedded phases all of which utilize various levels of water in the mobile phase. It is the focus of this presentation to compare and contrast the HPLC stationary phases developed for the analysis of polar compounds. In addition, we will introduce new stationary phases optimized for the separation of polar compounds. The ultimate goal of this work is to present a strategy for the HPLC analysis of polar compounds and to provide the chromatographer with the information necessary to match their polar compounds/samples with the appropriate HPLC technique.



POSTER ABSTRACT

#116

Unique Chemically Modified Carbohydrate Based Chiral Stationary Phases to Improve Chiral Separations

ES Industries

The chromatographic separation of chiral compounds is an important tool in the search for new pharmaceutical entities. Both HPLC and SFC separations of chiral chemicals are important tools for analytical determination and preparative isolation of enantiomeric mixtures. Existing chiral stationary phases can separate a many chiral mixtures. Many of these chiral stationary phases are based on chemically modified carbohydrates. However, even with the existing chemically modified carbohydrates stationary phases there are still many enantiomeric mixtures that are difficult to separate limiting the ability to characterize and purify chemical mixtures containing chiral compounds. In this study we are chemically modifying carbohydrates, such as cellulose and amylose, with functional groups that have not been routinely employed. Chemical modifications of the carbohydrates include halogenated, aromatic and hetero-aromatic functional groups. We will present information on the chiral separation characteristics and overall separation capabilities for these chemically modified carbohydrate based chiral stationary phases.



POSTER ABSTRACT

#117

The Development of Unique HPLC and SFC Stationary Phases that Utilize Advanced Particle Technologies

ES Industries

Both Reversed-phase HPLC and SFC is widely used for separation of many chemical compounds. A majority of these separations are based on ODS type columns. However, retention and separation of various compounds have proven to be a challenge. Many of these types of compounds are unretained, poorly retained or unseparated on most conventional ODS reversed-phase columns, even when these ODS column are packed with highly efficient sub 2 particles. Fortunately, to deal with these types of analytes we can employ alternative modes of chromatography that use unique stationary phases containing polar groups, organic bases, fluorinated groups and other non-hydrocarbon functional groups. These columns can to be used in the traditional reverse phase mode as well as both SFC and “hydrophilic interaction chromatography” or HILIC. SFC uses supercritical CO₂ along with an organic modifier such as methanol. HILIC chromatography uses mobile phases containing between 5 - 20 % water for the retention of polar compounds. These unique stationary phases are bonded to support materials that utilize advanced particle technologies. It will be demonstrated that the combination of unique stationary phases bonded to advanced particle technologies will improved separations and add flexibility to operating conditions.



POSTER ABSTRACT

#118

An integrated Chip-based LC-MS System for High Performance Microspray.

Helena Svobodova, Amanda Berg, Peter Wang, Gary Valaskovic

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Packed-tip columns have successfully enabled nanoflow LC-MS applications by delivering optimal chromatographic performance at nanoflow rates. Incorporating packed-tip-columns into a chip-like device enables ease-of-use while preserving the performance of this unique column format. Coupling this chip-based column solution to pneumatically enabled source hardware expands the functional application window, delivering a wider range of operating flow rates (100 nl/min. to 10 μ l/min.) in a single device. Using pneumatically enabled source hardware and chip-based columns, the effect of pneumatically assisted ESI was evaluated for 150 μ m ID chip-based consumables at 1 μ l/min. to 5 μ l/min. The effect of heat, previously investigated for 75 μ m ID columns, will be explored for microflow LC-MS using 150 μ m and 200 μ m ID columns.

