Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2017, **15**(7): 0550–0560
Chinese Journal of Natural Medicines

Global identification of chemical constituents and rat metabolites of Si-Miao-Wan by liquid chromatography-electrospray ionization/ quadrupole time-of-flight mass spectrometry

LU Jin-Jin, HU Xue-Wen, LI Ping, CHEN Jun *

State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China

Available online 20 Jul., 2017

[ABSTRACT] Si-Miao-Wan (SMW), a tradiational Chinese medicinal formula consisting of Atractylodis Rhizoma, Phellodendri Chinensis Cortex, Coicis Semen, and Achyranthis Bidentatae Radix, has been used for the treatment of gout and gouty arthritis for many years. In the present study, a liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-Q-TOF/MS) method was established to identify the multiple constituents of SMW and its metabolites in rat biological samples after oral administration. A total of 48 compounds in SMW, including 21 alkaloids, 12 organic acids, 2 terpenes, 3 lactones, 2 phytosterols, and 8 other compounds, were tentatively characterized with the diagnostic-ion filtering strategy. Based on the diagnostic ions applied to identify compounds in SMW, 28 prototype compounds and 10 metabolic compounds were detected in the biological samples. This was the first comprehensive drug metabolism investigation of SMW in rats. The developed method could be a useful means for identifying the multi-components in SMW and the metabolic components. The results may help explore the possible metabolic processes and mechanism of action for SMW *in vivo*.

[KEY WORDS] Si-Miao-Wan; LC-Q-TOF/MS; Chemical constituents; Metabolites

[CLC Number] R917 **[Document code]** A **[Article ID]** 2095-6975(2017)07-0550-11

Introduction

Si-Miao-Wan (SMW), a famous traditional Chinese medicinal formula composed of Atractylodis Rhizoma, Phellodendri Chinensis Cortex, Coicis Semen, and Achyranthis Bidentatae Radix, has been used as a heat-clearing and diuresis-promoting drug and as an adjuvant to chemotherapy for gout and gouty arthritis in the clinic for many years. The underlying mechanism of its efficacy in treating gouty diseases has been intensively investigated and proven to be related to its hypouricemic process and nephroprotective actions via regulating renal urate transporters, enhancing antioxidant enzymes activities [1-2], increasing Sirt1 expression, and suppressing NF-κB/NLRP3 inflammasome activation [3]. Recently, alkaloids such as menisperine, jatrorrhizine, palmatine, and berberine, organic acids such as 3-*O*-feruloylquinic acid, lactones such as atractylenolide I, and terpenoids such as obacunone and obaculactone, have been reported to be the major components of SMW [4]. However, the effective substance of SMW responsible for its therapeutic efficacy remains unknown. It has been shown that phytomedicines including traditional Chinese medicine (TCM) exert their efficacies from the synergistic actions of multi-components in most cases [5]. Chemical components in TCM are not always directly correlated with their effects *in vivo* since pre-systemic elimination and hepatic metabolism would alter the exposure types and levels of natural constituents in the body [6-8]. A detailed knowledge of drug biotransformation in different biological matrices, such as blood, urine, bile, and tissues, is crucial for better understanding of the metabolic process and possible mechanisms of action for TCM *in vivo* [9-12]. Knowledge concerning the systemic identification of chemical and metabolic components of SMW is still lacking. Thus, it is of significance to develop a valid method for characterization of the comprehensive constituents and exploration of the possible

[Received on] 13-Aug.-2016
[Research funding] This work was supported by the National Natural Science Foundation of China (No. 81573705), the Natural Science Foundation of Jiangsu Province (No. BK20171392) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.
[*]**Corresponding author]** Tel: 86-25-83271382, Fax: 86-25-8327-1379, E-mail: chenj2002cpu@126.com.
These authors have no conflict of interest to declare.

metabolic mechanism of SMW.

