



POSTER SESSION ABSTRACTS

Inspiration and Education

POSTER HALL OPEN

Thursday, April 16 9:00 am - 5:00 pm

Friday, April 17 9:00 am - 5:00 pm

*Submissions for General Poster
including Young Scientist Excellence
Award and Innovator Award*

CPSA Shanghai 2015
April 15 - 18, 2015
Renaissance Pudong Hotel Shanghai

Where Technology and Solutions Meet

Where East Meets West

***Where Technology and Solutions Meet
Where East Meets West***

Poster #	Abstract
101	Development of a High Sensitivity Quantitative SPE-LC/MS/MS Assay for Bradykinin in Human Plasma Using IonKey /MS Technology. Jianming Cao, Waters Corporation
102	Practical Considerations for LC/MS Bioanalysis of Proteins via the Surrogate Peptide Approach. Erin Chambers, Waters Corporation
103	Determination of Natamycin Content in Cake Using Ultra Performance Liquid Chromatography-Mass/Mass Spectrometry. Fuxin Chen, Xi'an University of Science and Technology
104	Quantitative Analysis of OATP1B1 in Human Hepatocytes of Chinese Population Using Liquid Chromatography Tandem Mass Spectrometry. Tao Chen, Guangdong Pharmaceutical University/CarysBio
105	Development of a Physiologically-based pharmacokinetic model to predict systemic and cerebrospinal icotinib exposure in cancer patients. Jia Chen, Peking Union Medical College Hospital
106	Is incidence of skin rash driven by peak concentration or wild type EGFR IC50 cover by pharmacokinetics for EGFR-TKIs? Kan Chen, AstraZeneca
107	Population pharmacokinetic/pharmacodynamic/survival modeling analysis of icotinib in Chinese non-small cell lung cancer subjects. Jia Chen Peking Union Medical College Hospital
108	Quantitative determination of Nucleoside Triphosphate in Rat liver using liquid chromatography-tandem mass spectrometry. Wenqi Cui, Covance
109	Application of a novel sample preparation method in pharmacokinetics research about total and free paclitaxel among solid tumor patients after intravenous infusion of paclitaxel liposome. Xinge Cui Peking Union Medical College Hospital
110	Characterization of a highly selective ratiometric two-photon fluorescent probe for human cytochrome P450 1A and its biological applications. Ziru Dai, Chinese Academy of Sciences
111	Comparative disposition of intravenous catechols, derived from Salvia miltiorrhiza roots (Danshen), in rats and mechanisms governing their systemic exposure. Jiajia Dong, Shanghai Institute of Materia Medica, Chinese Academy of Sciences
112	Single and Multiple Dose Pharmacokinetics, pharmacodynamics and safety of darapladib (a novel Lp-PLA2 enzyme inhibitor) in healthy Chinese subjects . Chaoying Hu, Shanghai Xuhui Central Hospital
113	Investigation on ISR failure and the subsequent solutions to overcome the assay variability. Liang Huang, Covance.
114	What Quality of a Commercial Kit (Immunoassay) Needed - Key parameters in a method for quantitative determination of target protein. Bo Li Frontage Laboratories (Shanghai) Co., Ltd
115	Adaptation of An in Vitro Model for Assessing the Reactivity of Acyl Glucuronides in Drug Discovery. Jerry Li, AstraZeneca
116	A mechanism-based model to describe oral glucose tolerance test data in Chinese type 2 diabetics. Yang Liu, Peking Union Medical College Hospital and Chinese Academy of Medical Sciences

- 117 Colonic Metabolites of Flavonols in Rats and Dogs after Oral Administration of GBE50, A Standardized Ginkgo biloba Leaf Extract. Xinwei Liu, Shanghai Institute of Materia Medica, Chinese Academy of Sciences
- 118 Human Cytochrome P450 Enzymes and Hepatic Microsomal Thiol Methyltransferase Involved in Stereoselective Formation and Methylation of Pharmacologically Active Metabolite of Clopidogrel. Cai Liu, Shanghai Institute of Materia Medica
- 119 High Sensitivity Bioanalysis for Small Drug-Like Compounds in Human Plasma using Microflow LC and High Resolution Mass Spectrometry. Russell Mortishire-Smith, Waters Corporation
- 120 Evaluation of drug-induced hepatotoxicity with five biomarkers in sandwich cultured rat hepatocytes. Long Na, CarysBio Holdings Limited
- 121 Applying LC/MS Natural Product Application Solution with UNIFI for the Identification of the Chemical and Herbal Components from an Unknown Traditional Chinese Medicine Product. Lirui Qiao, Waters Corporation
- 122 Extraction of Telmisartan from Human Plasma Utilizing Cleanert SLE Plate with High Capacity. Suzi Qin, Bonna-Agela Technologies, Tianjin
- 123 Ease-of-use and sensitivity with nanospray: An integrated chip-based solution incorporating column temperature control, long columns, and nebulization. Gary Valaskovic, New Objective
- 124 Development and Validation of an HPLC-MS/MS Method for Determination of Suramin in Monkey Plasma. Yi Tao, WuXi AppTec Co.
- 125 Intestinal Lymphatic Absorption into Consideration When Constructing Physiologically Based Pharmacokinetic Model (PBPK). Xun Tao, Shanghai Chempartner
- 126 PicoFuze: Integration enables high-flow nanospray/microspray with conventional LC-ESI-MS. Gary Valaskovic, New Objective, Inc.
- 127 Aristolochic acid I associated target identified by a monoclonal antibody against aristolochic acid I and II using immunoprecipitation and LC-MS/MS. Dan Wang, Peking university
- 128 Comparison of Various Sample Pretreatment Methods for Monitoring Arachidonic Acid in Plasma. Wan Wang, Bonna-Agela Technologies, Tianjin
- 129 Characterization of gomisin A as a novel isoform-specific probe for selective detection of human cytochrome P450 3A4. Jing-Jing Wu, Dalian Institute of Chemical Physics
- 130 Evaluation of the relative bioavailability of dabigatran etexilate mesylate in beagle dogs. Zhixiong Xiang, Shanghai Pharmaceuticals Holding Co., Ltd.
- 131 An LC/MS/MS Based Strategy for Quantification of Monoclonal Antibodies and Antibody-conjugated Drugs for Developing Antibody Drug Conjugates. Ling Xu, Takeda International Pharmaceutical Inc.
- 132 Impact of incubation time on determination of unbound fractions in blood and brain homogenate. Yumei Yan, AstraZeneca
- 133 Development of an Efficient Screening Method for Screening Potential Bioactive Compounds Binding to Mitochondria from Herbal Medicines by Combining Centrifugal Ultrafiltration with Liquid Chromatography/Mass Spectrometry. Xingxin Yang, Peking University, Beijing, China
- 134 Development and Validation of LC-MS/MS Method for the Quantitation of Infliximab in Human Serum. Hui Wang, Waters

- 135 **Systemic exposure to and disposition of phthalides from Ligusticum chuanxiong rhizomes and Angelica sinensis roots after intravenous administration of XueBiJing injection in human subjects and rats. Nating Zhang, Shanghai Institute of Materia Medica, Chinese Academy of Sciences**
- 136 **Utilization of MDCK permeability and short oral absorption model for GI restricted drug discovery. Jinqiang Zhang, AstraZeneca**
- 137 **A simplification of population pharmacokinetic/pharmacodynamic model based time-dependent kinetics of Red Blood Cell (RBC) binding model and its application to drug CPRC-1 in human. Xin Zheng, Peking Union Medical College Hospital**
- 138 **Chemical Profiling of Ginkgo Biloba Leaves and Extracts. Chenchun Zhong, Shanghai Institute of Materia Medica, Chinese Academy of Sciences**
- 139 **Oxidative and Reductive Activation of Nimesulide: Possibly Irrelevant with Hepatotoxicity. Lei Zhou, Shanghai Institute of Materia Medica**
- 140 **In Vitro and In Vivo Metabolism of TPN729 and Assessment of Its Drug-Drug Interaction Potential. Yunting Zhu, Shanghai Institute of Materia Medica**
- 141 **A simple, rapid and reliable liquid chromatography-mass spectrometry method for determination of methotrexate in human plasma and its application to therapeutic drug monitoring. Dan Wu, Fudan University**
- 142 **In Silico Modeling Can Predict the Unforeseen Renal Failure Caused by SGX523, a c-MET Kinase Inhibitor. Tao Chen, PharmGo Co., Limited**
- 143 **Population pharmacokinetics and Monte Carlo dosing simulation of HR7056 anesthesia in Chinese healthy subjects. Ying Zhou, Peking Union Medical College Hospital**
- 144 **Organic Anion-Transporting Polypeptides Contribute to the Hepatic Uptake of Berberine. Chen Chen, Jiangsu University**

Development of a High Sensitivity Quantitative SPE-LC/MS/MS Assay for Bradykinin in Human Plasma Using IonKey /MS Technology

Mary E. Lame, Erin Chambers, Kenneth J. Fountain, Waters Corporation

Bradykinin is a nine amino acid peptide that causes blood vessel dilation, and therefore a decrease in blood pressure. The ability to measure changes of this peptide hormone with high sensitivity, selectivity and accuracy, as a function of disease progression or drug treatment is thus highly advantageous. Accurate quantification in plasma is particularly challenging because it is present in low pg/mL levels, is rapidly metabolized, and is also artificially produced during blood sampling and sample preparation via proteolytic processes. This study utilizes specifically designed blood collection techniques to inhibit bradykinin formation *ex vivo*, takes advantage of mixed-mode solid phase extraction (SPE) and uses novel integrated microfluidic LC/MS technology for selective, sensitive, robust detection and quantification of bradykinin in human plasma. The method easily distinguishes 2.5 pg/mL changes in bradykinin over the basal level. Standard curves were accurate and precise from 2.5-8,000 pg/mL. All QC samples easily met recommended FDA regulatory criteria with mean accuracies ranging from 92.7-104.0 and mean %CV's of 1.2-4.31, indicating an accurate, precise and reproducible method.

Practical Considerations for LC/MS Bioanalysis of Proteins via the Surrogate Peptide Approach

Erin Chambers, Waters Corporation

Intro

Proteins and peptides represent a growing class of therapeutics due to their target specificity, lower toxicity and higher potency. Historically, these compounds have been quantified using ligand binding assays (LBAs). Recently, there has been a growing trend towards the use of LC/MS which offers the benefits of multiplexing, improved specificity, broader linear dynamic range and faster method development times. In addition, LC/MS avoids common LBA shortcomings such as cross-reactivity and anti-drug antibody effects. However, one inherent challenge associated with protein quantification proteins by LC/MS is that there is no single standardized workflow, and the multitude of options within a workflow makes optimization difficult and time-consuming. This research aims to provide practical method development guidance and comparative data for choice of surrogate peptide, protein-level pre-fractionation, pellet digestion, peptide-level clean-up, internal standard (IS) selection, and digestion conditions. Data is presented for infliximab, bevacizumab, and trastuzumab as examples.

Methods

Human or animal plasma containing the antibody drugs of interest and a labeled antibody IS (SiluMab) were denatured, reduced, alkylated and digested using trypsin. In some cases, the antibody drugs were isolated from other plasma components prior to digestion using an agarose-based Protein A clean-up step. Precipitation using various types and ratios of organic solvents was also tested in an effort to reduce the endogenous background prior to digestion. In particular, peptides arising from human serum albumin were monitored to compare the effectiveness of each treatment in albumin removal. After digestion, the resultant peptides were separated from digest reagents and phospholipids using mixed-mode cation exchange SPE. Finally, LC/MS peptide quantification was performed using tandem quadrupole MS and a low-dispersion, high pressure capable LC system with a 2.1 X 150 mm chromatographic column packed with sub-2 μ m C18 particles.

Results

In a direct digestion workflow, precipitation with the proper ratio of an optimized organic increased surrogate peptide signal by 2-3X. A single, generic mixed-mode cation exchange SPE method provided equivalent, high recovery for both generic and unique surrogate peptides from trastuzumab, bevacizumab, and infliximab whilst removing digest reagents and phospholipids. Generic affinity purification at the protein level increased sensitivity for humanized antibodies by 10-20X in preclinical species. Linearity, accuracy and the precision of quantification based on generic or unique human peptides were equivalent in preclinical species.

Average LLOQ values were 100 ng/mL. A common labeled antibody IS provided equivalent results for the antibody drugs tested. The greatest accuracy and precision was achieved when the IS peptide retention time was closest to that of the drug. Similar performance was observed when extended tag labeled peptides were used as IS.

Conclusion

This work evaluates and compares analytical options for each step of several common protein bioanalysis workflows. These data enable scientists to understand the magnitude of the effect each choice has on data sensitivity and specificity, thus allowing for more efficient method development based on study need. The data also suggest that a few standardized workflows could satisfy the requirements of a preclinical environment.

