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Original article

Pharmacokinetics and pharmacodynamics study of rhein treating renal fibrosis based on metabonomics approach

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ABSTRACT

Background: The selection of effect indicators in the pharmacokinetic/ pharmacodynamic study of complex diseases to describe the relationship between plasma concentration and effect indicators is difficult.
Purpose: Three effect indicators of renal fibrosis were successfully determined. The relationship between pharmacokinetics and pharmacodynamics of rhein in rhubarb was elucidated.
Study design: The study was a metabolomics analysis of rat plasma and pharmacokinetics/ pharmacodynamics of rhein.
Methods: A sensitive and simple ultra performance liquid chromatography-tandem triple quadrupole mass spectrometry (UPLC-MS/MS) method was applied to determine the rhein plasma concentration in the rat model of renal fibrosis and rat sham-operated group after the administration of rhubarb decoction. Then, the ultra performance liquid chromatography-Micromass quadrupole-time of flight mass spectrometry (UPLC-QTOF/MS) metabolomics method was used to screen biomarkers of renal fibrosis in rat plasma. Furthermore, the relationship between the plasma concentration of rhein and the concentration of three biomarkers directly related to renal fibrosis were analyzed.
Results: The three screened biomarkers could represent the effect of rhein treatment on renal fibrosis. Increasing the plasma concentration of rhein tended to restore the concentration of the three biomarkers in the model group compared with that in the sham-operated group. Evident differences in the area under the plasma concentration-time curve (AUC) of rhein were also observed under different pathological states. The results provide valuable information for the clinical application of rhubarb.
Conclusion: Rhein intervention could recover the physiological balance in living organisms from the pharmacokinetic/pharmacodynamic levels. New information on the pharmacokinetic/pharmacodynamic study of complex diseases is provided.

Introduction

Pharmacokinetic-pharmacodynamic (PK-PD) models are effective tools for investigating the quantitative relationship between dosage and drug effect. They have important reference value for selecting clinical drug dosage, improving efficacy and reducing side effects. Since traditional Chinese medicines (TCMs) often have multiple ingredients, multiple targets and integral regulation, there are two main challenges in the study of a PK/PD model: (1) it is difficult to choose testable components that represent full pharmacokinetic characteristic; (2) it is also hard to estimate the treatment effect with one or several indicators, especially for many chronic diseases such as chronic kidney disease, that involve interactions among multiple genes and environments. For these reasons, examples of successful PK-PD study involving TCMs are rare. Breakthroughs in theories and techniques are urgently needed in these fronts.

The focus in metabolomic research has been on variation in the content of endogenous molecules, which reflects the pathological status of living organisms and responses under external stimulation, such as drugs (Mastrangelo et al., 2014; Nicholson et al., 1999). Up-regulation and down-regulation of the concentration of endogenous biomarkers reflects perturbation of physiological balance. In essence, the result of drug intervention should be the restoration of equilibrium concentration of endogenous biomark-

Abbreviations: UPLC-QTOF/MS, Ultra performance liquid chromatography - micromass quadrupole - time of flight mass spectrometry; UPLC-MS/MS, Ultra performance liquid chromatography - triple quadrupole tandem mass spectrometry; AUC, Area under the plasma concentration-time curve; PK-PD, Pharmacokinetic-pharmacodynamic; TCMs, Traditional Chinese medicines; UUO, Unilateral ureteral obstruction; SOG, Sham-operated group; PCA, Principal component analysis; PLS-DA, Partial least square discriminant analysis; α-SMA, α-smooth muscle actin; CTGF, Connective tissue growth factor; Col-I, Collagen type I; LLOQ, Lower limit of quantitation; LOD, Limit of detection).

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ers at a metabolic level. Thus, the endogenous biomarkers can be regarded as diagnostic or effect indicators at the molecular level (Xiang et al., 2012). The endogenous metabolites in the living organism will make a response after the administration of drugs, and this response can be accurately characterized by the metabolomics method. Hwang et al. screened the biomarkers of disease based on metabolomics method, which was used to predict the pharmacokinetics characteristics of tacrolimus (Phapale et al., 2010). Zhou et al. develop a pharmacometabonomic approach to predict individual differences in pharmacokinetics of atorvastatin and therefore to facilitate individualized drug therapy (Huang et al., 2015). In this sense, the metabolic profiling of the organism indirectly reflects the characteristics of pharmacokinetics, and even can characterize the pharmacokinetic differences in the individual drug therapy. These results indicated that there was a close and dynamic interaction between metabolomics and the characteristics of drug pharmacokinetics.

Recent application of metabolomics in PK studies has attracted the attention of many scholars (Huang et al., 2013; Lan and Jia, 2010). Jia et al. proposed a strategy for an integrated metabolomics and PK study for herbal medicines (Lan and Jia, 2010). Luo et al. used PK and metabolomic methods to investigate the influence of caffeine on the sedative effects of promethazine. The results suggest that caffeine counteracts the sedative effects of promethazine and improves the decreased cognitive function, which might be induced by the accelerated metabolism of promethazine and the altered dopamine-, norepinephrine-, and epinephrine-related pathways. Their study provides a new approach for the research of combined promethazine and caffeine (Huang et al., 2013). To the best of our knowledge, few literature has researched the problem about metabolomics in the application of PK/PD of TCMs.

