Journal of Ethnopharmacology 173 (2015) 280–286

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Comparative pharmacokinetics of the main compounds of Shanzhuyu extract after oral administration in normal and chronic kidney disease rats

Min Zhao, Dawei Qian, Er-xin Shang, Shu Jiang*, Jianming Guo, Pei Liu, Shu-lan Su, Jin-ao Duan**, Leyue Du, Jinhua Tao

Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing University of Chinese Medicine, 138 Xianlin Road, Nanjing 210023, PR China

ARTICLE INFO

Article history:
Received 22 December 2014
Received in revised form
26 July 2015
Accepted 27 July 2015
Available online 29 July 2015

Keywords:
UPLC–MS
Morroniside
Loganin
Shanzhuyu extract
Chronic kidney disease
Pharmacokinetic study

ABSTRACT

Ethnopharmacological relevance: Pharmacokinetic studies on traditional Chinese medicine are useful to evaluate and predict the drug efficacy and safety. The renal impairment may affect drug clearance and other pharmacokinetic processes which can increase toxicity and drug to drug interactions or cause ineffective therapy. Pharmacokinetic studies in pathological status rats might be meaningful for revealing the action mechanism and improving clinical medication of the herb medicine.

Materials and methods: A highly sensitive and rapid ultra-performance liquid chromatography–mass spectrometry (UPLC–MS) method with multiple-reaction monitoring (MRM) mode was developed and validated for simultaneous quantitation of morroniside and loganin in normal and doxorubicin-induced chronic kidney disease (CKD) rat plasma after oral administration of Shanzhuyu (fruit of *Cornus officinalis*) extract.

Results: Both calibration curves gave satisfactory linearity ($r > 0.99$) at linear range of 1.96–1962.5 ng mL^{-1} for morroniside, 1.53–1531.25 ng mL^{-1} for loganin. The precision and accuracy of the *in vivo* study were assessed by intra-day and inter-day assays. The percentages of relative standard deviation (RSD) were all within 9.58% and the accuracy (RE) was in the −6.02% to 8.11% range. The extraction recoveries of morroniside, loganin and internal standard (IS) were all > 67.62% and the matrix effects ranged from 95.07% to 102.75%.

Conclusions: The pharmacokinetic behavior of morroniside and loganin in normal and CKD rat plasma was determined in this paper. The significant different pharmacokinetic parameters might partly result from the changes of P-glycoprotein and metabolic enzymes in the pathological state. The pharmacokinetic research in the pathological state might provide more useful information to guide the clinical usage of the herb medicine.

1. Introduction

Recently, many plant-derived natural products have been used in traditional medicine for the treatment of various diseases. *Cornus officinalis*, a species of dogwood, has been used for thousands of years as an important folk medicine, and it is also considered as one of the 25 vegetable drugs that are most frequently applied in PR China, Japan and Republic of Korea (Cao et al., 2011; Han et al., 2014; Ma et al., 2014). *Cornus officinalis* exhibits a number of biological activities, including immunological regulation, reducing blood glucose, antishock, antiarrhythmia and antibiosis (Lee et al., 2011; Park et al., 2011). Pharmacological studies have demonstrated that the fruit of *Cornus officinalis*, called 'Shanzhuyu', possesses immune regulation, anti-hyperglycemia, anti-aging, anti-oxidant, renal and neural protection effects. It is widely used for treatment of kidney diseases, including diabetic nephropathy. Recently, an aqueous extract prepared from the fruit has been reported to show strong anti-proliferative activity on estrogen receptor-positive breast cancer cells (Jeong et al., 2012; Telang et al., 2012; Zhang et al., 2013).

Characterization of the pharmacokinetic properties is essential for monitoring and prediction of drug disposition *in vivo* because of its special significance in the evaluation of drug therapeutic effect, dose adjustment and the rational use of the drug in the clinic (Feng et al., 2013). Drugs are used to treat diseases and only

* Corresponding author. Fax: +86 25 85811516.
** Corresponding author. Fax: +86 25 85811116.
E-mail addresses: jiangshu2000@163.com (S. Jiang), dja@njutcm.edu.cn (J.-a. Duan).

http://dx.doi.org/10.1016/j.jep.2015.07.037
0378-8741/

patients are the ultimate consumers. The pathological status and severity of disease seriously affects the drug absorption, which is directly related to drug efficacy and severity of side effects (Kang et al., 2014; Reid and Carlson, 2014; Schuetz et al., 2014).

Iridoid glycosides are the major active components widely distributed in Shanzhuyu (Yamabe et al., 2010; Liang et al., 2013; Zhou et al., 2013). Exploring dynamic of the iridoid glycosides may provide a helpful chemical proof for further pharmacology and active mechanism research of the herb medicine. Based on these points of view, a simple and accurate method was firstly developed and validated for simultaneous determination of the two main iridoid glycosides loganin and morroniside in normal and doxorubicin-induced chronic kidney disease (CKD) rats which were subjected to oral administration of shanzhuyu extract.