The effect of sheath gas on the spray stability was evaluated by calculating the relative standard deviation (RSD) for the extracted ion chromatogram of the angiotensin MH^{3+} ion (349.9 Da). The optimal conditions for 1 and 2 μ l/min. were achieved at a sheath gas setting of 0 (RSD 10.0 and 5.1 respectively). The addition of sheath gas resulted in increased RSD values, with a maximum RSD of 26.6 at 1 μ l/min. and 10.4 at 2 μ l/min., observed at a sheath gas setting of 10. The addition of sheath gas was beneficial at the higher flow rates of 4 and 5 μ l/min. At 4 μ l/min. the spray was most stable with a sheath gas setting of 20 resulting in an RSD of 3.4 compared with an RSD of 17.7 without sheath gas. The best spray stability at 5 μ l/min was achieved with a sheath gas setting of 30 (RSD 2.8), an eight-fold improvement when compared to a sheath gas setting of 0 (RSD 23.1). Extracted ion chromatograms for the MH^{2+} (524.2 Da) and MH^{3+} (349.9 Da) angiotensin II ions showed changes in the intensity ratios between these two ions which was parameter dependent. Increasing the flow rate from 1 μ l/min. to 5 μ l/min. resulted in the ratio of MH^{3+}/MH^{2+} increasing from 0.9 to 2.4 at 5 μ l/min. with a gas setting of 0. Increasing the gas from 0 to 30 resulted in a decreased MH^{3+}/MH^{2+} ratio from 1.2 to 0.6 at 3 μ l/min. The spray stability data will be used to optimize the conditions for the chromatographic runs and the chromatographic data presented.



POSTER ABSTRACT

#119

Pharmacokinetics study of XGX injection in Chinese non-cancer pain patients

Wen Xu - Clinical Pharmacology Research Center, PUMCH

XGX injection is an opioid receptor analgesic. It is registered as a type 1.1 innovative drug in China Food and Drug Administration (CFDA). Currently phase I clinical trial of XGX is being evaluated in Peking Union Medical College Hospital. In this thesis we investigated the safety, tolerance and single-dose pharmacokinetics of XGX in Chinese non-cancer pain patients.

The major objective of this part was to develop and validate a UPLC-MS/MS method for the determination of XGX, XGX-2 and XGX-3 in human plasma. The UPLC-MS/MS method was fully validated based on the bioanalytical guideline issued by CFDA. The validation parameters included specificity, linearity, sensitivity, accuracy, precision, stability, extraction recovery and matrix effect. The study results showed that the UPLC-MS/MS method was accurate, rapid and sensitive. With this high-throughput bioanalytical method, the plasma concentrations of XGX, XGX-2 and XGX-3 were simultaneously determined. This method was completely suitable for the human pharmacokinetic study of XGX in Chinese subjects. Then, the plasma concentrations of XGX, XGX-2 and XGX-3 were determined using the UPLC-MS/MS developed above.

In order to fully understand the pharmacokinetics of XGX in patients, and to identify the variability affecting the pharmacokinetics, a population covariate pharmacokinetic model of XGX was established by means of non-linear mixed-effects modeling using Phoenix™ NLME software. The following covariates were tested: creatinine (CR), direct bilirubin (DBIL), albumin (ALB), Aspartate transaminase (AST), total bilirubin (TBIL), gender, total body weight (TBW), height and lean body weight (LBW). This population pharmacokinetic model would be greatly helpful in the future clinical evaluation of XGX.



POSTER ABSTRACT

#120

Complementary *in vitro* tools to investigate renal drug transport

Laszlo Szilagyi, SOLVO Biotechnology

Renal excretion is an important pathway for the elimination of endogenous and xenobiotic substances. A wide range of efflux and uptake transporters are expressed in the renal epithelial cells to regulate the excretion and the reabsorption of various kinds of organic anions, cations, peptides and nucleosides.

The primary proximal tubule cell (PTC) monolayer assay, established by Dr Colin Brown (Newcastle University, UK) is a unique *in vitro* approach for investigating renal drug handling, identifying drug-transporter interactions, potential nephrotoxicity and transporter mediated drug-drug interactions. Unlike other primary renal cell models, this monolayer assay maintains the full complement and expression level of endogenous renal transporters, resulting in a more physiologically relevant, and therefore predictive, model of drug handling in the clinical setting.

While the PTC model serves as a holistic approach to study renal handling of drug molecules and endogenous substrates, double transfected monolayer assays could complete the results gained in PTC model with functional characteristics, such as substrate specificity and transport mechanisms involved in the renal elimination. Certain double transfected monolayer assays have been developed to investigate the underlying mechanisms of renal elimination of both organic anion (MDCKII-OAT1/BCRP and MDCKII-OAT3/BCRP) and cation (MDCKII-OCT2/MATE1 and MDCKII-OCT2/MATE2-K) compounds. Transport of selected drugs and physiologically relevant endogenous substrates such as metformin, methotrexate, PAH, urate, TEA and E3S has been investigated with both PTC and appropriate double transfectant monolayers. The results highlight the complementary nature of the two models.