Determination of Natamycin Content in Cake Using Ultra Performance Liquid Chromatography-Mass/Mass Spectrometry

Fuxin Chen¹, Xiaohuan Bai², Xiaoxian Ma¹, Pin Gong^{2*}

¹School of Chemistry and Chemical Engineering, Xi'an University of Science and Technology, Xi'an, 710054, China;

²College of Life Science and Technology, Shaanxi University of Science and Technology, Xi'an, 710021, China

Natamycin is a normal used food additive in many food, but the additive content have a strict standard in many countries. In this study, a methods based on the use of ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) were devised for determination of natamycin content in cake samples. Sample preparation included extraction of fat and protein removal by congelation, and then extract was concentrated by SPE.

UPLC was performed on an Acquity UPLC BEH C18 (100mm_2.1 mm). UPLC-MS/MS data acquisition was achieved using multiple reaction monitoring (MRM). The elaborated methods for determination of natamycin in cake were validated. Mobile phase components were acetonitrile and water, each containing 0.1% formic acid. Gradient profile is shown in Table 1. The flow rate was 0.2ml/min and column temperature was 30 °C. Injected volume was 5 uL. Desolvation Gas is N2 at a flow rate of 650 L/min. Capillary Voltage is 2.5 KV, Extractor Voltage is 4 V, Source Temperature is 120°C, Desolvation Temperature is 350 °C.

Table 1. Gradient of Mobile Phase for UPLC-MS/MS

Time	Water (0.1% FA)	Acetonitrile (0.1% FA)	Curve
0	80%	20%	6
1	40%	60%	6
2	20%	80%	6
3	0%	100%	6
4	80%	20%	6
5	80%	20%	1

Amphotericin B is the IS for our experiment. Quantification was performed using the pair ion 664.5-137.2 m/z for natamycin and 923.5-183.4m/z for the IS. Some parameters are list in Table 2

Table 2. Validation Parameters of UPLC-MS/MS Method

1	Limit of detection	5 ng/g
2	Liner range	5 -1000 ng/g
3	Retention time	2.3 + 0.03 min.
4	Analysis time	5 min.
5	Correlation coefficient	0.9925
6	Repaetability	6%

In this study, methods based on UPLC-MS/MS were developed for the determination of natamycin in cake. In the MRM model, UPLC-MS/MS have a good performance for the trace natamycin, it is a excellent methods for other food with natamycin as a additive.

Quantitative Analysis of OATP1B1 in Human Hepatocytes of Chinese Population Using Liquid Chromatography Tandem Mass Spectrometry

Tao Chen^{1,2}, Na Long², Hong Yu², Suxing Zhang², Lingmei Zhi², Feipeng Zhang², Laiyou Wang¹, Kezhi Zhang² and John J. Baldwin²

¹ Key Unit of Modulating Liver to Treat Hyperlipidemia SATCM, Level 3 Lab of Lipid Metabolism SATCM, Guangdong TCM Key Laboratory against Metabolic Diseases, Institute of Chinese Medical Sciences, Guangdong Pharmaceutical University, Guangdong Province, PRC

²CarysBio Holdings Limited, Foshan, Guangdong Province, PRC

Purpose: to establish a highly sensitive, selective and reproducible method to absolutely quantify organic anion transporting polypeptide (OATP) ,OATP1B1.

Method: it is universally acknowledged that membrane transporters have a significant influence on the absorption, distribution, metabolism and extraction of drugs. The greatest challenge of the quantification drug transporter is the intrinsic hydrophobic property and low abundance of membrane proteins. In our present study, we have developed a suitable pretreatment method considering the following effect factors on extract efficiency and protein abundance: (i) the ratio of extract buffer volume to hepatocytes number; (ii) the selection of solvents to solubilize the membrane proteins; (iii) the ratio of trypsin quantity to membrane protein for proteolysis; (iv) the reaction time of membrane proteins with trypsin. With the optimized process, OATP1B1 of 3 batches of human hepatocytes of Chinese population isolated from liver fragments resected for medical purposes with proper informed consent were quantified with Liquid Chromatography Tandem Mass Spectrometry. First, the hepatocytes were prepared with a two-step collagenase perfusion, and then plated in sandwich-culture for uptake activity study. And protein quantification of OATP1B1 were conducted with cryopreserved hepatocytes as follow steps before analysis with LC-MS/MS: 1). thawed hepatocytes and washed with PBS for three times; 2). extracted the total membrane protein with Triton X-114 and enriched with acetone; 3). re-solubilized the membrane protein and digested to peptides with trypsin; 4). terminated the reaction with acetic ether containing 0.5%TFA before the addition of internal standard. Finally, the samples were analyzed with the tryptic peptide NVTGFFQSFK as the surrogate peptide of OATP1B1 for quantification and a stable isotope-labeled peptide with the same sequence as the internal standard.

Result: the optimization of process method revealed that Triton X-114 could be used as a cheap and available extract reagent in membrane protein extraction. And though SDS had the strongest solubilization, it was not compatible with the trypsin and MS analysis, while another two solvents, the sodium laurate and deoxycholate, were more suitable for the solubilization of membrane proteins. And as the result of protein quantification of OATP1B1 analyzed with LC-MS/MS, the surrogate peptide abundance was highly sensitive , selective, reproducible and comparable to uptake activity.

Conclusion: the present analytical method and pretreatment process were suitable for the further and in-depth quantification of OATP1B1.

POSTER ABSTRACT

#105

Development of a Physiologically-Based Pharmacokinetic Model to Predict Systemic and Cerebrospinal Icotinib Exposure in Cancer Patients

Jia Chen, Dongyang Liu, Qian Zhao, Zheng Xin, Ji Jiang, Pei Hu.
Phase I Unit, Clinical Pharmacology Research Center, Peking Union Medical College Hospital and Chinese Academy of Medical Sciences, Beijing, China

Objective Icotinib is a novel epidermal growth factor receptor (EGFR)-tyrosine kinase (TKI) to treat non-small cell lung cancer (NSCLC). Now, icotinib is being tested as a new therapy to treat metastatic brain tumor. However, the relationship between patient circulating and cerebrospinal pharmacokinetics is unknown, which limited development of the new therapy. Our goal was to develop a physiologically-based pharmacokinetic (PBPK) model for the prediction of drug concentration-time profiles in systemic and cerebrospinal exposure of patients.

Methods MDR1-MDCK cell transwell assay was carried out to obtain the CL_{transport}. Cells were planted in a transwell for 5 days until the cell confluent. The assay was started when Trans-epithelial electrical resistance (TEER, Ω/cm^2) exceed 300 Ω . Transwell devices were made of different concentrations of icotinib added in the upper chamber and the blank Hank's solution in the down chamber. After incubation in 37°C for half hour, concentration of solution in down chamber was detected. CL_{transport} was calculated as the slope of transport velocity versus original concentration profile. Passive permeability-surface area product on BBB (PSB) was estimated using QSAR method with molecular weight and logP_{o:w} and Passive permeability-surface area product on BCSFB (PSC) was predicted as one in ten fold of PSB. Affinity constant rate (K_p) was also estimated using QSAR with polar surface area (PSA). Besides these parameters, other parameters for systemic PBPK were provided by Beta Pharm. Co. Ltd. (such as logP_{o:w}, pK_a, et al.) And cancer population physiological information was from reference of Jin Y. Jin et al. , which was used to simulate 100 virtual Chinese cancer patients using Monte Carlo methods. Predicted profiles were verified by observed plasma and cerebrospinal concentration in NSCLC patients with metastatic brain tumor from two clinical trials.

Results and Discussion TEERs of most wells planting MDCK cells were increased with cell growth and were more than 300 after 5 days. The transporter-mediated clearance CL_{transport} was 0.0009 $\mu\text{L}/\text{min}$. PSB finally estimated to 36.84 L/h and PSC was 3.7 L/h. fuCSF was examined and equaled to 0.72. Systemic exposure of icotinib predicted by PBPK modeling based on Jin's population data could well fit the observed data. Differences of parameters including AUC, C_{max}, CL and T_{max} between observed data and predicted data were less than 2 folds. Cerebrospinal profiles also can be well predicted and fitted the observed data in different doses group. From the parameters, we found that AUC_{ss} in cerebrospinal was 1/8 fold of that in plasma, which showed it is easy to come into the brain and worth to use in treatment of metastatic brain tumor.

Conclusion PBPK model was successfully developed to describe the icotinib pharmacokinetic profile and parameter of systemic and cerebrospinal exposure in Chinese NSCLC patients, which can be used in future to make simulation under the different physiological conditions

POSTER ABSTRACT

#106

Is Incidence of Skin Rash Driven By Peak Concentration or Wild Type EGFR IC50 Cover By Pharmacokinetics For EGFR-TKIs?

Kan Chen, Li Zheng, Jessie Xu, and Pamela Yang, Zack Cheng Asia and Emerging Markets
iMed, AstraZeneca, Shanghai, China

Abstract: The development of small molecular inhibitors against Epidermal Growth Factor Receptor (EGFR) leads to new therapy options for several common malignancies.[1] Patients with activating mutations showed selectivity and clinical benefits, however for currently approved EGFR-TKIs skin rash affecting 65% patients (and up to 90% for afatinib) are thought to be induced by direct EGFR inhibition. This hypothesis is supported by the observation that EGFR inhibition leads to expression of chemokines and consequent skin inflammation through leukocyte recruitment, vascular dilation and edema.[2-3] Skin rash is a main side effects of erlotinib therapy at 150 mg daily oral dose, and all grade rash occurring 33-79% and >grade 3 rash occurring 3-10%. Clear PK/PD and PK-rash/outcome relationship to erlotinib was demonstrated in preclinical animal models at a clinically relevant dose. Investigation of skin rash incidence in preclinical animals of EGFR inhibitors is a critical assessment of safety margins and projection of dose limiting toxicity (DLT) in Phase I clinical trial. Four compounds were tested in han wistar rats with diversified pharmacokinetic properties and varied margins of inhibitory potency (IC50) between wild type EGFR (EGFRwt) and activating mutant EGFR. Incidence of rat skin rash with Cmax or pharmacokinetic coverage was systemically evaluated, indicating there was a clear co-relationship between incidence of skin rash and PK cover against EGFRwt IC50. All the data supports that longer PK cover against EGFRwt IC50 is likely to develop higher grade skin rash. Based on in vitro EGFRwt inhibition data and clinical human PK, a maximum tolerated dose due to DLT for >grade 3 skin rash could be projected.

POSTER ABSTRACT

#107

Population Pharmacokinetic/Pharmacodynamic/Survival Modeling Analysis of Icotinib in Chinese Non-Small Cell Lung Cancer Subjects

Jia Chen, Dongyang Liu, Qian Zhao, Ji Jiang, Pei Hu.

Phase I Unit, Clinical Pharmacology Research Center, Peking Union Medical College Hospital and Chinese Academy of Medical Sciences, Beijing, China

Objective Icotinib is a potent small-molecule inhibitor of epidermal growth factor receptor (EGFR)-tyrosine kinase (TKI), which was designed for the treatment of non-small cell lung cancer (NSCLC). It is the first novel anti-cancer drug developed by Chinese pharmaceutical industry and has been approved to market in China in 2009. Up to date, the influence factor affecting PK profile in Chinese non-small cell lung cancer (NSCLC) patients was not well characterized, and the relationship of icotinib and tumor shrinkage was also not quantitatively described. In order to make clear on relationship and influence factor of PKPD/survival of icotinib, we developed a PKPD/survival model to describe pharmacokinetics and tumor size evolution in patients.

Methods The icotinib PK concentration data from 212 patients was received from two phase I studies and one phase III study over the dose range from 125mg tid to 625mg tid. PKPD model parameters were fitted sequentially using NONMEM (v7.2) interfaced with PIRANA and kinds of plots were conducted using R (v3.1.2). Firstly, one-compartment model with first order absorption and elimination as structure model was developed was constructed by FOCEI method. Kinds of PD model was tested and optimized. Then, we developed a model incorporating tumor-growth parameter K_{growth} , treatment-efficacy parameter K_{drug} , drug resistance-related parameter $K_{tolerance}$ and exposure-related parameter AUC_{SS} using PK parameters: $dTS/dT = K_{in} \cdot TS - K_{out} \cdot TS \cdot AUC_{SS} \cdot EXP(-K_1 \cdot T)$ when $TS(0) = \text{Baseline}$. Survival analysis constructed by conditional laplace methods using weibull distribution model for hazard ratio and survival distribution. Individual variability of PK was assessed using mixed model and PD was assessed using proportional model. Possible covariates were explored using forward stepwise method ($p < 0.05$) and backward stepwise method ($p < 0.01$). The final Pop-PK model was validated by visual predict check (VPC) method.

