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^{1}H NMR based pharmacometabolomics analysis of metabolic phenotype on predicting metabolism characteristics of losartan in healthy volunteers

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ABSTRACT

Inter-individual variability in drug metabolism and disposition is common in both preclinical and clinical researches. Losartan and its active metabolite EXP3174 present a high degree of inter-individual differences in blood concentrations that affect drug efficacy and side effect. Pharmacometabolomics has been increasingly applied on predicting the drug responses by analyzing the differences in metabolic profile. A pre-dose metabolic phenotype was investigated to interpret inter-individual variations in the metabolism characteristics of losartan. ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy-based metabolic profiles were performed on 36 healthy Chinese male volunteers by measuring their pre-dose plasma samples. After oral administration of losartan, the concentrations of losartan and its bioactive metabolite EXP3174 were monitored by liquid chromatography-mass spectrometry (LC-MS). Orthogonal partial least-squares (*O*-PLS) model was conducted to select potential biomarkers that substantially contributed to the inter-individual variations in the metabolism features *via* analyzing the ratio of pharmacokinetics (PK) parameters of its metabolite to parent drug. Potential metabolites such as glycine, phosphorylcholine, choline, creatine, creatinine, lactate, citrate, α-glucose, and lipids showed strong correlations with metabolism features of losartan. In addition, the pathway analysis revealed that baseline lipid metabolism, the glycine, serine and threonine pathway, and glycolysis or gluconeogenesis metabolism pathway were significantly associated with the ratio of PK parameters of EXP3174 to losartan. Step-wise multiple linear regression (MLR) was constructed to investigate the potential roles of the selected biomarkers in predicting individualized metabolism characteristics of losartan. These results showed that the pre-dose individual metabolic traits may be a useful approach for characterizing individual differences in losartan metabolism characteristics and therefore for expediting personalized dose-setting in further clinical studies.

1. Introduction

Drug treatment is challenged by the fact that the responses of drugs varied from person to person. Individual differences can come from physio-pathological conditions, diet, environment and gene, all of which affect the metabolism and disposition of drugs [1]. There is growing awareness that drug therapy has to be chosen based on personal characteristics that are determined by certain tests revealing drug responses. Personalized medicine has become increasingly important in clinical treatment to improve drug efficacy and reduce adverse drug reactions. Pharmacogenomics has been used for decades of years in clinic to select patient subsets for whom best efficiency with minimal adverse drug reactions can be achieved when using with a particular drug [2, 3]. Warfarin treatment is a good example of using pharmacogenomics to monitor dose-setting in clinical. According to the comprehensive genotypic and basic clinical information, integrative approach was developed to predict optimal warfarin dose for individuals [4, 5]. However, due to confounding factors, it's still difficult to quantify the individual differences only based on genetic variations.

In view of the limitation of pharmacogenomics, pharmacometabolomics, a different approach to predicted drug response, has been proposed recently [6]. Pharmacometabolomics focuses on the analysis of the pre-dose biofluid metabolite profiles, which could reflect the complex interactions among physio-pathological conditions, gene

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expression, gut microbiome and drug responses [7]. Clayton et al. [4] first proposed the new concept of "pharmacometabolomics" by using pre-dose metabolism profiles to predict drug response. A gas chromatography–mass spectrometry (GC–MS) based pharmacometabolomics study suggested that several endogenous metabolites in pre-dose plasma could better explain the PK parameters of atorvastatin in healthy volunteers than a conventional physiological index [8]. A recent study of pharmacometabolomics demonstrated that the cisplatin nephrotoxicity of rats could be predicted by the contents of branched-chain amino acids (BCAAs) in pre-dose serum [9]. These studies demonstrated the potential of pharmacometabolomics in predicting effectiveness, PK parameters and toxicity of drugs in preclinical investigations and clinical trials.

Losartan, the first active nonpeptide angiotensin II receptor blockers (ARBs), is widely used in the treatment of hypertension and congestive heart failure [10, 11]. Losartan is primarily metabolized by hepatic cytochrome P450 enzymes (such as CYP2C9, CYP3A4 and CYP2C8, et al.) to the active 5-carboxylic acid metabolite EXP3174 which has higher activity and longer half-life [12, 13]. After oral administration, losartan would reach the peak concentration during 2 h, and peak concentrations of EXP3174 is reached in 3–4 h [12–14]. Losartan and EXP3174 have significant inter-individual differences in plasma concentration [12–14]. There are considerable differences among individuals in response to losartan treatment concerning treatment efficacy, toxicity and drug metabolism [14–16]. Thus, it's necessary to predict individual variations in the metabolism characteristics of losartan for the purpose of individualized medication.

In this study, ^{1}H Nuclear Magnetic Resonance (NMR)-based pharmacometabolomics was applied to select metabolites in pre-dose plasma to predict individualized metabolism characteristics of losartan. The integrative approach, including NMR analysis, *O*-PLS modeling, pathway analysis, and step-wise MLR analysis, was able to effectively predict individual differences in the metabolic outcomes of losartan administration.