POSTER ABSTRACT

#121

Determination of the liposome and nonliposome vincristine in human plasma by Ultra Performance Liquid Chromatography-tandem mass spectrometry

Ye Tian

Beijing Union Medical College Hospital Clinical Pharmacology Center

Background:

Liposomal encapsulation of vincristine sulfate is designed to increase safety and tolerability by decreasing neurotoxicity and gastrointestinal toxicity. Liposomes can offer the potential of selective delivery to the site of action, thus increasing the exposure profile to disease cells or tissues. EMA and FDA have pointed out that should developed an effective biological analytical methods of separating the liposome and non-liposome drug. Here, we developed an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method to quantify the free vincristine (F-VCR) and total vincristine (T-VCR) in human plasma after intravenous administration of vincristine sulfate liposome injection (VSLI).

Method:

The analytical column was HSS T3 1.8 μm (2.1 \times 50mm) , and the mobile phase was composed of methanol- water containing 0.2% formic acid. The flow rate was 0.4 mL/min. Detection was performed with multiple reactions monitoring (MRM) using positive electrospray ionization (ESI).

Result:

It was indicated that the calibration curve was linear over the concentration range of 0.2-50 ng/mL for F-VCR and 0.5-400 ng/mL for T-VCR. Inter-and intra-day precision were less than 15% and accuracy was within 85-115% about F-VCR and T-VCR.

Conclusion:

This method proved to be rapid, sensitive and specific, and suitable for the pharmacokinetic study of VSLI in humans.



POSTER ABSTRACT

#122

High Quality Bioanalytical Services for TK/PK/BE Studies

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Since Frontage Laboratories (Shanghai) Co., Ltd. was established in 2006, the Department of Biologics Services has been dedicated to supply high quality bioanalytical services on TK/PK/BE Studies and on biomarker/immunogenicity evaluations for preclinical and clinical studies. About 50 projects were conducted including method transfer, method validation, and sample analysis by using immunoassays and biochemical assays on platforms of SpectraMax M2 and MSD Sector Imager 6000. The drugs of the projects covered from proteins (including antibodies, polypeptides or hormones), polysaccharides, to small molecules (for tests on PD/biomarkers).

We have high capability on bioanalytical services:

1. Method Development

Not only using methods supplied by sponsors when working well, or modifying methods to make them work better, but also developing/establishing methods when no qualified methods supplied by sponsors

Recently we developed methods for anti-drug-antibody detection successfully with good sensitivity and specificity by using MSD platform.

2. Method Transfer and Validation

After we get methods and reagents, methods transfer was normally done within 1 ~ 2 weeks, and method validation done within 2 ~ 3 weeks according to approved validation plan.

3. Sample Analysis

Normally one scientist runs 4 ~ 6 plates, about 100 ~ 200 samples on one assay day.

Good performance is always required.

For example on a TK study by using MSD platform, over 3000 samples were analyzed in more than 150 runs, less than 0.5% of runs failed, less than 0.8% of samples were reassayed due to CV>20%, and more than 97% of ISR samples met requirements.

Department of Biologics Services at Frontage Laboratories (Shanghai) Co., Ltd. will continuously supply high quality services using GLP principles as a quality standard.



POSTER ABSTRACT

#123

Development of the in vitro fluorescence-based human OATP1B1 and OATP1B3 Model for DDI evaluation

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The objective of the current study was to establish the fluorescence-based assays for the assessment of drug interaction with the human transporters OATP1B1 and OATP1B3 stably expressed in HEK-293 cells using 8-fluorescein-cAMP (8-FcA) as a substrate. The OATP1B1 or OATP1B3-mediated transport of 8-FcA was time dependent and saturable (K_m was $3.49 \pm 0.734 \mu\text{M}$ for OATP1B1, and $1.70 \pm 0.190 \mu\text{M}$ for OATP1B3, respectively) under the conditions tested. Each of positive inhibitors for OATPs, including Cyclosporine A, Bromosulphophthalein, Ritonavir and Rifampicin, demonstrated a concentration-dependent inhibition of 8-FcA transport by OATP1B1 and OATP1B3. The in vitro fluorescence-based assays described here using 8-FcA as the substrate are convenient, rapid and have utility in screening drug candidates for potential drug–drug interactions with OATP1B1 and OATP1B3.