Results and Discussion The basic one-compartmental pharmacokinetic model was constructed to describe the inter-individual variability of icotinib in Chinese NSCLC patients. The typical value of CL, V and K_a were 11.2L/h (7%), 28.2L (21%) and $0.139h^{-1}$ (10%), respectively. Population pharmacokinetic analysis results showed that LBW and GGT were the influential covariate for clearance: the clearance increased when the LBW increased and the clearance decrease when GGT increased. As to PD model, typical value of K_{growth} , K_{drug} and $K_{tolerance}$ equaled to $8.48 \cdot 10^{-4} \text{ week}^{-1}$, $1.02 \cdot 10^{-3} \text{ week}^{-1} / AUC$ and $2.28 \cdot 10^{-3} \text{ week}^{-1}$ respectively. Scale factor LAM and shape factor SHP of survival model were 0.0017 and 1.95 respectively. Stepwise method was introduced to be found that baseline of tumor size affected scale factor and AUC_{SS} affected shape factor. Individual plots exhibited that the model can describe the tendency of tumor change. Final model was evaluated that the model can fit most observed data by GOF plots and verified by the VPC plots.

Conclusion The pop-PK model can acceptably captured the pharmacokinetics behavior of icotinib and discovered source and extent of variabilities of pharmacokinetic character in Chinese NSCLC patients. The developed pop-PD model can basically describe the change of tumor size while icotinib administration. And the survival model was proved by VPC to be used in simulation with the predictors: Tumor baseline and AUC_{SS}

Quantitative determination of Nucleoside Triphosphate in Rat liver using liquid chromatography–tandem mass spectrometry

Wenqi Cui¹ and Luke Bi²

1. Department of Drug Metabolism 2. Department of Bioanalytical Chemistry, Covance Pharmaceutical R&D (Shanghai) Co., Ltd., Building #3, No.3377 Kangxin Road, SIMZ Pudong, Shanghai 201318, China

Nucleoside analogs have been widely used as antiviral and oncology treatment. This class of drug goes through sequential cellular activation to form active moiety; nucleoside triphosphate; which terminates the DNA and RNA chain elongation. A sensitive and selective LC-MS/MS method was developed for the quantitative analysis of Nucleoside Triphosphate in rat liver tissues. The tissue exposure level is correlated to the therapeutic efficiency of nucleoside-analog drugs. Compared with other small molecules, Nucleoside Triphosphate poses significant challenges to bioanalysis, mainly due to very high polarity, poor stability and presence of other form of nucleoside analogs, such as mono-phosphate and di-phosphate and other endogenous nucleotides.

In order to determine nucleoside triphosphate, the liver tissue samples were extracted with solid phase extraction (SPE) using a weak anion-exchange (WAX) 96-well plate to remove, nucleoside analog, Mono-, Di- phosphates first; nucleoside triphosphate fraction was eluted from SPE and subsequently convert to the corresponding nucleoside analog through dephosphorylation by alkaline phosphatase; and lastly analysed by tandem mass spectrometry (MS/MS) detection in negative ion multiple reaction monitoring (MRM) mode. A sensitive method was validated to achieve a lower limit quantitation (LLOQ) of 10.0 ng/mL, which is corresponding to 50.0 ng/g tissue for Nucleoside Triphosphate with a linearity of 50.0 - 25000 ng/g ($r^2 > 0.99$). This method was successfully used in the analysis of nucleoside triphosphate in liver tissues and was effective in the compound selection and development for hepatitis C program.

POSTER ABSTRACT

#109

Application of a Novel Sample Preparation Method in Pharmacokinetics Research About Total and Free Paclitaxel Among Solid Tumor Patients After Intravenous Infusion of Paclitaxel LiposomeXinge Cui¹, Xin Zheng¹, Yiming Zhang², Xinxiang Zhang², Ji Jiang¹

1. Peking Union Medical College Hospital 41 Damucanghutong, Xicheng, Beijing, 100032, China

2 Peking University. 5 Yiheyuan Road, Haidian, Beijing, 100871, China

Object:

In this report, we compared the pharmacokinetics of total paclitaxel and free paclitaxel in plasma from solid tumor patients after infusion of paclitaxel liposome.

Method:

We developed and validated a sensitive and specific LC-ESI-MS/MS method to determine the concentration of total paclitaxel and free paclitaxel in plasma. The preparation of plasma samples containing total paclitaxel was performed by a liquid-liquid extraction method, while plasma samples containing free paclitaxel were prepared by a novel SPE method with columns made by our own lab. Both of the two preparation methods used docetaxel as internal standard. Analytes were separated on a BEH C18(1.7 μ m, 2.1 \times 50mm) column with a flow rate of 0.4m/min and detected by a triple quadrupole tandem mass spectrometer equipped with electrospray ionization (ESI) source in MRM mood. The detection limits for free paclitaxel and total paclitaxel were 2-2000ng/mL and 5-5000ng/mL, respectively.

Result:

The results of entrapment rate testing of 100% entrapped paclitaxel liposome by SPE columns made in our own lab and commercial SPE columns under the same condition were 97% and 75%, which indicated that SPE columns made in our lab are more selective and specific for free paclitaxel compared with commercial SPE columns. This validated method was successfully applied for the pharmacokinetic study concerning about paclitaxel liposome. Six solid tumor patients were divided into two groups receiving single dose of paclitaxel liposome 135mg and 175mg via intravenous infusion, respectively. We determined the concentration of total paclitaxel and free paclitaxel in plasma from these six patients by this validated method. The main pharmacokinetic parameters for total paclitaxel in dose group 135mg and dose group 175mg of paclitaxel liposome are as follows: the areas under the plasma concentration-time curve ($AUC_{0-\infty}$) are 10530.2 \pm 1296.1 h*ng/mL and 29461.4 \pm 2847.5 h*ng/mL, respectively; the plasma elimination half-life ($t_{1/2}$) are 50.0 \pm 8.2 h and 44.7 \pm 9.1h, respectively; the apparent volumes of distribution (V_z) are 933786.6 \pm 186794.6mL/m² and 381541.8 \pm 62093.9mL/m², respectively. The main pharmacokinetic parameters for free paclitaxel in dose group 135mg and dose group 175mg of paclitaxel liposome are as follows: the areas under the plasma concentration-time curve ($AUC_{0-\infty}$) are 4807.6 \pm 571.0 h*ng/mL and 6039.1 \pm 634.0 h*ng/mL, respectively; the plasma elimination half-life ($t_{1/2}$) are 31.0 \pm 2.5 h and 28.3 \pm 8.0 h, respectively; the apparent volumes of distribution (V_z) are 1270144.0 \pm 208656.9 mL/m² and 1206628.45 \pm 460895.8 mL/m², respectively.

Conclusion:

By the novel free paclitaxel preparation method we developed, we determined the plasma concentration of free paclitaxel after infusion of paclitaxel liposome for the first time. It was confirmed that there exists approximately 45.7% free paclitaxel and 20.5% free paclitaxel in plasma from solid tumor patients after infusion of paclitaxel liposome 135mg and 175mg, respectively. The circulation exposure of total paclitaxel was less than free paclitaxel at about 11 hours post-dose.

POSTER ABSTRACT

#110

Characterization of a Highly Selective Ratiometric Two-Photon Fluorescent Probe For Human Cytochrome P450 1A and its Biological ApplicationsZi-Ru Dai,^{‡^a} Lei Feng,^{‡^{a,b}} Guang-Bo Ge,^{‡^{a,b}} Jing Ning,^a Liang-Hai Hu,^c Dan-Dan Wang,^a Xia Lv,^a Jing-Nan Cui^{*^b} and Ling Yang^{*^a}^a Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China. E-mail: yling@dicp.ac.cn^b State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian, 116024, China^c Research Center for Drug Metabolism, College of Life Science, Jilin University, Changchun, 130012, China

Human cytochrome P450 1A (CYP1A), one of the most important phase I important drug-metabolizing enzymes in human, plays a significant role in metabolism of therapeutic drugs and activation of environmental contaminants or other xenobiotics to toxic or carcinogenic species [1]. By far, most of CYP1A fluorescent probes were intensity-based and work with one photo microscopy, which may be disturbed in quantitative detection by many factors [2]. Therefore, a more simple, sensitive and practicable method based on fluorescence probe for CYP1A is highly desirable. Herein, a ratiometric two-photon fluorescent probe (**NCMN**) for the selective and sensitive detection of CYP1A has been designed, synthesized and fully evaluated. **NCMN** can be selectively dealkylated by CYP1A, accompanied with the remarkable changes in absorption and fluorescence spectra (red-shifted emission 114 nm), allowing the naked-eye visible and fluorescence analysis. Reaction phenotyping and chemical inhibition assays demonstrate that **NCMN** is highly selective for CYP1A over other human CYPs. Meanwhile, the probe also exhibits good reactivity and ideal kinetic behaviors towards CYP1A in human biological samples. **NCMN**-O-demethylation in both HLM and CYP1A displayed high affinity ($K_m < 12 \mu\text{M}$) and good reactivity ($k_{cat}/K_m > 1.0 \text{ nmol}\cdot\text{min}^{-1}\cdot\text{nmol}^{-1}$ CYP). The probe has been applied successfully for real-time monitoring the real activities of CYP1A in complex biological systems, and for rapid screening CYP1A inhibitors by using tissue preparation as enzyme source. Furthermore, the two-photon excited fluorescence properties make this newly developed probe can serve as a powerful tool for bio-imaging of endogenous CYP1A in living cells and exploring the related biological functions.

Keywords: Two-photon ratiometric probe; CYP1A; **NCMN**-O-demethylation**References:**

- [1] D.L.Eaton, E.P.Gallagher, T.K.Bammler, K.L.Kunze, Role of cytochrome P4501A2 in chemical carcinogenesis: implications for human variability in expression and enzyme activity, *Pharmacogenetics* 5(5) (1995) 259-274.
- [2] Huang,C.S, Jia.T, Tang, M.F, Yin, Q, Zhu, W.P, Zhang, C, Yang, Y, Jia,N.Q, Xu, Y.F, Qian, X.H, Selective and Ratiometric Fluorescent Trapping and Quantification of Protein Vicinal Dithiols and in Situ Dynamic Tracing in Living Cells, *J Am Chem Soc* 2014, 136, 14237.

POSTER ABSTRACT

#111

Comparative Disposition of Intravenous Catechols, Derived from *Salvia Miltiorrhiza* Roots (Danshen), in Rats and Mechanisms Governing Their Systemic Exposure**Jiajia Dong¹, Weiwei Jia¹, Meijuan Li¹, Feifei Du¹, Fang Xu¹, Fengqing Wang, Chuan Li^{1,2}**¹Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China; ²*Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China*

Danshen catechols, the pharmacologically active hydrophilic components in the cardiovascular herb *Salvia miltiorrhiza* (Danshen). Except for tanshinol and protocathechuic aldehyde, the other Danshen catechols, such as salvianolic acids A, B, and D, rosmarinic acid, and lithospermic acid, were either poorly absorbed from the gastrointestinal tract or extensively eliminated. Intravenous administration is a major way of Danshen catechols taking effects. We investigated the disposition and mechanisms governing their systemic exposure of these Danshen catechols following intravenous administration to rats. Due to the highly hydrophilic and organic anion nature of Danshen catechol compounds, their distribution in the body is highly dependent on the presence of appropriate uptake transporters. We examined the role of OATPs and OATs in the transport of Danshen catechols in the HEK 293 cells after transfection. On the other hand, we also investigated the possible herb-drug and herb-herb interactions associated with Danshen catechols and found that Danshen catechols interact very weakly with OATP1B1, OATP1B3, OAT1, and OAT3 at physiological concentration.

POSTER ABSTRACT

#

**Single and Multiple Dose Pharmacokinetics,
Pharmacodynamics and Safety of Darapladib (a novel Lp-PLA₂
enzyme inhibitor) in Healthy Chinese Subjects**

Chaoying Hu¹, Debbie Tompson³, Mindy Magee⁴, Qian Chen¹, Yanmei Liu¹, Wenjing Zhu², Hongxin Zhao², Annette Gross⁵, Yun Liu^{1*}

Affiliations: ¹Phase I Clinical Research Unit, Shanghai Xuhui Central Hospital, Shanghai, China; ²GlaxoSmithKline (China) R&D Company Limited, Shanghai, China; ³GSK Medicines Research Centre, UK; ⁴Clinical Pharmacology Modeling and Simulation, GlaxoSmithKline, King of Prussia, PA, USA ⁵Ethnopharmacology, GlaxoSmithKline, Sydney, Australia

***Corresponding Authors:**

Yun Liu, Phase I Clinical RU, Shanghai Xuhui Central Hospital, 966 Middle Huaihai Road, Shanghai 200031, China. Email: yliu@shxh-centerlab.com Phone: +86 21 31270810-63014 Fax: +86 21 54036058

Debra Tompson, GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK
Email: debra.j.tompson@gsk.com Phone +44 1438 766698 Fax: +44 1438 762798;

Background and Objectives

Darapladib is a lipoprotein-associated phospholipase A₂ (Lp-PLA₂) inhibitor. This study evaluated pharmacokinetics, pharmacodynamics and safety of darapladib in healthy Chinese subjects.

