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Research Paper

Comparative pharmacokinetics of rhein in normal and loperamide-induced constipated rats and microarray analysis of drug-metabolizing genes

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ABSTRACT

Ethnopharmacological relevance: Rhein is a pharmacological active component found in *Rheum palmatum* L. that is the major herb of the San-Huang-Xie-Xin-Tang (SHXXT), a medicinal herbal product used as a remedy for constipation. Here we have investigated the comparative pharmacokinetics of rhein in normal and constipated rats. Microarray analysis was used to explore whether drug-metabolizing genes will be altered after SHXXT treatment.

Materials and methods: The comparative pharmacokinetics of rhein in normal and loperamide-induced constipated rats was studied by liquid chromatography with electrospray ionization tandem mass spectrometry (LC-MS/MS). Gene expression profiling in drug-metabolizing genes after SHXXT treatment was investigated by microarray analysis and real-time polymerase chain reaction (RT-PCR).

Results: A validated LC-MS/MS method was applied to investigate the comparative pharmacokinetics of rhein in normal and loperamide-induced constipated rats. The pharmacokinetic results demonstrate that the loperamide-induced constipation reduced the absorption of rhein. C_{max} significantly reduced by 2.5-fold, the AUC decreased by 27.8%; however, the elimination half-life ($t_{1/2}$) was prolonged by 1.6-fold. T_{max} and mean residence time (MRT) were significantly prolonged by 2.8-fold, and 1.7-fold, respectively. The volume of distribution (Vss) increased by 2.2-fold. The data of microarray analysis on gene expression indicate that five drug-metabolizing genes, including Cyp7a1, Cyp2c6, Ces2e, Atp1b1, and Slc7a2 were significantly altered by the SHXXT (0.5 g/kg) treatment.

Conclusion: The loperamide-induced constipation reduced the absorption of rhein. Since among the 25,338 genes analyzed, there were five genes significantly altered by SHXXT treatment. Thus, information on minor drug-metabolizing genes altered by SHXXT treatment indicates that SHXXT is relatively safe for clinical application.

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1. Introduction

Constipation is a frequent condition that is often treated pharmacologically (Muller-Lissner, 2013). Based on the Rome II diagnostic criteria, constipation affects at least 8.5% of the individuals in Taiwan, occurring more often in females and older persons (Chey et al., 2011; Jong et al., 2010). The aim of treating constipation is to treat the underlying causes, improve symptoms, and resume the normal physiological function of the bowel. Drugs commonly used include bulk laxatives, osmotic laxatives, non-absorbable sugar, stimulant laxatives, cholinergic agents and other prokinetics agents. In East Asian countries, traditional Chinese medicine (TCM) is another option in addition to or instead of Western medicine for treating constipation (Camilleri and Bharucha, 2010; Candy et al., 2011; Chey et al., 2011). In many of these countries, especially Taiwan, TCMs are frequently prescribed for the treatment of many chronic diseases (Jong et al., 2010; Lai et al., 2012; Shih et al., 2012). According to a survey from a cohort of one million randomly sampled cases from the National Health Insurance database in Taiwan, the Chinese herbal formula San-Huang-Xie-Xin-Tang (SHXXT) is widely used for the treatment of constipation (Jong et al., 2010). This herbal formulation

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consists of rhizomes of *Rheum palmatum* L. (rhubarb), roots of *Scutellaria baicalensis* Georgi and rhizomes of *Coptis deltoidea* C. Y. Cheng & P. K. Hsiao, with a weight ratio of 2:1:1, respectively. Using the ancient process of TCMs preparation, the herbs are mixed and decocted with water before oral administration (Trivedi et al., 2007). However, convenient pharmaceutical herbal products are also used instead of the traditional herbal decocting process. Pharmaceutical herbal products are mainly made by industrial manufacturing methods of decoction, filtration, extraction, concentration, spray or fluid bed granulation, coating and filling (Jong et al., 2010; Lai et al., 2012; Shih et al., 2012).

Analytical methods have been reported to investigate the pharmacokinetic screening of multiple constituents in Chinese herbal formulae by HPLC-UV (Shia et al., 2011; Yin et al., 2009), and HPLC-MS/MS (Shaw et al., 2012; Shi et al., 2011; Zan et al., 2011). Furthermore, the assessment of rhein and/or its metabolites from various Chinese herbal formulae in human and animals has been studied (Jiang et al., 2012; Shia et al., 2011; Zan et al., 2011). In our previous study, the pharmacokinetic data of rhein demonstrate that the herbal formulae or the single herbal extract provides significantly higher absorption rate than the pure compound (Hou et al., 2014). This phenomenon suggests that the other herbal ingredients of SHXXT and rhubarb extract significantly enhance the absorption of rhein in rats. In addition, the herbal formulae (SHXXT) are more efficient than the single herb (Rhubarb) or the pure compound (rhein) in rhein absorption (Hou et al., 2014). According to our pharmacokinetic results for rhein, Chinese herbal formulae containing various ingredients may have an obvious impact on the contribution of a specific chemical to absorption, producing additive, synergetic, or antagonistic effects. In addition, studies on the comparative pharmacokinetic profiles of drugs with the various body conditions, including acute blood stasis, thrombotic focal cerebral ischemia, ulcerative colitis, and acute pancreatitis have been reported (Dai et al., 2014; Feng et al., 2013; Han et al., 2013; Zhu et al., 2014). Our hypothesis is that whether may influence the pharmacokinetics of rhein after SHXXT treatment. However, there is still limited information on the comparative pharmacokinetic studies of Chinese herbal formulae in normal and constipated rats and the impacts on pharmacokinetics of rhein in constipated animals.