POSTER ABSTRACT

Young Scientist Excellence Submission #124

Relationships between ilaprazole/esomeprazole treatment and plasma asymmetric dimethylarginine level in healthy subjects and patients with duodenal ulcer

Xuemei Liu, Hongyun Wang, Pei Hu, Ji Jiang*

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Abstract

Background: Proton pump inhibitors (PPIs) have demonstrated a superior efficacy profile in the management of acid-related disorders. Recently, much more concerns have been raised about the potential for PPIs to increase the cardiovascular disease risk, which may be illustrated by the elevation of plasma asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of endothelial nitric oxide synthase (eNOS). However, there is little clinical data evaluating the relationship between plasma ADMA and PPIs exposure, and whether the use of PPIs directly causes cardiovascular harm.

Method: A total of two prospective clinical trials were carried out to investigate the pharmacokinetics of ilaprazole and esomeprazole in Chinese healthy individuals (ILA-I) and patients with duodenal ulcer (ILA-IIA). For ILA-I study, 16 individuals with 18-45 years of age in general good health were randomized into 4 treatment sequences, which were separated by 3 periods of 1-week washout intervals to evaluate both 3 ascending doses (5 mg, 10 mg, 20 mg) of ilaprazole with injection formulation (iv) and 10 mg of ilaprazole with enteric-coated tablet (ETC) formulation. For ILA-II, 20 duodenal ulcer active volunteers, confirmed by gastroscopy within an ulcer diameter ≤ 15 mm, mild, non-consolidated ulcer bleeding, were included to receive either 20 mg ilaprazole (iv) or 40 mg esomeprazole (iv). In both two studies, the plasma samples collected for PK studies would also be utilized to assess the fluctuation of ADMA in plasma, ranging from baseline to 24 h after administration. A simple, sensitive and rapid hydrophilic interaction chromatographic method coupled to tandem mass spectrometry was developed and validated for the determination of plasma ADMA. The potential correlation between the ADMA and concentration of PPIs (either ilaprazole or esomeprazole) were evaluated using spearman correlation coefficient.

Results: The primitive ADMA plasma levels were 0.44 ± 0.05 $\mu\text{mol/L}$ for healthy volunteers ($n=15$) and 0.53 ± 0.09 $\mu\text{mol/L}$ for patients with duodenal ulcer ($n=20$), and Mann-Whitney U-test show statistically significant difference between two studies ($p < 0.05$). However, the relationship between ilaprazole and esomeprazole plasma levels and change (%) in ADMA from baseline show marginal correlation ($r=0.036$ for healthy volunteers and 0.085 for patients) with no statistical significance ($p=0.599$ and 0.903).

Conclusion: In those two studies conducted among healthy subjects and patients with duodenal ulcer, the ADMA baseline were much more higher in patients, and PPI use did not significantly influence plasma ADMA level. Larger, long-term and blinded trials are needed to mechanistically explain the correlation between PPI use and adverse clinical outcomes, which has recently been reported in retrospective cohort studies.



POSTER ABSTRACT

Young Scientist Excellence Submission #125

A Semi PBPK model constructing the transporter mediated clearance and P450 induction in liver compartment, to demonstrate the drug drug interaction when co-administration of Rifampin, Lopinavir and Ritonavir in HIV-Infected Adults