The metabolomics method has been widely used in the pathological prediction of chronic kidney disease and the discovery of biomarkers (Zhao, 2013). Kobayashi et al. screened plasma biomarkers of CKD patients based on metabolomics method. These biomarkers were then used to construct a multivariate regression equation to predict CKD stages with 81.3% accuracy (Kobayashi et al., 2014). Zhao et al. investigated the metabolic profiling of urine and serum samples from chronic renal failure (CRF) model rats to find potential disease biomarkers (Zhao et al., 2013, 2012a, b). In a brief, these biomarkers can accurately characterize the pathological process of CKD or CRF.

Rhei Rhizoma (RR), the rhizome of *Rheum palmatum* L., is a popular herb in clinical Chinese medicine. It was well and widely used for treating chronic kidney disease and renal fibrosis in China and Japan (Wang et al., 2009; Wei et al., 2002; Zhang and el Nahas, 1996). Zhao et al. investigated the therapeutic effect of different rhubarb extract on adenine-induced chronic kidney disease and anti fibrosis in rats by using metabolomics method. The result demonstrated that different rhubarb extract had the nephroprotective effects against tubulo-interstitial fibrosis (Zhang et al., 2015). Results of our previous study also indicated that the efficacy of rhubarb in treating renal interstitial fibrosis was related to anthraquinone compounds (Xiang et al., 2015). Rhein is one of the main active compounds of rhubarb extracts and was the mainly absorbable anthraquinone derivative into systemic circulation after oral administration (Fang et al., 2011; Song et al., 2010; Wu et al., 2014a). It is reported that rhein has well anti-fibrosis properties in some literatures (Gao et al., 2010; Guo et al., 2001; He et al., 2011). But recent studies reported that the anthraquinone derivatives contained in rhubarb had nephrotoxicity (Wang et al., 2009; Yan et al., 2006). Therefore, PK/PD study on rhubarb extracts is necessary for clinical rational and safety drug use. In this work, the relationship between plasma concentration of rhein in rhubarb extracts and efficacy of treating renal interstitial fibrosis was further investigated. UPLC-QTOF/MS and UPLC-MS/MS were used to conduct metabolic profilings of plasma samples and quantitative determination of rhein in plasma samples, respectively. In addition, pharmacokinetic parameters of rhein in the model group and sham-operated group in different physiological states were compared to determine pharmacokinetic differences. Furthermore, biomarkers of renal fibrosis (pharmacodynamic indexes), which were screened via metabolomics, were used to descript the PK-PD relationship between rhein and three biomarkers. Thus, findings of this study define the PK-PD characteristics of rhein in rhubarb and provide a practical basis for the establishment and improvement of PK-PD investigation methods for TCMs.

Materials and methods

Experimental reagents

The rhizomes of *Rheum palmatum* was purchased from traditional Chinese medicine pharmacy of the First Affiliated Hospital of Wenzhou Medical University (Wenzhou, Zhejiang Province, China), and were identified by Professor Chongliang Lin (The First Affiliated Hospital of Wenzhou Medical University). Rhein (98%) and neohesperidin dihydrochalcone (Internal standard IS, 98%) were purchased from Chengdu MUST Biotechnology Co., Ltd (Chengdou, Sichuan Province, China). Methanol and acetonitrile were purchased from American Merck company (Kenilworth, USA). Formic acid was purchased from Aladdin Industrial Inc. (Shanghai, China). All other reagents were of analytical grade or higher purity.

Experimental animals

14 male Sprague-Dawley rats (8 weeks) with an average weight of 200 ± 20 g were purchased from Laboratory Animal centre of Wenzhou Medical University. These rats were randomly but equally divided into two groups (model group and sham operation group). They were fed in a specific pathogen-free environment (temperature: 22 ± 2 °C, relative humidity: 55 ± 10%) for one week with free access to food and water. The experimental protocol was approved by the ethics committee of our institute (Wenzhou Medical University) complied with the guidelines of the responsible government agency and with international standards (NIH publications No 80-23) revised 1996.

Construction of animal models and preparation of rhubarb decoction

Unilateral ureteral obstruction (UUO) is an ideal experimental model for renal interstitial fibrosis. Rats in the model group and sham-operated group (SOG) were prepared according to our previous operating procedure described (Xiang et al., 2015). Rhubarb decoction (0.5 ml/g, containing 2.0 mg/g of rhein) was prepared according to our procedure described previously (Xiang et al., 2015). The concentrate was stored in a refrigerator at 4 °C.

Therapy evaluation of rhubarb decoction

The curative effect of rhubarb decoction treating renal fibrosis was evaluated by biochemical tests of creatinine and blood urea nitrogen, histopathology (Hematoxylin-eosin and Masson staining) and immunohistochemistry (α-SMA, CTGF, and Col-I). Detailed information can be obtained in our previous work (Xiang et al., 2015).

Preparation of standard solution and quality control samples (QC)

An appropriate amount of rhein was weighed precisely and dissolved in methanol to prepare 80 μg/ml of stock solution. Samples for IS were weighed accurately and dissolved in methanol to