Methods

Twenty-four subjects received darapladib 160 mg orally, approximately 1 hours after a standard breakfast, as a single dose and once daily for 28 days. Non-compartmental methods were used to determine the single and multiple dose pharmacokinetics of darapladib and its metabolite SB-553253. Repeat dose Lp-PLA₂ activity and safety were evaluated.

Results

Systemic exposure (AUC₍₀₋₇₎, C_{max}) of darapladib was higher after multiple-dosing (519 ng.h/mL, 17.9 ng/mL) compared to single-dose administration (153 ng.h/mL, 34.4 ng/mL). The steady-state accumulation ratio was less than unity (R_s=0.80), indicating time-dependent pharmacokinetics of darapladib. Steady-state of darapladib was reached by Day 14 of once daily dosing. Systemic exposure to SB-553253 was lower than darapladib with median (SB-553253 : darapladib) ratios for AUC₍₀₋₇₎ of 0.0786 for single dose and 0.0532 for multiple dose administration.

On Day 28, pre-dose and maximum inhibition of Lp-PLA₂ activity were approximately 70% and 75%, respectively. The most common adverse events (≥ 21 % subjects) were abnormal faeces, abnormal urine odour, diarrhoea and nasopharyngitis.

Conclusion

In healthy Chinese subjects single dose systemic exposure to darapladib was consistent with that observed previously in Western subjects whereas steady-state systemic exposure was approximately 65% higher in Chinese than Western subjects. The pharmacodynamic activity and adverse event profile were similar in healthy Chinese and previous reports in Western subjects. Ethnic-specific dose adjustment of darapladib is not considered necessary for the Chinese population.

Investigation on ISR failure and the subsequent solutions to overcome the assay variability

Liang Huang, Covance (Shanghai)

Incurred sample reanalysis has been a regulatory requirement for studies with pharmacokinetics as end point. ISR test has been implemented in bioanalysis since the publication of the AAPS/US FDA Bioanalytical Workshop of 2006. ISR test is very effective way to evaluate the reliability of the data and assay performance.

Indeed, a successful assessment of ISR gives bioanalytical scientists confidence in the analytical method and the associated bioanalytical results; whereas a failed ISR may requires an immediate investigation to determine root causes for the variability of the assay. This poster will summarize the ISR failures we encountered in the past few years; discussed the investigation process to identify the root causes of ISR failures and the subsequent solutions to overcome the assay variability.

The root causes for the ISR failures range from metabolite insource fragmentation converting into analyte (Compound A), metabolite conversion into parent drug in the presence of methanol (Compound B) during extraction, to low recovery of the analyte with analog internal standard. In all three cases, the method was developed and validated with analyte. The method performance issue was identified when analyzing study samples in which the metabolites were present. These assays were modified to overcome the assay variability. The modified assays were used in study samples reanalysis and ISR test successfully. All three cases illustrated the importance of the method development with the consideration of metabolites, and also the value in ISR test.

What Quality of a Commercial Kit (Immunoassay) Needed —Key parameters in a method for quantitative determination of target protein

XR Li, XJ Wang, TY Zhang, B Li*, Frontage Laboratories (Shanghai) Co., Ltd

Quality, ease of use, short processing time, and costs are the main characteristics of consideration when choose a commercial kit. Of these, high quality is the most important element. High quality can be demonstrated in parameters such as sensitivity, dynamic range, P&A, specificity/cross reactivity, selectivity, matrix effect, MRD, stability, robustness, standard curve regression model etc. They are key parameters for a commercial kit to determine protein concentrations (drug, antibody or biomarker) in preclinical/clinical studies (TK/PK/PD/BE). Based on our experiences, sometimes it is difficult to find a right commercial kit for determination of a target protein. For example, from package insert of a commercial kit, an assay seems not bad, but kit producer does not supply enough data to show that the kit could meet requirements.

Requirements on several parameters are discussed briefly below:

1. Sensitivity and dynamic range

Sensitivity and dynamic range are two fundamental parameters for a commercial kit. A good one should be capable of measuring the lowest target level of samples for a study. The basic information, such as physiological concentrations of biomarkers in related species, drug concentrations in PK/TK studies, and the trends (up-/down regulation) of target protein concentration after treatments should be considered during a method development. Even If sensitivity of an immunoassay is good enough to measure physiological level of a biomarker, it might not be sufficient when the biomarker is down-regulated after a treatment. On the other hand, it is not always true that the higher sensitivity, the better for an immunoassay. Too sensitive assay could be prone to errors and problems due to very low concentration and extra dilution step. Suitable sensitivity and dynamic range is desired when we consider a commercial kit.

2. QCs and P&A

P&A could be the next parameters we search in package insert of a commercial kit. For a preclinical/clinical study, P&A data of ULOQ, High, Mid, Low and LLOQ QCs are required during a method validation. In sample analysis, at least three QC levels, High, Mid and Low QCs, are required. HQC within 75% of ULOQ and LQC within 3×LLOQ concentration levels, relative Error (RE) within ±20~±25% and coefficient of variation (CV) within 20~25% for QC samples (intra-/inter-plate P&A) are normally required for an immunoassay. Most of commercial kits were produced for R&D purpose, there were no QC data supplied or QC concentrations not at appropriate concentration levels, which can be a concern in a regulated study. We normally look into the data in the list of several samples spiked with high/mid/low concentrations of target protein in a package insert to evaluate RE and CV. So we expect that the concentration levels of spiked samples are close to the range of High QC, Mid QC and Low QC under certain dynamic range.

A commercial kit with good sensitivity, dynamic range and P&A are not enough. More parameters, as aforementioned, will need to be evaluated and tested before a commercial kit can be used in bioanalysis to support a regulated preclinical/clinical study.

*Bo Li, Corresponding author; Email: boli@frontagelab.com.cn
Tel: +86 (21) 50796566 Ext 306; Mobil: +86 152 1688 8692

POSTER ABSTRACT

#115

Adaptation of An *in Vitro* Model for Assessing the Reactivity of Acyl Glucuronides in Drug Discovery

Jerry Li, Kan Chen, Yumei Yan, Zhen Zhang, Bonnie Zhang, Jinqiang Zhang, Zack Cheng*DMPK , Asia & Emerging Markets iMed, AstraZeneca, Shanghai, China***Abstract**

Carboxylic acid containing drug molecules that are known to form acyl glucuronides (AGs), which will cause the irreversible covalent binding to protein target and probably leading to potential toxicity. The purpose of this study is to adapt a screening model for the reactivity of acyl glucuronide, to assess degradation and isomerization kinetics of AGs. AGs from corresponding carboxylic acids were produced in the formation phase of the assay with the UGT enzymes present in liver microsomes. The degradation and isomerization half lives were calculated based on data from every time point at degradation phase. The AG isomers was chromatographically separated by UPLC using gradient elution mode. Zomepirac was used as the positive control whose degradation and isomerization half lives were calculated to be 21.3 and 36.5 min, respectively. Application results indicated that three test AZ compounds assessed by this model undergone AGs, whereas no subsequent isomerization and degradation during the degradation phase thus demonstrating AGs of the test compounds were stable under the current conditions.

POSTER ABSTRACT

#116

A Semi-Mechanistic Model to Describe FSIGT Data in Obese Chinese People

Yang Liu, Dongyang Liu, Ji Jiang, Pei Hu

Phase I Unit, Clinical Pharmacology Research Center, Peking Union Medical College Hospital and Chinese Academy of Medical Sciences, Beijing, China

Objectives: An insulin-glucose system reciprocal feedback model updating from minimal model was developed to describe the insulin-modified frequently sampled intravenous glucose tolerance test data. This model aims at finding more reliable pharmacodynamics parameters after diagnostic test or administration of anti-diabetic drugs, and to quantify disease progression in Chinese people.

Methods: The dataset applied to establish the model was derived from Chinese obese subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes mellitus (T2DM). 220 subjects underwent 75g oral glucose test (OGTT) and insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT). Then the feedback model was used to capture the glucose and insulin kinetic characteristics using ADAPT 5 and Berkeley Madonna. The indirect response model was applied to describe the plasma glucose and insulin production, elimination, and interaction. The increase of insulin concentration stimulated the utilization of glucose. Similarly, the increase of plasma glucose concentration stimulated the secretion of insulin. Finally, we could estimate the related parameters of pharmacodynamics and disease characteristics.

Results: The feedback model can successfully capture the characteristics of the data and obtain the suitable parameters. At the same time, the CV% of the parameters was small.

Conclusions: A semi-mechanistic minimal model was established to quantitatively explore disease progression in Chinese T2DM people.

Keywords: Type 2 diabetes mellitus (T2DM); semi-mechanism model; FSIGT; Chinese obese subjects

POSTER ABSTRACT

#117

Colonic Metabolites of Flavonols in Rats and Dogs after Oral Administration of GBE50, A Standardized Ginkgo Biloba Leaf Extract

Xinwei Liu, Shanghai Institute of Materia Medica

The study characterized the metabolic profile of the ginkgo flavonols produced from microbial reductive metabolism and PK properties of the reduction products in rats and dogs after orally administration of GBE50.

POSTER ABSTRACT

#118

Human Cytochrome P450 Enzymes and Hepatic Microsomal Thiol Methyltransferase Involved in Stereoselective Formation and Methylation of Pharmacologically Active Metabolite of Clopidogrel

Cai Liu, Xiaoyan Chen, Dafang Zhong*
Shanghai Institute of Materia Medica, Shanghai, P. R. China

Abstract

Clopidogrel is a thienopyridine antiplatelet prodrug widely used for management and prevention of cardiovascular and cerebrovascular thrombotic events. Clopidogrel is first oxidated to 2-oxo-clopidogrel, followed by metabolism to the pharmacologically active thiol metabolite which can consist of four isomers (H1-H4) *in vitro*. After oral administration of clopidogrel to humans, only H3 and H4 are observed in plasma with similar exposure levels, and only H4 is active in humans. The present study aims to investigate the mechanism of stereoselectivity in the formation and S-methylation of H3 and H4 *in vitro*. The two diastereomers of 2-oxo-clopidogrel were epimerized rapidly at physiological pH. In human liver microsomes, H3 and H4 were further S-methylated and the S-methylation was inhibited by 2,3-dichloromethyl benzylamine indicating the involvement of thiol S-methyltransferase. The intrinsic clearance (CL_{int}) for H3 formation from 2-oxo-clopidogrel in human liver microsomes was 3.1-fold higher than that for H4 formation, indicating stereoselectivity of metabolism. Results from kinetic studies using human cDNA-expressed CYP450s demonstrated that the contribution to the formation of the active H4 from 2-oxo-clopidogrel followed the order of CYP3A4 > CYP2C19 > CYP2B6 > CYP2D6 > CYP2C9 > CYP3A5. The CL_{int} values for H3 formation from 2-oxo-clopidogrel by CYP2B6, CYP2C9, and CYP3A4 were 2.2-, 1.7-, and 1.7-fold higher than that for H4 formation, respectively, whereas the CL_{int} values for H3 and H4 formation by CYP2C19 and CYP3A5 were similar. However, the CL_{int} value for H4 formation by CYP2D6 was 3.5-fold higher than that for H3 formation. The CL_{int} value for the S-methylation of H3 in human liver microsomes was 98.1-fold higher than that for H4. The more stereoselective formation of H3 from 2-oxo-clopidogrel and the stereoselective S-methylation of H3 may lead to the previously reported similar exposure levels of H3 and H4 in humans.

High Sensitivity Bioanalysis for Small Drug-Like Compounds in Human Plasma using Microflow LC and High Resolution Mass Spectrometry

Yun Alelyunas, Mark Wrona, Jim Murphy, Kelly Doering

Introduction:

Bioanalysis to support PK-PD studies and clinical trials can be challenging especially when quantifying highly potent compounds. In this study, quantitation attributes were determined for a set of drug-like compounds in human plasma using microflow LC and high resolution mass spectrometry (HRMS) with ionKey/MS and the Xevo G2-XS Time of Flight (ToF) mass spectrometer. The iKey is a microflow separation device that is fitted into the source of Xevo family of mass spectrometers. Xevo-G2XS QToF is the latest QToF instrument with enhanced resolution and sensitivity compared to its predecessor. The combined use of both technologies yields an extremely sensitive LC/HRMS instrument. Addition of a trap-and-elute configuration enables the system to handle analytical scale sample volume injections while maintaining excellent peak shape, which further enhances the system's sensitivity.

Methods:

Test compounds, including buspirone, propranolol, verapamil, and clopidogrel were prepared in human plasma. The LC/MS system used was the AQUITY M-Class UPLC system, coupled with the Xevo G2-XS QToF mass spectrometer and the ionKey/MS source. The iKey tile used was the HSS T3 1.8 μ 150 μ m x 50 mm at 45°C. The trap column used was the HSS T3 1.8 μ 300 μ m x 50 mm. A generic, linear gradient from 5-60 B% in 3.5 minutes at flow rate of 3 μ L/min was used (mobile phase A was water + 0.1% formic acid; mobile phase B was acetonitrile + 0.1% formic acid (v/v)).