2. Experimental

2.1. Materials and reagents

Shanzhuyu raw material was purchased from Nanjing Guoyao Pharm Co. Ltd (Nanjing, China). Loganin, morroniside and chloramphenicol were purchased from Shanghai Winherb Medical S&T Development Co. Ltd (Shanghai, China). HPLC-grade acetonitrile was obtained from TEDIA Company Inc. (Fairfield, USA); formic acid and methanol were obtained from Merck KGaA (Darmstadt, Germany); Ultra-pure water was purified by an EPED super purification system (Nanjing, China). The distilled water was used for the extraction and preparation of samples. All other reagents and chemicals were of analytical grade and commercially available.

2.2. Instrument and analytical conditions

Chromatographic experiments were performed on an Acquity UPLC system (Waters Corp., Milford, MA, USA). Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm) was employed with a flow rate of 0.4 mL/min. The mobile phase consisted of 0.1% formic acid in water as solvent A and acetonitrile as solvent B. The gradient condition of the mobile phase was: B increased from 3% to 97% in 9 min. The sample injection volume was 5 μL. All separations of standards and serum samples were performed at room temperature. All sample extracts were maintained in the autosampler at 4 °C while awaiting injection.

Mass spectrometry detection was carried out using a Xevo Triple Quadrupole MS (Waters Corp., Milford, MA, USA) equipped with an electrospray ionization source (ESI) in multiple reaction monitoring (MRM) mode. Following optimization of the setting parameters, the instrument was operated in the negative mode. The parameters in the source were set as follows: capillary voltage 3.0 kV; source temperature 120 °C; desolvation gas flow 600 L/h; desolvation temperature 350 °C; cone gas flow 50 L/h. The cone voltage and collision energy were optimized for each analyte and selected values were given in Table 1. Dwell time was automatically set by MassLynx (Waters Corp., Milford, MA, USA).

Table 1
MS/MS detection parameters for morroniside and loganin.

Analyte	Retention time (min)	MRM transitions	Cone voltage (V)	Collision energy (eV)
Morroniside	4.41	451.0→179.0	18.0	15.0
Loganin	4.87	435.0→227.0	20.0	10.0
IS	6.06	320.8→151.9	20.0	20.0

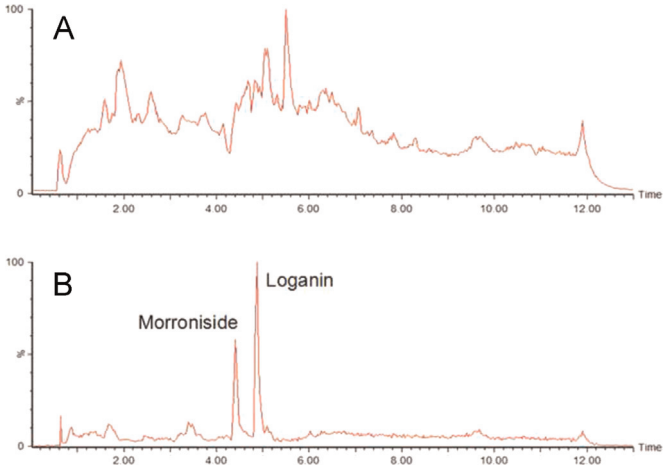

Fig. 1. Total ion chromatogram of shanzhuyu extract (A); extracted ion chromatogram of morroniside and loganin (B).

2.3. Shanzhuyu sample preparation

The shanzhuyu raw material (100 g) were extracted with 1000 mL water for 2 times and 2 h per time. The extraction solutions were combined and concentrated to 100 mL. The effective components in shanzhuyu were analyzed and evaluated before the pharmacokinetic experiment. Morroniside and loganin standards were dissolved in water at a series concentration to construct calibration curves. According to peak areas of the morroniside and loganin in the shanzhuyu extract sample, the contents of morroniside and loganin in the extract were 1.06% and 0.91%, respectively. The UPLC chromatograms of Shanzhuyu extract were displayed in the Fig.1. Peaks of all the compounds detected in UPLC–MS conditions were shown in the total ion chromatogram (Fig.1a). When the m/z of morroniside and loganin were entered into the total ion chromatogram to select their peaks, extract ion chromatogram was obtained as the Fig.1b.

2.4. Animals and induction of CKD in rats

Pathogen-free male Sprague-Dawley rats (180–220 g) were bought from the Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China) and the certificate number was SCXK (Jing) 2012-0001. The animal experiment was carried out according to the Regulations of Experimental Animal Administration (State Committee of Science and Technology of the People's Republic of China). The rats were housed in an air-conditioned animal quarter with 12 h light/12 h dark cycle at a temperature of 22 ± 2 °C and a relative humidity of 50 ± 10%.

According to previous reports, doxorubicin was dissolved in physiological saline at a concentration of 2.0 g/L. The CKD group was established by tail vein injection with 5.0 mg/kg doxorubicin for one time. After 7 days, rats with total amount of urinary protein in 24 h more than 100 mg were classified as the CKD group and used in the experiment (Chen et al., 2013; Hrenak et al., 2014).

2.5. Pharmacokinetic experiment

The rats were randomly divided normal rats oral administration shanzhuyu group and model rats oral administration shanzhuyu group ($n=6$). Before the test, the rats were fasted for 12 h, but were allowed water ad libitum. Then, shanzhuyu extracts were administered to the rats at a dose of 4.0 mL/kg body weight once, respectively.

The blood sample (about 0.3 mL) from the rat was drawn into a heparinized haemospasia tube at the time points of 5, 15, 30, 45,