Xun Tao - Chempartner

Lopinavir-Ritonavir Cocktail has been widely applied in the therapy for HIV-infected patients. Both Lopinavir and Ritonavir have been proven as substrate of CYP3A4 with extensive metabolism rate in human liver microsomes. However, neither Lopinavir nor Ritonavir has the good passive permeability into hepatocyte, On the other hand, Lopinavir and Ritonavir are reported as the substrate of OATP. Hence, the in vivo clearances of Lopinavir and Ritonavir likely are limited by the step of hepatic uptake rate. The co-administration of Lopinavir and Ritonavir utilized the competition of OATP occupancy to slow down the hepatic uptake rate, and then achieve the AUC boost. On the other hand, Rifampin coadministration dramatically reduces plasma lopinavir and Ritonavir concentrations, probably because of its potent CYP3A4 induction. A recent clinical trial demonstrates that doubling the dose of a lopinavir-ritonavir capsule formulation overcame the interaction of Rifampin. Based on that clinical data, as well as other published in-vitro data, a semi-PBPK model is established, which constructing the transporter mediated clearance, and P450 induction progress in liver compartment, to demonstrate the aforementioned drug drug interaction. The model will be helpful for the protocol optimization in future clinical trial.



POSTER ABSTRACT

Young Scientist Excellence Submission #126

Modeling and Simulation of pharmacokinetics for Trastuzumab emtansine from pre-clinical species to human

Xian Pan - Chempartner

The research and development of therapeutic biological products have been surging over last three decades. Human dose and pharmacokinetics (PK) prediction based on the PK data of pre-clinical species shows great importance in bridging pre-clinical and clinical research, however, prediction of human PK profile can be very challenging, especially for the biologics showed nonlinear PK caused by target-mediated drug disposition (TMDD). Trastuzumab emtansine (T-DM1) is approved for patients with human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer. T-DM1 binds to non-human primate HER2 but not the rodent homolog. Therefore, target-dependent and non-target-dependent drug disposition can be evaluated in monkeys and rats, respectively. In this study, total antibody plasma concentration in rats and monkeys was measured by generic enzyme-linked immunosorbent assay. Two-compartment model was applied to obtain T-DM1 specific parameters in rats. Then two-compartment model with TMDD approximations was developed to predict T-DM1 PK profile in NHPs upon different dose regimens using Berkeley Madonna 9.0 software. The predicted PK in NHPs of TDM1 was in good agreement with the observed nonlinear PK. Using human physiological parameters and estimated PK parameters via allometric method, the model successfully predicted the human PK profile of T-DM1. Taken together, the model applied in the present study was proved to be an effective approach in human PK prediction for T-DM1, which in turn, could be helpful in the PK prediction for the biologics with TMDD-caused nonlinear elimination.



POSTER ABSTRACT

Young Scientist Excellence Submission #127

Determination of major clearance pathway of ilaprazole in human by profiling and characterizing in vitro and in vivo metabolites of non-radiolabeled ilaprazole

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Abstract

Ilaprazole, a new proton pump inhibitor (PPI), is developed in China and approved by CFDA recently for the treatment of peptic ulcers. Ilaprazole has significant advantages over traditional PPIs such as omeprazole, including favorable longer half-life and no association with polymorphic CYP2C19. Metabolism study in human liver microsomes (HLM) showed that ilaprazole mainly undergoes CYP3A4-mediated sulfur oxidation to form the sulfone metabolite. However, there is no correlation between the CYP3A phenotype and ilaprazole exposure in human. Furthermore, co-administration of ilaprazole with clarithromycin, a potent CYP3A4 inhibitor, and clarithromycin does not increase the exposure of ilaprazole in human. Although ilaprazole sulfide and its further metabolite are found in human urine, radiolabeled human ADME study has not been conducted. Thus, metabolism and disposition of ilaprazole in human, especially the major clearance pathway and associated enzyme or transporter, remains unclear. The primary objective of this study was to determine the major clearance pathway of ilaprazole in humans. Three sets of experiments were carried out to accomplish the objective: (1) identification, characterization and quantitative estimation of ilaprazole metabolites in HLM, (2) determination of metabolic rates and profiles of ilaprazole sulfide and ilaprazole sulfone in HLM, and (3) detection and identification of ilaprazole-related components in human urine and feces. In vitro and/or in vivo metabolites of test compounds (ilaprazole, ilaprazole sulfide, and ilaprazole sulfone) were detected and characterized using high resolution mass spectrometry and quantitatively analyzed using LC/UV or LC/MS with chemical standards. Results showed that ilaprazole sulfide and its demethylated product were major metabolites in HLM, while previously reported ilaprazole sulfone and monohydroxylated metabolites were minor metabolites. The reduction of ilaprazole to sulfide in HLM did not require NADPH and occurred after HLM enzymes were deactivated. A majority of these ilaprazole metabolites were also observed in HLM incubation with ilaprazole sulfide. Metabolic stability testing showed that T_{1/2} values were 34 min for ilaprazole, 144 min for ilaprazole sulfone metabolite and 8 min for ilaprazole sulfide in HLM. A large number of ilaprazole metabolites were found in human urine but no parent drug after a single oral dose (10 mg), most of which were associated with ilaprazole sulfide. Based on these observations, we propose that non-enzymatic reduction of ilaprazole to the sulfide metabolite followed by fast oxidative metabolism is the major clearance pathway of ilaprazole in human. If metabolite profiling data in human feces support the proposal, ilaprazole would have no drug-drug interactions when co-administered with an inhibitor of a metabolizing enzyme or transporter. The information would greatly help the safe use of ilaprazole in patients.