Results:

Serially diluted samples of test compounds in human plasma were quantified via direct injection onto the LC/MS system using ToF-MRM mode of acquisition. Results showed excellent linearity ranging from log = 3.6 to 4.2 for verapamil and clopidogrel, respectively. LLOQs ranged from 0.8 fg to 3.0 fg on column. The signal/noise ratio at LLOQ ranged from 9 to 79. These attributes suggest the system is well-suited to meet the needs of routine bioanalysis. In the second set of experiments, a trap valve manager was installed, and the system was configured for trap-and-elution. Increasing injection volumes from 1 to 5, 10, and 20 μ L showed excellent peak shape and peak resolution. A linear response with $R^2 = 0.9987$ was observed, indicating complete sample recovery using the trapping column. For a 20 μ L injection of samples containing 20% acetonitrile, which is equivalent to a 4 mL injection at analytical scale using a 50 x 2.1 mm column, polar compounds such as buspirone and propranolol were well-retained and showed no peak distortion. These data suggest the microflow LC/MS system is well-suited to support routine bioanalytical sensitivity requirements. Additional benefits for using the system include ease-of-use and a 90% reduction in solvent usage compared with analytical LC.

Novel aspect:

Novel aspects of this paper include the use of microflow LC/HRMS and dual pump trap-and-elute for high sensitivity small molecule quantitation in human plasma at analytical scale injection volumes.

Evaluation of Drug-induced Hepatotoxicity with Five Biomarkers in Sandwich Cultured Rat Hepatocytes

Na Long, Suxing Zhang, Hong Yu, Lingmei Zhi, Feipeng Zhang, Enya Li, Kezhi Zhang and John J. Baldwin

CarysBio Holdings Limited, Foshan, Guangdong Province, PRC

Purpose: to evaluate drug-induced hepatotoxicity in sandwich cultured rat hepatocytes and explore the biomarkers to evaluate drug-induced hepatotoxicity in vitro.

Methods: fifteen compounds including compounds known associating with liver injury (such as Cyclosporin A, Labetolol, Diclofenac, Sorafenib, Chlorpromazine, Verapamil, Benzbromarone, Acetaminophen) were tested with sandwich cultured rat hepatocytes (SCRH) and following points were investigated: (i) concentration-dependent effect of drug-induced hepatotoxicity; (ii) effect of culture time of SCRH on drug-induced hepatotoxicity evaluation; (iii) effect of treatment time of test compounds on drug-induced hepatotoxicity evaluation in SCRH; (iv) five bio-markers including the leakage of ALT, AST, LDH and ALP and the generation of urea to evaluate hepatotoxicity; (v) correlation of 5 biomarkers in hepatotoxicity evaluation. Briefly, on day-0, fresh rat hepatocytes were isolated from male SD rat based on a two-step collagenase perfusion method and seeded on 48-well plate pre-coated with rat tail collagen at density of 120,000 viable cells/well. After 4 hour incubation, the medium was discarded and overlaid with ice cold BD Matrigel™ to form 2-layer configuration and bile canaliculi network. The hepatotoxicity study was performed on day-1 and day-3, when cells were treated with test compounds at different concentrations for 24 hours. After 24h-treatment, pipetted the supernatant media to determine the leakage of AST, ALT, LDH and ALP. And then incubated the cells with HBSS supplemented with ornithine and ammonium chloride for another 1 hour to determine the urea generation after rinsing twice with regular HBSS. The effect of treatment time of test compounds on hepatotoxicity was studied in day-1 SCRH with 3 days continuous incubation and evaluated with urea generation.

Result: The results demonstrate that sandwich cultured rat hepatocytes was a useful tool to evaluate drug-induced hepatotoxicity and five bio-markers could be used as the evaluation indicators. And culture time of SCRH will affect the drug-induced hepatocytes evaluation which may be caused by the change of enzymes and transporters expression as reported. And drug-induced hepatotoxicity will be aggravated with the treatment time of drugs in SCRH.

Conclusion: The sandwich cultured rat hepatocytes are an excellent model to characterize drug-induced hepatotoxicity and urea generation is a good and sensitive biomarker in hepatotoxicity evaluation.

Applying LC/MS Natural Product Application Solution with UNIFI for the Identification of the Chemical and Herbal Components from an Unknown Traditional Chinese Medicine Product

Lirui Qiao, Waters Corporation

The critical foundation for Traditional Chinese Medicine (TCM) effectiveness originates from the chemical ingredients of the raw herbal materials. It is very difficult to identify chemical ingredients and deduce possible herbal composition for complete unknown products, especially the available sample background information is close to none. Here, we present a novel workflow that enables researchers to quickly identify chemical ingredients and deduce possible herbal content from unknown TCM product from a single LC/MS injection. The informatics platform processing result shows that the unknown sample commercially named XinKeShu, which contains DanShen, SanQi, GeGen, ShanZha, and MuXiang.

Extraction of Telmisartan from Human Plasma Utilizing Cleanert SLE Plate with High Capacity

Suzi Qin¹, Wan Wang¹, Linsen Li¹

¹ Bonna-Agela Technologies, Tianjin 300462, China

ABSTRACT

This research has established a method for the determination of telmisartan in human plasma by employing Cleanert SLE Plate for sample purification. The plate was packed with 600mg of diatomaceous earth, allowing a maximum load volume to 600 μ L which was larger than that of normal commercial SLE plate. The recovery for telmisartan was ranged from 83.6% to 98.9% with RSD less than 4.5%. Linearity checks across the full concentration range were good. LOD of telmisartan was 0.1ng/mL.

KEY WORDS

Telmisartan; Human Plasma; Cleanert SLE Plate; LC-MS/MS



POSTER ABSTRACT

#123

Ease-of-use and Sensitivity with Nanospray: An Integrated Chip-Based Solution Incorporating Column Temperature Control, Long Columns, and Nebulization.

Helena Svobodova; Amanda Berg, Peter Wang, Gary Valaskovic

New Objective, 2 Constitution Way, Woburn, MA 01801 USA

New Objective Shanghai Instrument Company, 789 ZhaojiaBang Rd Shanhai 200032 PRC

Packed-tip columns have successfully enabled nanoflow LC-MS applications by delivering optimal chromatographic performance at nanoflow rates. Assembling a high-performance system is at present time the domain of the expert user. Challenges in pre- and post-column plumbing, sample preparation of complex matrices, and mass spectrometer tuning requires training and persistence in method development. Incorporating short (10 cm) or long (25 cm) 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 a heated and pneumatically enabled modular "docking station" source platform expands the functional application window, delivering a wider range of operating flow rates (100 nL/min. to 10 μ L/min.) and column temperature (40 to 60 C) 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 column heating is investigated for 75 μ m ID and 150 μ m ID columns.

Development and Validation of an HPLC-MS/MS Method for Determination of Suramin in Monkey Plasma

Ying Han, Lili Xing, Weiqun Cao, Wenzhong Liang, Yi Tao*, Xin Zhang

WuXi AppTec Co., Department of DMPK/Non-GLP Bioanalytical Service, 90 Delin Rd, Waigaoqiao Free Trade Zone, Shanghai, P.R. China, 200131

Abstract:

Suramin was originally used as an anti-parasitic drug against African sleeping sickness. Later, this drug was tested as a reverse transcriptase inhibitor to treat AIDS patients. Subsequently, it was evaluated as an anti-cancer drug and multiple clinical trials have been conducted to test suramin for treatment of prostate cancer, renal cancer, and ovarian cancer, either given alone or in combination with other chemotherapeutic agents.

The concentrations of Suramin in preclinical or clinical biological samples were mostly determined using HPLC-UV and no LC-MS/MS method has been reported previously. Suramin is a highly hydrophilic and ionic drug due to the presence of six sulfonic groups. Under conventional reverse phase condition, suramin is eluted at void volume. In addition, serious protein binding and low extraction recovery from bio-matrix were observed during LC-MS/MS analysis.

In present study, a rapid, specific, and reproducible ion-pairing reversed phase LC-MS/MS method for the quantitative determination of suramin in cynomolgus monkey plasma has been developed. The dynamic range of the method is 0.1-20 µg/mL. By using ion-pairing agents, satisfactory peak shape and retention were achieved. The method was validated successfully for linearity, sensitivity, selectivity, within-run accuracy and precision, matrix effect, carryover, and recovery.

Novel Aspect:

The first reported LC-MS/MS method for the determination of Suramin in monkey plasma.

Preliminary Results:

Figure 1. Representative LC-MS/MS chromatogram of Suramin (0.1µg/mL) in Monkey plasma

POSTER ABSTRACT

#125

Intestinal Lymphatic Absorption into Consideration When Constructing Physiologically Based Pharmacokinetic Model (PBPK)

Xun Tao, Chempartner

Intestinal lymphatic absorption represents an absorption pathway alternative to the absorption via portal vein. However, when constructing the oral absorption model, the conventional Physiologically Based Pharmacokinetic Model (PBPK) generally takes the portal uptake into consideration, whereas the lymphatic absorption pathway is always absent. On the other hand, more and more compounds, particularly lipophilic ones, have been proven subjecting to the lymph uptake. Via reviewing the historical studies of these compounds, the lymphatic absorption scarcely presents in the aqueous based formulation, while much more significant in lipid based formulation. It is because that the intestinal lymphatic absorption is closely associated with drug partitioning into chylomicron, which is just digestion product of lipid. The absorption via lymph takes the significant advantages in terms of bypassing the intestine and hepatic first pass elimination, and consequently, enhancing the oral absorption of drug. Therefore absence of lymphatic absorption consideration may underestimate the oral absorption of these drugs when constructing PBPK modeling. Our study have selected several lymph uptake compounds, which are either metabolic unstable, or subjecting to the significant efflux according to in-vitro ADME data in reference. The in vivo PK data in reference basically have one IV group and two PO groups (aqueous based formulation vs lipid based formulation). Here we employ Berkeley Madonna as the modeling tool. The IV PK and metabolic stability data will be utilized for modeling to fit the hepatic extraction and tissue distribution. The PO PK (aqueous formulation) and permeability data will be utilized for modeling (without lymph uptake) to fit the absorption constant. And then the % of lymphatic absorption is estimated by chylomicron partitioning data. The simulated PO profile (lipid formulation) will be based on foregoing PO modeling, with additional lymph uptake drug directly into venous compartment. And ultimately, the goodness of fit will be determined by the comparison between simulated PO profile and authentic profile (lipid formulation).

PicoFuze: Integration enables high-flow Nanospray/Microspray with conventional LC-ESI-MS

Gary Valaskovic

New Objective Inc., 2 Constitution Way, Woburn MA 01801 USA

Chad Christianson, Jennifer Zimmer, Shane Needham

Alturas Analytics, Inc. 1324 Alturas Drive, Moscow, ID 83843 USA

Advances in genomics data, mass spectrometry, and separation science places mass spectrometry at a unique time, one poised to make significant and sustained contributions to fundamental life science and applied human health. Liquid chromatography-mass spectrometry (LC-MS) has experienced significant technical evolution, having established trends toward decreasing column diameter, lower flow rates, and packing particle sizes. State of the art "conventional" LC has evolved from 4.6 mm inside diameter columns (ID), operating at ca. 1 mL/min, to 1 – 2 mm ID columns, operating at less than 200 μ L/min. Even smaller micro- (0.2 – 0.3 mm ID) and nano-scale (< 0.2 mm ID) column formats, operating at 10 and 0.3 μ L/min respectively, have strong application specific roles, particularly when high sensitivity is required and/or sample volumes are strictly limited. We have developed a nano/micro bore LC column ("PicoFuze" technology, 200 or 250 μ m ID x 5cm length) integrated directly inside a conventional ESI electrode/probe assembly (SCIEX Turbo V). Advantages of this integrated approach include: ease-of-use, a new high-voltage contact with each column, control or elimination of pre-and post-column columns, a new ESI spray assembly with each column, and preservation of LC performance. Examples of this technology in the quantitative bioanalysis of small molecule drugs and targeted peptides/proteins in combination with triple-quadrupole mass spectrometry will be shown. This technology yields a sustainable sampling advantage, yielding the security of multiple injection volumes, an increase in analytical sensitivity, while preserving the culture of LC method development.

Aristolochic acid I associated target identified by a monoclonal antibody against aristolochic acid I and II using immunoprecipitation and LC-MS/MS

Dan Wang,^{†,‡} Xuan Wang,[‡] Ming-Ying Shang[†], Shao-Qing Cai[†]

[†]Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, P.R. China

[‡]Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, P.R. China Wangdankuaile800@126.com

Abstract: Aristolochic acid I (AA-I), a major constituent of the carcinogenic, is known to be nephrotoxic, carcinogenic and mutagenic. This study aimed to gain insight into the uptake and distribution of AA-I in human kidney cell line (HK-2) using an immunocytochemistry technique, and to identify possible intracellular binding partner using immunoprecipitation (IP) and LC-MS/MS, to provide more information about nephrotoxic effect of AA-I. After incubation of HK-2 cells with AA-I, primarily cytoplasm and nucleus staining were observed, especially the nucleus, which intensified with increasing exposure times. Immunoprecipitation of cells lysates (treated with AA-I) revealed binding of AA-I to a fraction of protein with a molecular weight about 100 kDa. Further analysis of the protein mixture via LC-MS/MS (LTQ Orbitrap Velos) resulted in the identification of the protein. The identity of this protein was confirmed using immunodetection with a specific antibody. To the best of our knowledge, this is the first report that this protein as possible target of AA-I in HK-2 cells.