2. Methods and materials

2.1. Healthy Chinese volunteers

Thirty-six Chinese male volunteers were selected on the basis of their age, body mass index (BMI), routine clinical laboratory test, and medical history. We excluded the subjects who had any history or indication of cardiovascular, gastrointestinal, hepatic, renal, pulmonic, endocrinic and nervous system diseases. In addition, participants with any hematology, immunology, pathergasiology disease, or metabolic disturbance were also excluded from the study. Before participation in the trial, subjects who had used any other drugs within 2 weeks, or joined any drug clinical practices within 1 month, or donated blood within 3 months, were not included. Subjects were hospitalized 24 h before and 48 h after drug administration. Informed consent was signed by all participants before the initiation of study. The experimental protocol was in accordance with the ethical standards of the Ethics Committee Board of the Third Xiangya Hospital of Central South University (Changsha, China) and with the Helsinki declaration.

2.2. Study design

This was a single-center, open-label, randomized clinical trial. All subjects enrolled in this study received a single 50 mg of Losartan Potassium Tablet (MSD Pharmaceutical Co. Ltd., Hangzhou, China) with 250 mL warm water. The participants were given the same standardized diet with strict control. The blood samples (5 mL) were collected into sodium-heparin-containing tubes before (0 h) and at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36 and 48 h after administration of Losartan Potassium Tablet. All blood samples were centrifuged at 4, 000 rpm for 5 min immediately and stored at −40 °C until analysis.

2.3. Determination of losartan and EXP3174 concentrations

A sensitive and validated LC-MS/MS method was applied for measurements of losartan and EXP3174 concentrations. Brief details are presented as follows. The reference standard of losartan and the internal standard (IS, irbesartan) were both purchased from NIFDC (National Institutes for Food and Drug Control, Beijing, China). EXP3174 was purchased from Toronto research chemicals. A 500 μL aliquot of plasma was added to a 5 mL disposable Eppendorf tube, followed by 100 μL of 1 M HCl and 100 μL of IS (114.9 ng/mL). After vortexed for 30 s, 2 mL diethyl ether was added to each tube. The mixture was vortexed for 10 min and centrifuged at 4000 rpm for 10 min. The upper organic layer was separated and evaporated to dry at 37 °C under a gentle stream of nitrogen. The residues were redissolved in 150 μL of acetonitrile-water (4:1, v/v), and centrifuged at 13,000 rpm for 5 min before analysis. An aliquot of 20 μL was injected into the LC–MS/MS system.

Liquid chromatography was carried out on Waters 2695 HPLC system (Waters, USA), using a Thermo Hypurity C18 column (150 mm × 2.1 mm, 5 μm) at 40 °C. The mixture mobile phase consisted of phase A (0.2% formic acid in water) and phase B (acetonitrile) with a flow rate of 0.3 mL/min. The MS/MS system was carried out on Micromass QuattroMicro triple quadrupole mass spectrometer (Waters, USA) in positive electrospray ionization mode. The data analysis and processing software was MassLynx version 4.1 (Waters, USA). Ionization conditions was optimized as follows: the capillary voltage was 3.5 kV, ion source temperature was 100 °C, the cone gas was 50 L/h, desolvation temperature and gas were 350 °C and 500 L/h, respectively. The multiple reaction monitoring (MRM) transitions of precursor ions to product ions were 422.5 → 207.1(*m/z*) for losartan, 437.1 → 235.0 (*m/z*) for EXP3175, 429.3 → 207.0 (*m/z*) for IS, respectively, with a dwell time of 300 ms.

The LC-MS/MS method was validated according to the Food and Drug Administration guidelines [17], including linearity, sensitivity, selectivity, precision, accuracy, matrix effect, extraction recovery, and stability. Calibration curves were established over the range of 1.446 to 520.7 ng/mL for losartan, and 1.408 to 507.0 ng/mL for EXP3174. The intra-day and inter-day accuracy and precision of the analytical method were determined by the replication of five sets of QC samples at low, medium, and high concentrations within 1 day or on three consecutive days. The stabilities of losartan and EXP3174 in human plasma were evaluated under different storage conditions, using triplicated of low and high QC samples. Extraction recovery was measured by comparing the peak areas of the spiked analytes (losartan, EXP3174) extracted from the plasma samples with those from post-extracted blank plasma at low, medium, and high concentrations. The matrix effect of the method was measured by comparing the peak areas of the spiked analytes (losartan, EXP3174) spiked post-extracted with those of pure reference standard solutions at low, and high concentrations.

2.4. Pharmacokinetic analysis

The PK parameters of losartan and EXP3174 were analyzed by non-compartmental assessment of data using Drug and Statistics Software (DAS, version 3.2.2, Mathematical Pharmacology Professional Committee of China). The concentration-time curves were plotted, and the maximum drug concentration (C_{max}), and the time to reach C_{max} (T_{max}), the area under curves (AUC_{0-t}) and the area under the plasma concentration-time curve to time infinity ($AUC_{0-\infty}$) were calculated from the measured data. The PK parameter ratio values were calculated as follows: ratio of AUC = $AUC_{0-\infty\text{-E}}$ / $AUC_{0-\infty\text{-L}}$, ratio of C_{max} = $C_{max\text{-E}}$ / $C_{max\text{-L}}$.

2.5. Metabolic sample preparation and data acquisition

After thawed at room temperature, plasma samples were vortexed for 1 min. Then, 300 μL of plasma sample and 300 μL of $Na_2HPO_4{\cdot}7H_2O$