POSTER ABSTRACT

Young Scientist Excellence Submission #128

Prediction of In Vivo Hepatic Uptake of Rosuvastatin in Chinese People Based on Transporter Protein Quantification and In Vitro Hepatocyte Uptake Model

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Purpose: This research is to explore the correlation between transporter expression and its biological functions, and predict the in vivo transporter mediated hepatic uptake of Rosuvastatin based on transporter protein quantification and In Vitro Hepatocyte Uptake Model.

Method: Drug transporters including OATP1B1, OATP1B3, OATP2B1 and NTCP play an important role in Rosuvastatin hepatic clearance. However, no effective method could be used to evaluate the uptake activities in vivo. So, this research aimed to establish a model based on PBPK model through determination of transporter protein expression and uptake activity in vitro to predict transporter-mediated uptake in vivo. Firstly, the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for simultaneous determination of OATP1B1, OATP1B3, OATP2B1 and NTCP expression level in 23 donors of Chinese human cryopreserved hepatocytes and HEK293 cells stably transfected with human OATP1B1/OATP1B3/OATP2B1/NTCP gene, with synthesizing surrogate peptides of OATP1B1, OATP1B3, OATP2B1 and NTCP as calibrated standard, and corresponding stable isotope labeled surrogate peptides as internal standard and optimizing the chromatograph and mass spectrometry conditions.

Second, K_m and V_{max} values of Rosuvastatin were determined with stable transfected cell lines including hOATP1B1-293, hOATP1B3-293, hOATP2B1-293 and hNTCP-293, and applied in prediction of uptake activity in vivo combined with the result of protein quantification based on Michaelis-Menten equation.

Besides, with the model of sandwich cultured human hepatocytes, hepatic uptake activities of Rosuvastatin were also determined in different batches of human hepatocytes. And the result was used to analyze the correlation between transporter protein expression and uptake activity in vitro and the differences of predicting activity in vivo and determined data in vitro.

Result: The LC-MS/MS method was established and validated for simultaneous quantification of OATP1B1, OATP1B3, OATP2B1 and NTCP. Both the intra-day and inter-day precision and accuracy of analytes were within the acceptable criterion of macromolecules bioanalysis. The standard curves were demonstrated linear in the range of 0.2-50 nM for OATP1B1, OATP1B3 and OATP2B1 and 0.4-100 nM for NTCP, respectively ($r > 0.995$). As the result, the protein expression levels of OATP1B1, OATP1B3, OATP2B1 and NTCP in 23 donors of Chinese human cryopreserved hepatocytes were 4.74 ± 1.26 , 1.39 ± 0.82 , 3.02 ± 0.71 and 3.69 ± 1.15 fmol/ μ g, respectively. The results showed that these four transporters had different expression level on hepatocytes membrane in which OATP1B1 was the highest. Besides, transporter protein expression had individual differences. Both could be useful for drug research and clinical individualized medication.