Keywords: Immunocytochemistry; Immunoprecipitation; HK-2; Aristolochic acid

Comparison of Various Sample Pretreatment Methods for Monitoring Arachidonic Acid in Plasma

Wan Wang¹, Suzi Qin¹, Linsen Li¹

¹ Bonna-Agela Technologies, Tianjin 300462, China

ABSTRACT

This poster presents a comparative study about 3 kinds of sample pretreatment processes to extract arachidonic acid from plasma, which involve conventional Protein precipitation 96-well plate, Cleanert PEP plate and Bonna-Agela Technologies, Inc.'s Cleanert MAS-M 96-well plate. Protein precipitation method enjoyed the convenience due to its minimum procedures, but its recoveries of arachidonic acid were 129.32%~149.02%, implying the bad purification effect which caused the matrix enhancement on mass spectrum. The recoveries of arachidonic acid on Cleanert PEP 96-well plate were 44.09%~70.15% which was insufficient, while the recoveries of Cleanert MAS-M 96-well plate were 99.19%~106.38, thus showing that it is an effective extraction product to support a rapid, high throughput assay of arachidonic acid analysis in plasma.

KEY WORDS

Arachidonic Acid; Plasma; Cleanert MAS-M 96-well plate; LC-MS/MS

POSTER ABSTRACT

#129

Characterization of Gomisin A as a Novel Isoform-Specific Probe for Selective Detection of Human Cytochrome P450 3A4

Jing-Jing Wu, Guang-Go Ge, Ping Wang, Zi-Ru Dai, Jing Ning, and Ling Yang

Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, 116023, Dalian, China.

Half of prescription medicines are metabolized by human cytochrome P450 (CYP) 3A. CYP3A4 and 3A5 are two major isoforms of human CYP3A, and share most substrate spectrum. There was no previous study distinguishing the activity of CYP3A4 and CYP3A5, making the trouble to predict CYP3A-mediated drug clearance and drug-drug interaction. In the present study, we introduced gomisin A (GA) with a dibenzocyclooctadiene skeleton as a novel selective probe of CYP3A4. Via LC-MS and NMR spectrums, 8-hydroxylated GA was identified as the predominant metabolite of GA. Multiple methods proved that CYP3A4 was the major isozyme involved, while contribution from CYP3A5 could be ignored. GA 8-hydroxylation in both recombinant human CYP3A4 and human liver microsomes followed the Michaelis-Menten kinetics. Intrinsic clearance values indicated that CYP3A4 contributed a dozen times higher than CYP3A5 on GA 8-hydroxylation (2.20 $\mu\text{l}/\text{min}/\text{pmol}$ CYP vs. 0.17 $\mu\text{l}/\text{min}/\text{pmol}$ CYP). In addition, in silico molecular docking studies showed a shorter proton-iron distance in GA-CYP3A4 than GA-CYP3A5 model, indicating that GA could orient itself better in the active site of CYP3A4 than CYP3A5. Furthermore, GA 8-hydroxylation was significantly inhibited by three structurally different CYP3A4 substrates: midazolam, nifedipine and bufalin. More importantly, GA has been successfully applied for real-time monitoring the modulation of CYP3A4 activities by the CYP3A4 inducer rifampin in hepG2 cells. Taken together, these results suggest that GA can be used as a novel probe to represent the catalytic function of CYP3A4 in various biological samples such as microsomes and living cell

POSTER ABSTRACT

#130

Evaluation of the Relative Bioavailability of Dabigatran Etexilate Mesylate in Beagle Dogs

Xiang Zhixiong, MA Wendi
(Central Research Institute, Shanghai Pharmaceuticals Holding Co., Ltd., Shanghai, 201203, China)

Objective: To evaluate the relative bioavailability of test formulations of dabigatran etexilate mesylate in beagle dogs and provide the reference for the clinical trial of dabigatran etexilate mesylate.

Methods: A pilot study was designed to estimate the possible effecting of gastric fluid pH on drug absorption. Six beagle dogs were orally dosed with dabigatran etexilate mesylate or dabigatran etexilate mesylate combined with pantoprazole sodium enteric-coated tablets in crossover design. After then, a randomized single dose crossover design was used to evaluate the relative bioavailability of test and reference dabigatran etexilate mesylate combined with pantoprazole sodium enteric-coated tablets in eight beagle dogs. The plasma concentrations of dabigatran were measured by a validated UPLC-MS/MS method, and the pharmacokinetic parameters were calculated using Kinetica5.1, SPSS 11.5 was used for the statistic analysis.

Results: In pilot study, the main pharmacokinetic parameters of dabigatran etexilate mesylate alone or combined with pantoprazole sodium enteric-coated tablets after single oral dose were shown as follow: T_{max} (2.600 ± 1.949) and (1.900 ± 0.742) h, C_{max} (660 ± 773) and (748 ± 723) ng/ml, AUC_{0-t} (3926 ± 3654) and (3692 ± 2997) ng · h/ml, MRT (9.4 ± 3.2) and (6.4 ± 1.9) h, $T_{1/2}$ (6.8 ± 2.5) and (4.9 ± 2.3) h. In the relative bioavailability study, the main pharmacokinetic parameters of test or reference dabigatran etexilate mesylate after single oral dose administration were shown as follow: T_{max} (2.0 ± 0.8) and (1.8 ± 0.3) h, C_{max} (90.1 ± 47.5) and (113.8 ± 65.0) ng/ml, AUC_{0-t} (565.4 ± 300.0) and (657.4 ± 340.8) ng · h/ml, MRT (9.7 ± 2.3) and (8.7 ± 2.2) h, $T_{1/2}$ (6.4 ± 1.6) and (5.9 ± 1.9) h. The relative bioavailability of the test formulations versus the reference was $89.8 \pm 40.4\%$ ($P > 0.05$).

Conclusion: According to the result, there are no apparent PK property changes with or without pantoprazole sodium enteric-coated tablets combined administration, which means that the beagle dogs model can be used to predict dabigatran etexilate mesylate's potential absorption in human. The test dabigatran etexilate mesylate formulation is bioequivalent to the reference formulation.

An LC/MS/MS Based Strategy for Quantification of Monoclonal Antibodies and Antibody-conjugated Drugs for Developing Antibody Drug Conjugates

Ling Xu, Shoaxia Yu, Jing-Tao Wu, and Mark Qian

DMPK Department, Takeda Pharmaceuticals International Co., 35 Landsdowne St., Cambridge, MA 02139, USA

For the development of antibody-drug conjugates (ADCs), specific, rugged, and reliable multi-species quantification assays of the total antibody are critical for preclinical *in-vivo* PK, TK, and in-vitro plasma ADC stability assessment. LC-MS/MS based antibody assays have recently emerged as a promising assay platform for this purpose with unique features comparing with more traditional ligand binding assay (LBA) methodologies.

A sensitive and highly specific hybrid immuno-capture LC/MS/MS method has thus been developed at Takeda to quantitate total antibodies (conjugated and unconjugated antibodies) and antibody conjugated drug. The hybrid immune-capture method has been compared head-to-head with an LBA method for quantification of the total antibody levels in mouse plasma samples from a PK study. It was found that the two methods matched very well. Currently, the methodology is being applied to more ADC programs. The strategy of the LC/MS/MS based quantitative analytics will be presented.

POSTER ABSTRACT

#132

Impact of incubation time on determination of unbound fractions in blood and brain homogenate

Yumei Yan, Kan Chen, Zack Cheng

DMPK, Asia and Emerging Markets iMed, AstraZeneca, Shanghai, China;

Abstract: Free drug hypothesis is a fundamental principle for in vivo pharmacology, thus free brain concentrations at site of action leads to pharmacological activities. Unbound fraction in blood and brain tissue are critical parameters to estimate free drug concentrations in bio-phase. In vitro equilibrium dialysis is routinely used for unbound fraction measurement, however the outcome could be affected by several experimental conditions, such as frozen or fresh tissue used and incubation time. There is a good correlation of rat $f_{u,blood}$ and $f_{u,brain}$ using either frozen or fresh collected tissues, and the ratio of $f_{u,brain}$ to $f_{u,blood}$ (K_{bb}) does not change with either frozen or fresh tissue. Blood to plasma ratio of selective compounds were close to 1. Thus, disrupted red blood cells didn't affect unbound fractions. Higher unbound fraction was observed after 16 hr incubation as compared to 4 hr incubation, which is plausible explained by high hydrophobic compounds (high LogD value) that need longer incubation time to reach equilibrium. 16 hr incubation should works well for reversible compounds since mass recovery is still kept reasonable range for most of them. High f_u value were obtained for irreversible compounds after 16 hr incubation. However, recovery was compromised by long incubation time as compared to 4 hr incubation. Caution should be taken for data interpretation for f_u value of irreversible compounds.

Development of an Efficient Screening Method for Screening Potential Bioactive Compounds Binding to Mitochondria from Herbal Medicines by Combining Centrifugal Ultrafiltration with Liquid Chromatography/Mass Spectrometry

Xing-Xin Yang,^{†,‡} Feng Xu,[†] Xuan Wang,[‡] Ming-Ying Shang,[†] Shao-Qing Cai[†]

[†]Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, P.R. China

[‡]Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, P.R. China

yxx78945@163.com (X.X. Yang)

ABSTRACT: Mitochondria are becoming of increasing interest as a highly significant pharmacological target for various disease remedy over the years, because damage to this organelle results in a range of human diseases and because mitochondria play a central role in such complex processes as energy-generation, adjustment of apoptotic cell death, reactive oxygen species (ROS) generation, maintenance of calcium homeostasis and regulation of lipidic metabolism. To promote the screening of natural bioactive compounds binding to mitochondria, we initially developed an efficient mitochondria-based screening method by combining centrifugal ultrafiltration (CU) with liquid chromatography/mass spectrometry (LC/MS), which is suitable for the search of mitochondrial ligands from complex samples such as herbal medicines extracts. The functionally active, structurally intact and pure mitochondria were obtained from rat myocardium with a protocol for mitochondrial isolation which is comprised of organelle release followed by differential and Nycodenz density gradient centrifugation. After evaluating the feasibility of the method using positive controls, this method was successfully applied for screening bioactive constituents from 5 different plant medicines extracts. Totally 43 active compounds were discovered and identified by LC/MS, among which 38 compounds were found as new mitochondrial ligands. The activity of 18 compounds among 38 ligands were confirmed by pharmacological experiments *in vitro*. The results indicated that our mitochondria-based CU/LC/MS method could be used for the efficient screening of mitochondrial ligands in complex matrixes such as medicinal herbals applied for remedying mitochondrial disorders, which may be useful for deep comprehension of drug action mechanism and drug discovery using medicinal herbs as lead compound.

KEYWORDS: Mitochondria; Bioactive constituents; Screening method; Centrifugal ultrafiltration; Liquid chromatography/mass spectrometry



POSTER ABSTRACT

#134

Development and Validation of LC-MS/MS Method for the Quantitation of Infliximab in Human Serum

Hui Wang, Waters Corporation

The liquid chromatography tandem mass spectrometry (LC-MS/MS), especially triple-quadrupole mass spectrometry, has become an attractive alternative method to ligand binding assays (LBAs) for therapeutic monoclonal antibody (mAb) quantification in biological samples, but the use of an internal standard with infliximab LC-MS/MS assays has not been reported yet. In this study, an improved LC-MS/MS method for quantification of infliximab in human serum was developed and validated. A surrogate peptide was used as a representative of infliximab which was cleaved for the quantification of infliximab based on LC-MS/MS assay. And a stable isotope labeled signature peptide was used as the internal standard (IS). The results showed the linearity in the range of 0.39-100 μ g/mL, the lower limit of quantification (LLOQ) and the lower limit of detection (LLOD) was 0.39 μ g/mL and 0.0975 μ g/mL, respectively. The quality control (QC) data showed that the within-run, between-run precision (%RSD) and accuracy (%RE) were conformed to the acceptance criteria of $\pm 15\%$ for calibration standards and QCs ($\pm 20\%$ at the LLOQ). Other validation parameters including selectivity, methanol precipitation efficiency, serum matrix effect, stability and auto-sampler carryover were also evaluated. This improved LC-MS/MS method might be a promising LC-MS-based methodology for pharmacokinetic studies of other recombinant monoclonal antibodies.