Studies on gene expression profiling as an initial approach for mechanistic studies of therapeutic prediction, drug development, and the safety evaluation of herbal plants, herbal dietary supplements and Chinese herbal formulae have been reported (Cheng et al., 2010; Guo et al., 2010b). Analysis of the gene expression profiles using microarrays, both in vitro and in vivo, has the potential to be a practical approach for understanding the mechanisms of toxicity and tumorigenicity (Wen et al., 2011). In addition, investigation on gene expression changes in liver samples of rodents treated with Chinese herbal formulae can provide information about its mechanism of action, such as the modulation of drug metabolizing enzymes, which can potentially cause herb–drug interactions and may be responsible for different types of liver injury. We have proposed that analysis of alterations in gene expression profiles can be an initial approach for determining the mechanisms by which Chinese herbal formulae induced hepatotoxicity. In the present study, gene expression changes were analyzed with the focus on drug metabolizing genes in the livers of rats treated orally with SHXXT.

Recently, using TCM for constipation treatment is an alternative approach to Western medicine. However, the impacts of constipation on the pharmacokinetic profiling of rhein after SHXXT treatment remain unknown. In addition, concerns on safety evaluation of the commercial pharmaceutical product, SHXXT, gene expression changes focus on drug metabolizing genes in the livers of rats after SHXXT treatment need to be further investigated. Hence, the aim of this study is (i) to investigate the comparative pharmacokinetics of rhein from SHXXT in normal and loperamide-induced constipated rats by LC-MS/MS methods; and (ii) to explore gene expression profiling in drug-metabolizing genes after SHXXT treatment.

2. Materials and methods

2.1. Chemicals and Reagents

The chemicals rhein and ibuprofen (internal standard) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). LC/MS grade solvents were obtained from J.T. Baker, Inc. (Phillipsburg, NJ, USA) and chromatographic reagents were obtained from Tedia Co., Inc. (Fairfield, OH, USA). Triply deionized water (Millipore, Bedford, MA, USA) was used for all preparations. The pharmaceutical herbal product SHXXT manufactured in accordance with Good Manufacturing Practice (GMP) for Chinese Crude Drugs was obtained from pharmaceutical companies in Taiwan and has been used medicinally for patients. The pharmaceutical herbal product, SHXXT (product lot no. CP1403040), was purchased from Sheng Chang Pharmaceutical Co., Ltd. (Taipei, Taiwan).

2.2. LC-MS/MS

The analytical method was carried out as in the previous study (Hou et al., 2014). Briefly, the LC-MS/MS analysis was performed using a Waters Acquity UPLC™ system (Waters, Manchester, UK) consisting of a binary solvent manager, an automatic liquid chromatographic sampler and a Waters Xevo™ tandem quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. Separation was achieved using a Waters Acquity UPLC type BEH C18 (100 × 2.1 mm, 1.7 μm) analytical column, maintained at 40 °C in a column oven. The mobile phase consisted of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) with a linear gradient elution of 15–85% (v/v) B at 0–8 min, and 15% B at 8–10 min. The flow rate was set at 0.25 mL/min, and the injection volume was 5 μL. For operation in the MS/MS mode, the electrospray ion source was operated with a negative ion mode. The ESI parameters were set as follows: source temperature, 150 °C; desolvation temperature, 500 °C; desolvation gas flow, 800 L/h. The optimized cone voltages (CV) were 49 V and 15 V for rhein and ibuprofen, respectively. The multiple reaction monitoring (MRM) mode using specific precursor/product ion transitions was employed for quantification. The molecular ions of rhein and ibuprofen were fragmented at collision energies of 22 and 8 eV using argon as collision gas. Ion detection was performed by monitoring the transitions: m/z 283.1→238.9 for rhein and m/z 205.2→160.8 for ibuprofen. Ibuprofen was used as the internal standard for negative ion mode determination. The software program providing the data platform for spectral acquisition, spectral presentation and peak quantification was the MassLynx 4.1 software package.

2.3. Method validation

The method validation assays for quantification of rhein in rat plasma were carried out according to the currently accepted US Food and Drug Administration (FDA) bioanalytical method validation guidance. The specificity was tested by screening six different batches of drug-free rat plasma for the exclusion of any endogenous co-eluting interference at the peak regions of rhein and internal standard. The method validation assays were performed as the previous study (Hou et al., 2014).

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