With the K_m and V_{max} result in stable transfected cell model, Rosuvastatin was the substrate of all four transporter with different affinity (K_m : OATP1B3 < NTCP < OATP1B1 < OATP2B1). Combined with transporter quantification,

With the K_m and V_{max} result in stable transfected cell model, Rosuvastatin was the substrate of all four transporters with different affinity (K_m : OATP1B3 < NTCP < OATP1B1 < OATP2B1). Combined with transporter quantification, NTCP showed a major contribution in the hepatic uptake of rosuvastatin with 68% of overall active uptake, and predicting activity was 56.9 ± 37.0 pmol/min/mg based on Michaelis-Menten equation. Besides, the result of hepatic uptake clearance with sandwich cultured human hepatocytes showed that $74.94\% \pm 15.04\%$ of hepatic uptake of Rosuvastatin was mediated by transporters, and the determined active uptake activity was 21.0 ± 19.7 pmol/min/mg. As the comparison of the values of predicting clearance and determined hepatic active clearance, linear regression correlation coefficient was 0.66 for Rosuvastatin. And the results with spearman analysis showed positive correlation with r_s was 0.3604.

Conclusion: protein quantification of hepatic transporter was very important for prediction and evaluation of drug hepatic clearance in vivo. And a preliminary PBPK model was established with transporter quantification and hepatic uptake model in vitro with well correlation between predicting and determined data.



POSTER ABSTRACT

Young Scientist Excellence Submission #129

A Systems Pharmacology Model for Predicting Anticoagulant Effects of FXa Inhibitors in Healthy Subjects: Assessment of Pharmacokinetic and Binding Kinetic Properties*

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For the last decade, the concept of drug-target binding kinetics has been in focus in the drug discovery, because there is mounting evidence that the often ignored kinetic aspects of the interaction between a small molecule drug and its protein target are highly relevant for *in vivo* efficacy and clinical success.

The exploration of pharmacokinetics and binding kinetics of drugs focusing on the duration of effect has been described using traditional PK/PD models. However, the complex biological process and detailed drug action are usually unclear, which makes it difficult to predict clinical outcome at the drug discovery stage.

In this study, we selected the target of Factor Xa (FXa) as an example, which emerged as a promising target for effective anticoagulation. A large-scale systems pharmacology model was developed based on the published data. It takes in to account the pathways of the coagulation network, and captures drug-specific feature: PK and binding kinetics. As the results, the model predicts the clotting time and anti-FXa effects and could serve as a predictive tool for the anticoagulant potential of a new compound.



POSTER ABSTRACT

Young Scientist Excellence Submission #130

Application of a multi-compartment model to predict the distribution of a steroid glycoside in gut, liver and plasma in rats.

Cuifeng Zhang^{1,2}, Hongcan Ren³, Yunfei Lin², Zhitao Wu², Xuan Ni², Le Wang², Haitang Xie^{*1}, Guoyu Pan^{*2}.

(1. Yijishan hospital of Wannan Medical College 2. Shanghai Institute of Materia & Medica, University of Chinese Academy of Sciences 3. Hutchison Medi Pharma)

Introduction: TX-1 is a major bioactive steroid glycoside isolated from traditional Chinese medicine. It has various pharmacological activities, such as anti-oxidative, anti-inflammatory, and anti-platelet aggregation. Previously study showed TX-1 was mainly metabolized into TX-2 and TX-3 by the deglycosylation from intestinal microbiota. This study aimed to establish a multi-compartment model to simulate the PK profile of TX-1 in gut, liver and plasma in rats, which may facilitate the understanding of the metabolic kinetics of TX-1 and its metabolites.

Methods: TX-1 metabolism in gut was determined by get SD rat colon contents incubated *in vitro*. After single oral administration in SD rats respectively, the plasma and liver concentration of TX-1, TX-2 and TX-3 were quantified by HPLC-MS/MS. Their individual PK parameters were calculated. A multi-compartment model was constructed for TX-1 and its metabolites TX-2 and TX-3 using Phoenix WinNonlin (6.1). Data from literature and our partner's group (Professor Chenggang Huang) were used to validate the accuracy of this model. The model was employed to predict TX-1, TX-2 and TX-3 liver concentration in rats.

Results: An integrated multi-compartment model was established successfully. Our model worked well in the dose range from 30-90mg/kg. When the dose increased to 180mg/kg, the predicted plasma concentrations were below the observed concentrations which may came from metabolic saturation.

Conclusions: An integrated multi-compartment model was successfully constructed, which was important to understand the disposition of TX-1, TX-2 and TX-3 in plasma, liver and gut.