POSTER ABSTRACT

#135

Systemic exposure to and disposition of phthalides from *Ligusticum chuanxiong* rhizomes and *Angelica sinensis* roots after intravenous administration of XueBiJing injection in human subjects and ratsNa-ting Zhang¹, Yan Sun¹, Chen Cheng¹, Feng-qing Wang¹, Fei-fei Du¹, Fang Xu¹, Chang-hai Sun², Gui-ping Zhang² and Chuan Li¹¹Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China; ²Tianjin Chase Sun Pharmaceutical Co., Ltd, Tianjin, China

XueBiJing injection, an emerging herbal injection approved by China FDA, has been widely used for sepsis and multiple organ dysfunction syndrome treatment and consists of five herbs, including *Ligusticum chuanxiong* rhizomes (Chuanxiong) and *Angelica sinensis* roots (Danggui). Phthalides from Chuanxiong and Danggui are believed to be responsible for their therapeutic effects. This study aimed to examine the systemic exposure to and disposition of these phthalides in human and rats receiving intravenous XueBiJing injection. A total of 12 phthalides were detected, with content levels of 0.056-326 μ M in the injection, and the major compounds included senkyunolide I, senkyunolide H, senkyunolide N, senkyunolide G and 4-hydroxy-3-butylphthalide with their daily doses \geq 1 μ mol/subject. After dosing, senkyunolide I and senkyunolide H exhibited exposure in human subjects due to their highest doses. Phthalides were eliminated quickly with half-lives within 0.4-1 h in human and 0.2-0.6 h in rats mainly through hepatobiliary excretion of their metabolites. Glucuronide and glutathione conjugates were found to be the major metabolites for most phthalides. Doubling dose by increasing the infusion rate or prolonging the infusion time increased the systemic exposure of phthalides in human subjects and the dose proportionality assessment in rats showed dose-dependent pharmacokinetics. No accumulation and change in disposition of phthalides was found after consecutive dose for seven days. Phthalides exhibited extensive tissue distribution in rats, which is beneficial to treat multiple organ dysfunctions. The information gained here is relevant to pharmacological research on and clinical application of XueBiJing injection.

POSTER ABSTRACT

#136

Utilization of MDCK permeability and short oral absorption model for GI restricted drug discovery

Jinqiang Zhang, Bonnie Zhang, Yumei Yan, Zhen Zhang, Jerry Li, Lifang Zhu, Jing Lv, Zack Cheng
DMPK, Asia&Emerging Markets iMed, AstraZeneca R&D, Shanghai, China
Abstract

Gastrointestinal (GI) restricted small molecule drugs work predominantly in the GI tract with minimal systemic exposure to avoid undesired side effects. One effective method avoiding systemic exposure is to limit the permeation of compounds through GI tract into bloodstream while maintaining high solubility to achieve efficacy in GI tract. We have employed MDCKII cell line with modified assay condition to improve compound mass balance. The very good correlation was observed between A>B absorptive permeability in MDCKII cell line and portal vein exposure in rats evaluated by short oral absorption (SOA) model. Absorptive permeability $< 0.5 \times 10^{-6}$ cm/s (preferably $< 0.1 \times 10^{-6}$ cm/s) and dose normalized AUC (DNAUC) < 20 (ng·h/mL)/(mg/kg) in portal vein were set as criteria for compound screening. Consistently low oral bioavailability was observed in rats and dogs IV&oral PK studies for selected compound. We have a high degree of confidence that the selected compound would have low oral absorption in human. Our results have demonstrated the value of utilizing permeability in MDCKII cell line and SOA model as effective tools to help select drug candidate in a rapid and cost-effective manner.

POSTER ABSTRACT

#137

A Simplification of Population Pharmacokinetic/Pharmacodynamic Model Based Time-Dependent Kinetics of Red Blood Cell (RBC) Binding Model and its Application to Drug CPRC-1 in Human

Xin Zheng¹, Kairui (Kevin) Feng², Wenyuan Qi¹, Hongzhong Liu¹, Pei Hu¹, Ji Jiang¹

1. Peking Union Medical College Hospital 41 Damucanghutong, Xicheng, Beijing, 100032, China

2. Pharsight, Cary, NC 27518, USA

Background: CPRC-1, a new chemical entity that possesses pharmacological activity on Platelet-Activating Factor (PAF). It was approved into phase I evaluation for treatment of ischemic stroke in China. A nonlinear red blood cell (RBC) binding/partition was observed in clinical trial within PUMCH. The clinical observation including 7 escalation dose studies of CPRC-1 shows that both partitioning and kinetic of binding happens at the same time and it possibly has active transporters involved. However, an In Vitro B/P experiment was conducted to determine the concentration and time dependent B/P relationship. It didn't show any evidence of RBC concentration or time dependent B/P.

Objective: A semi-mechanism population pharmacokinetic model with time-dependent kinetics of red blood cell binding and partitioning of CPRC-1 was developed. The model incorporates individual haematocrit level as a covariate and includes the kinetics of CPRC-1 in blood, erythrocytes and plasma. This semi-mechanism population pharmacokinetic model linked a pharmacodynamic model in order to describe the platelet inhibition rate as drug effect.

Methods: Firstly, an in vitro blood to plasma partition assay was conducted for investigating the relationship between B/P ratio and concentration within the range from 0.01 μM to 5 μM . Subsequently, a sub-compartment of blood model is built in the software phoenix NLME (Pharsight Company, St. Louis, Missouri, USA). This sub-compartment of blood, erythrocytes and plasma includes first-order kinetics of partitioning to RBC and ON/OFF rate binding process of RBC. A final two-compartmental pharmacokinetic model with this sub-compartment of blood model is developed for the pop-PK analysis of 7 escalation dose study. Further, this PK model linked PD model to fit the platelet inhibition rate in multiple dose study.

Results: In vitro B/P partition assay result indicated that the B/P ratio of CPRC-1 was close to 1 and was independent from concentration and incubation time. A two compartment pharmacokinetic model with a parallel RBC partitioning and kinetic RBC receptor binding best described the plasma and whole blood concentration data. The cross validation result indicated that the haematocrit level significantly improve the individual level of prediction for both plasma and blood concentration. An Emax model linked to this PK model and it well described the drug effects.

Conclusions: The RBC distribution of CPRC-1 involved two parallel processes and in Vitro B/P experiment is not possible to provide the In vivo time-dependent kinetic of RBC binding and partitioning. A simple semi-mechanistic blood compartment cannot predict the time-dependent kinetic of RBC binding and partitioning. This is due to a rapid ON rate and a slow OFF rate to the RBC. The true mechanism is still unknown because of the complexity of red blood cell structure. The developed semi-mechanistic population PK/PD model incorporated individual haematocrit best described plasma and whole blood profile of CPRC-1 in Chinese subject and the Cross validation indicates that using the individual haematocrit level can predict the whole blood concentration from the measured plasma concentration data and hence potentially to save the clinical trial.

POSTER ABSTRACT

#138

Chemical Profiling of *Ginkgo Biloba* Leaves and Extracts

Chenchun Zhong¹, Wei Niu¹, Li Li¹, and Chuan Li^{1, 2},¹Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China,²Institute of Chinese Materia Medica, China Academy of Chinese Medical Science, Beijing, China

As one of most popular botanical OTC medicines and/or dietary supplements in the world, *Ginkgo biloba* leaf extract products have been widely used for prevention or even treatment to cardiovascular disease and cerebral insufficiency. A novel and generally applicable approach was provided for comprehensive screening of target and non-target constituents from *Ginkgo biloba* leaf based on liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. After background subtraction from blank scan, all detected peaks were unambiguously identified by comparing accurate mass data/characteristic mass fragments and retention times with those of reference compounds, and/or tentatively assigned by matching empirical molecular formula with that of known compounds. A total of 150 components were identified, including 9 carboxylic acids, 4 flavanols, 9 terpene lactones, 68 glycosylated flavonols and flavones, 11 flavonol and flavone aglycones, 16 biflavones, 31 alkyphenols, and 2 unknown compounds in an aqueous methanol or hexane extract of the leaves and extracts. Not only most compounds literature-reported with significant different physicochemical properties (hydrophobic constant Log *P* values in range of -1.93~11.18) have been identified but also quite a few new glycosylated flavonols and alkyphenols components have been detected. This comprehensive strategy could be applied for effective evaluation on systemic quality of Ginkgo preparations.

Oxidative and Reductive Activation of Nimesulide: Possibly Irrelevant with Hepatotoxicity

Lei Zhou, Xiaoyan Pang, Cen Xie, Dafang Zhong, and Xiaoyan Chen*
Shanghai Institute of Materia Medica, Shanghai, P. R. China

ABSTRACT:

Nimesulide (NIM) is a classic nonsteroidal anti-inflammatory drug with idiosyncratic hepatotoxicity. The bioactivation of its reduced metabolite M2 has been elucidated and might underlie the hepatotoxicity. However, it was reported that M2 did not cause toxicity to primary rat hepatocytes, implying the presence of other bioactivation pathways of nimesulide. Herein, we investigated bioactivation pathways of nimesulide and their association with hepatotoxicity. After incubating nimesulide with isolated rat hepatocytes, two types of GSH conjugates were characterized: NIM-OH-GSH and NIM-NH₂-GSH, formed by the conversion of nitro to hydroxyl and amino, respectively. The yield of NIM-OH-GSH was much higher using nimesulide as the substrate than M7 (NIM-OH) in GSH and NADPH-supplemented human liver microsomes. The addition of microsomal epoxide hydrolase (MEH) in the nimesulide incubations decreased NIM-OH-GSH formation in a concentration-dependent manner, suggesting that epoxidation was involved in the formation of NIM-OH-GSH from nimesulide. NIM-NH₂-GSH could be formed not only by oxidation of M2, but also through the reductive activation of nimesulide independent of cytochrome P450s. The nitroso intermediate of nimesulide (NIM-NO) could readily react with GSH to form NIM-NH₂-GSH. P450 phenotyping studies showed that NIM-OH-GSH from nimesulide was primarily catalyzed by CYP1A2. The reductive metabolism of nimesulide could occur in HLC without any cofactors. Chemical inhibitor studies demonstrated that aldehyde oxidase (AO) was the main reductase mediating the reduction of nimesulide in the cytosol. The addition of non-specific CYP inhibitor ABT significantly increased nimesulide-induced toxicity in primary rat hepatocytes. The increase of reductive activation extent mediated by NADP did not attenuate the toxicity of nimesulide, and no increased toxicity was observed in nimesulide incubation system with additional NIM-NO or BSO (a GSH depletor). These results demonstrated that oxidative and reductive activation pathways of nimesulide were possibly irrelevant with the hepatocellular toxicity, which was induced by nimesulide per se.

In Vitro and In Vivo Metabolism of TPN729 and Assessment of Its Drug-Drug Interaction Potential

Yunting Zhu, Pan Deng, Liang Li, Zitao Guo, Xiaoyan Chen, Dafang Zhong*
Shanghai Institute of Materia Medica, Shanghai, P. R. China

ABSTRACT

TPN729 has been reported as a novel phosphodiesterase type 5 inhibitor for the treatment of erectile dysfunction, and is currently being tested in clinical trials in China. To date, its metabolic characteristics have not yet been revealed. This work aimed to investigate the in vitro metabolism of TPN729 in hepatocytes across species and characterize its metabolic profiles in vivo in dogs. In addition, studies were conducted to evaluate the potential of TPN729 for drug-drug interactions associated with drug metabolizing enzymes and transporters. A total of 17, 15, 13, and 13 metabolites were detected using UPLC/Q-TOF MS after incubations in human, dog, rat, and mouse hepatocytes, respectively. Dogs offered better coverage than rats and mice for humans, in terms of the metabolites identified. The predominant metabolites resulted from sulfonamide *N*-depyrrolidine-ethylation (M3), oxidative deamination and aldehyde oxidation (M8 and M14), and *O*-glucuronidation (M16). Structures of M3, M8, and M14 were confirmed by comparison with the references synthesized, with M16 confirmed by the β -glucuronidase experiment. The metabolic profile of TPN729 in vivo in dogs was qualitatively similar to that in dog hepatocytes. M8 and M14 were the primary metabolites in feces. M14 and the unchanged parent drug were the prominent drug-related components in urine. In plasma, the parent compound was the major circulating substance. The formation of TPN729 metabolites was NADPH-dependent, and the further in vitro phenotyping studies using human recombinant P450 isoforms and chemical inhibition studies demonstrated that CYP3A4 was principally responsible for the metabolism of TPN729. Pretreatment with ketoconazole, a potent CYP3A inhibitor, or dexamethasone, a potent inducer of CYP3A, significantly affected the plasma concentrations of TPN729 in dogs: ketoconazole increased the $AUC_{0-\infty}$ and C_{max} by 7.8 and 2.5 folds, respectively, whereas dexamethasone decreased these parameters by 75.1% and 63.7%, respectively, which indicated the high potential of TPN729 as a victim for drug-drug interactions with CYP3A. Moreover, TPN729 was a substrate and an inhibitor for the efflux transporter P-gp. The high intrinsic membrane permeability of TPN729 limited the impact of P-gp on its intestinal absorption. However, the P-gp mediated efflux was significantly inhibited in the presence of TPN729. The efflux ratio of imatinib, a known substrate for P-gp, with 10 μ M TPN729 co-treatment in human P-gp expressing MDCK cells was much lower (1.8) than that in the absence of TPN729 (19.0). Overall, results from this study demonstrated the pyrrolidine moiety of TPN729 as the active metabolic site, which underwent various metabolic reactions, including *N*-dealkylation, oxidative deamination, *N*-oxidation, dehydrogenation, lactam formation, and glucuronidation. TPN729 was a sensitive substrate of CYP3A, and a potent inhibitor for P-gp. The potential of TPN729 as a perpetrator or victim in drug-drug interactions should be considered in clinical practice.

POSTER ABSTRACT

#141

A simple, rapid and reliable liquid chromatography-mass spectrometry method for determination of methotrexate in human plasma and its application to therapeutic drug monitoring

Dan Wu, Department of Medical Oncology, Fudan University Shanghai Cancer Center; Department of Oncology, Shanghai Medical College, Fudan University, Shanghai

Methotrexate (MTX) was commonly used in the chemotherapy of osteosarcoma, acute lymphoblastic leukemia (ALL) and other malignant tumors. Because of its narrow therapeutic window, severe toxicity and individual pharmacokinetic differences, routine monitoring of MTX is crucial to improve the efficacy of individual patient treatment as well as minimize adverse effects.

A simple, rapid and reliable liquid chromatography-electrospray ionization tandem mass spectrometry method was established and validated for the determination of methotrexate in human plasma. The pre-treatment procedure used a one-step protein precipitation method by acetonitrile-water (70:30, v/v), with no need for evaporation and reconstitution. MTX and internal standard were separated on a Column C₁₈ column (50 × 2.1 mm, 3 μm; Column Technology, Fremont, CA, USA) using a gradient elution with mobile phase of acetonitrile and 0.03% acetic acid aqueous solution. The total chromatographic runtime was 5 min for each injection. Quantification detection was performed in a triple-quadruple tandem mass spectrometer under positive mode monitoring the following mass transitions: *m/z* 455.3→308.3 for MTX and *m/z* 136.1→94.4 for internal standard. The calibration curve was linear over the range of 0.05 – 25.0 μmol/L with a lower limit of quantification (LLOQ) of 0.05 μmol/L. The intra- and inter-day precisions were less than 5.2%, the accuracy varied from -4.1% to 4.5%. The recovery was greater than 94%.

The presented LC-MS/MS approach was compared with the HPLC method developed in our laboratory by measuring 42 clinical samples. The statistical results using Passing & Bablok Regression and Bland-Altman difference plot analysis showed that the two assays were in good agreement. The method was reliable and reproducible for the therapeutic drug monitoring of MTX. Herein, we offer a candidate reference method that is practically applicable in clinical laboratories.

In Silico Modeling Can Predict the Unforeseen Renal Failure Caused by SGX523, a c-MET Kinase Inhibitor

Michael Lawless¹, John DiBella¹, Tao Chen², and Michael B. Bolger¹

1-Simulations Plus, Inc., Lancaster, California, USA; 2-PharmoGo Co., Limited, Shanghai, China

SGX523 is a quinoline-containing molecule that was a promising c-MET kinase inhibitor with an IC₅₀ of 4 nM and >1,000-fold selectivity over other protein kinases. It is orally bioavailable and inhibited the growth of human glioblastoma lung and gastric cancer xenografts in mice. However, in a phase 1 clinical trial, the six patients that received ≥80 mg daily doses all developed renal failure as confirmed by a rise in serum creatinine and blood urea nitrogen. Hydration therapy returned these levels to baseline after 1 to 4 weeks. Follow-up studies revealed that the cause of renal toxicity was drug-induced nephropathy due to a metabolite of SGX523 that was not detected in preclinical studies. Aldehyde oxidase transforms the quinoline ring into a quinolinone. Generation of the quinolinone metabolite is species-dependent; it is formed in human and monkey liver S-9 but not in dog S-9 incubations. Here, we demonstrate the use of predicted physicochemical and biopharmaceutical properties from ADMET Predictor™ (Simulations Plus, Inc.) to predict the toxicokinetics of SGX523 and its oxidized quinolinone metabolite. These predicted properties were then used in a mechanistic oral absorption (ACAT™) and physiologically based pharmacokinetic (PBPK) model in GastroPlus™ (Simulations Plus, Inc.) to simulate the plasma and renal concentrations versus time. Oxidative metabolism results in conversion of the basic quinoline (predicted pK_a of 4.2) group in SGX523 to an acidic lactam ring with a predicted pK_a of 11.0. This also results in decreased solubility; the predicted aqueous solubility drops from 2.4 µg/mL in SGX523 to 0.56 µg/mL in its quinolinone metabolite. Our PBPK simulations show high concentrations of the quinolinone metabolite in the lumen of the kidney, beyond its solubility, creating the probability of precipitation. Additional population PBPK simulations were performed to assess inter-subject variability. Thus, our in silico analysis predicts the observed renal toxicity in humans and monkeys due to crystallization of the metabolite in the kidney.

Population pharmacokinetics and Monte Carlo dosing simulation of HR7056 anesthesia in Chinese healthy subjects

Ying Zhou, Hongyun Wang, Pei Hu, Ji Jiang*

Clinical Pharmacology Research Center, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100730, China. Corresponding author. Tel.: +86-10-6915-6576; fax: +86-10-6915-8365 E-mail: pk.frosh@gmail.com

Background

A new benzodiazepine, HR7056, which is rapidly metabolized by tissue esterases to an inactive metabolite, has been developed to permit a fast onset, a short duration of sedative action, and a more rapid recovery profile than currently available drugs. We aimed to develop a population pharmacokinetic model of HR7056 in Chinese healthy subjects undergoing the phase Ia clinical trial of the new drug. We report on modeling of the data and simulations of dosage regimens for future study.

Methods

A phase I, single-center, double-blinded, randomized, active controlled, single ascending-dose study of HR7056 (0.01-0.45 mg/kg) administered as a 1-minute IV infusion was conducted. A total of 1197 arterial plasma concentrations of HR7056 from 63 subjects were analyzed using non-linear mixed-effects modeling. The parameters obtained were used for Monte-Carlo simulations of different dosing regimens. Phoenix WinNonlin 6.3 (Pharsight Corp., USA) was applied for population pharmacokinetic modeling.

Results

A three-compartment model best described HR7056 pharmacokinetics. No covariates were identified. Total clearance was 1.49 L/min, central volume was 2.1 L, intercompartmental clearances were 0.96 L/min and 0.28 L/min, respectively, and peripheral volumes were 10.4 L and 22.7 L, respectively. We used data from phase Ib clinical trial (a total of 416 arterial plasma concentrations of HR7056 from 8 subjects who receiving an induction dose of 0.4 mg/kg (infusion within 1 min), followed by 1.5 mg/h/kg for 2 hours) to perform external validation for the model. We found that the simulated data from final model fitted the observed data very well.

Conclusions

Population pharmacokinetic model developed for HR7056 fitted the observed data well. The model simulation will enable maintenance doses to be more accurate for future study.

Organic Anion-Transporting Polypeptides Contribute to the Hepatic Uptake of Berberine

Chen CHEN¹, Zhi-tao Wu², Lei-lei MA², Xuan Ni², Yun-fei LIN², Le WANG², Ke-ping CHEN¹, Cheng-gang HUANG², Guo-yu PAN^{2,*}

¹Institute of Life Sciences, Jiangsu University, Zhenjiang 212013, China and ²Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China;

Abstract

1. The purpose of this study was to investigate the mechanism of hepatic uptake of berberine. Berberine accumulation in hepatocytes was found to be highly dependent on active uptake, which could not be explained by liver organic cation transporter (OCT) alone.
2. Our studies indicated that berberine uptake was significantly suppressed by rifampicin, cyclosporine A and glycyrrhizic acid, which act as specific inhibitors of different Oatp isoforms (Oatp1a1, Oatp1a4 and Oatp1b2) in rat hepatocytes. The combination of OCT and OATP inhibitors further reduced berberine accumulation in both rat and human hepatocytes. The uptake of berberine could be increased in human HEK293-OATP1B3 but not in OATP1B1-transfected HEK 293 cells.
3. Rifampicin could reduce the berberine liver extraction ratio (ER) and double its concentration in the effluent in isolated rat livers. Further *in vivo* study indicated that berberine plasma exposure could be significantly increased by co-administration of the OATP inhibitor rifampicin.
4. In conclusion, this study demonstrated that both OCT and OATP contribute to the accumulation of berberine in the liver. OATPs may have important roles in berberine liver disposition and potential clinically relevant drug-drug interactions.



WebMetabase: A Tool to Exploit Metabolite Identification Information in Drug Discovery

Ismael Zamora^{1,3}, Fabien Fontaine¹, Luca Morettoni², Blanca Serra¹, Guillem Plasencia¹, Xavier Pascual¹, Esra Nurten Çece^{1,3}, Tatiana Radchenko^{1,3}, Gabriele Cruciani²

1. Lead Molecular Design, S.L. Vallés 96-102 L 27, 08173 Sant Cugat del Vallés, Barcelona, Spain
2. Molecular Discovery Ltd. 215 Marsh Road, 1st Floor HA5 5NE, Pinner, Middlesex, United Kingdom
3. Pompeu Fabra University, Dr. Aiguader 80, 08008, Barcelona, Spain

The identification and structural elucidation of the metabolites is challenging and time consuming task, which traditionally has been done in the late drug discovery stages with few number of compounds. Computer assisted method can increase the speed of processing metabolite experiments, by changing the initial stage of the process closer to the final results: the peak finding and structure elucidation. Therefore, the increase in the throughput of the computer assisted metabolite identification makes it possible to analysis a higher number of experiments, accessing new workflows where the knowledge of the structure of metabolite may change the paradigm in the way this information is used in Drug Discovery.

In this presentation we will show how the structural information provided by the computer assisted metabolite identification can be used in: cytochrome reaction phenotyping, GSH adduct formation, soft spot identification analysis, matrix and cross species comparison. Moreover, the fact that the data is computer assisted make it possible to be used by different analysis tools that may help in hypothesis building and compound design to overcome issues related to the metabolism for a compound. In this case, the metabolite identification results will be used as a trigger for docking of the parent compound in the structure of several cytochromes P450, suggestion of bioisosteric replacement for the chemical moiety that has been identified as being metabolic labile, comparison of the metabolic pathways for compounds of the same chemical series and finally comparison of the metabolites obtained for the same compound across different experimental conditions. Examples for each work flow will be shown on several compounds. Showing the powerful of having metabolite identification data in an electronic format that makes possible the metabolic structure comparison.



Adding Immuno-purification to a Plasma Collection Device

¹Fred Regnier, ¹Tim Woenker, ¹JinHee Kim, ²Jiri Adamec, and ¹Tim Schlabach.

¹Novilytic, West Lafayette, IN 47906 and ²University of Nebraska, Lincoln, NB 68598.

Blood specimens are at the heart of clinical diagnostics and DMPK studies. The conventional approach is to draw blood by venipuncture and remove the red blood cells by centrifugation. This dated technology requires multiple medical professionals and produces far more plasma than required by techniques such as LC/MS. Newer approaches to plasma collection require only a drop or two of blood and can be performed quickly by the subject with minimal help from professionals. Because these newer devices typically collect less than 10 μ L of plasma, even LC/MS assays can be challenging if the level of background interference is high. Mobile Sorbent Affinity Chromatography (MSAC) has been developed to address this potential shortcoming.

Mobile affinity sorbents are a complex of specific antibodies attached to a larger structure, such as nanoparticles. These sorbents stay suspended in solution and can be pipetted onto the plasma collection disc of a commercial, plasma preparation device. The antibodies in the sorbent specific bind analyte(s) from the collection disc. The extraction solution will also non-specifically remove plasma compounds from the collection disc. The extraction solution with the mobile affinity sorbents is collected and injected onto a restricted access media (RAM) column. The mobile affinity sorbents are excluded from the RAM column along with large proteins. Small molecules are trapped by the reversed-phase in the RAM column. The nanoparticles are collected in the void volume of the column and then spun down. After removing the liquid fraction the mobile affinity sorbents are treated with a low pH solution which releases the analyte from the immuno-complex. This highly purified solution can be directly introduced into the LC/MS system or quickly chromatographed with a ballistic gradient on a short column.

The MASC system has been used in conjunction with a plasma preparation card and tandem LC/MS to analyze vitamin D in whole blood. The MASC system improves detection limits for vitamin D compared to direct extraction of the plasma disc. The MASC approach also improves the throughput for vitamin D analysis by either eliminating or reducing the chromatographic run time.