In this work, the chemical components of SMW and its metabolites in rat biological matrices after oral administration were investigated by a newly developed liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-Q-TOF/MS) method. The chemical constituents in SMW and their metabolites in blood, urine, and bile were identified by the diagnostic-ion filtering strategy [13-14].

Materials and Methods

Reagents and materials

Methanol from Merck (Darmstadt, Germany), formic acid (99%) and acetonitrile from ROE Scientific Inc. (New Castle, DE, USA.) were of HPLC grade. Water used in this experiment was purified through a Milli-Q ultrapure water system (Millipore, Billerica, MA, USA). The solid-phase extraction (SPE) cartridges (Oasis HLB 1 cc, 30 mg) were purchased from Waters (Milford, MA, USA). The compound preparation of SMW was obtained from Jilin Zixin Pharmaceutical Industrial Co., Ltd. (Jilin, China). The reference compounds, including chlorogenic acid, ferulic acid, neochlorogenic acid, magnoflorine, phellodendrine, berberine, palmatine, ecdysterone, atractylenolide II, and atractylenolide III were purchased from Chengdu Must Bio-Technology Co., Ltd. (Sichuan, China).

Sample preparation

The compound preparation SMW was crushed into powder until uniformly mixed. The SMW powder (0.2 g) was accurately weighed and immersed in 10 mL of 50% methanol. The mixture was then sonicated in an ultrasonic bath for 45 min. The extract was then centrifuged at 13 000 r·min^{-1} for 10 min and the supernatant was applied for LC-Q-TOF/MS analysis. The reference standards were dissolved in methanol to a final concentration of 1 mg·mL^{-1}. All the sample solutions were filtered using a 0.22-μm filter before analysis.

Animals and drug administration

Male Sprague-Dawley rats (weighing 250 ± 15 g) used in this experiment were obtained from the Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). The animals were housed in cages with free access to food and tap water under the standard conditions of humidity (50% ± 10%), temperature (25 ± 2°C) and light (12 h light/12 h dark cycle). All the animals were handled with humane care throughout the experiment. The rats were acclimated to the laboratory for two weeks prior to the start of the experiment and were fasted with free access to tap water for 12 h before administration.

The animals were randomly divided into two groups: group A (n = 9) dosed with SMW and group B (n = 9) dosed with the blank solvent. Group A was then divided into three subgroups: bile sample collection group (n = 3), urine sample collection group (n = 3), and blood sample collection group (n = 3). Group B was also divided into three subgroups in the same way. The powder of SMW was suspended in 0.5% sodium carboxymethylcellulose (CMC-Na) solution (0.2 g·mL^{-1}) and orally administrated to rats at a dose of 1.0 mL/100 g body weight, while the control group received the same volume of 0.5% CMC-Na solution. The treatment dose of SMW to rats at 2.0 g/kg body weight was designed and transformed based on the conversions from clinical dosages and was doubled for the first dose.

Biological sample collection and preparation

The whole blood samples (about 500 μL each) were collected from the ophthalmic venous plexus of rats into 1.5-mL heparin-coated tubes at 15 min, and 0.5, 1, 2, 4, 6, 8, 10, and 12 h, respectively, after a single oral administration. Heparinized blood samples were then centrifuged at 4 000 r·min^{-1} for 10 min to obtain the plasma. The urine samples were collected using metabolic cages for a period of 24 h after the administration. For bile samples collection, rats were anesthetized with 5% urethane by intraperitoneal injection and then placed on a wooden plate. An abdominal incision was then made and the common bile duct was cannulated with polyethylene tubing. Bile samples were collected during the 0−24 h period after the dose. Blank samples were collected from the control animals in the same way. All the samples were stored at −80 °C until analysis.

The plasma samples at different time points (150 μL each) were mixed to obtain a comprehensive metabolic profile of SMW, considering that different types of compounds in SMW may process a time-different metabolism. Phosphoric acid (30 μL) was added to the mixed plasma sample (1 mL) to stop the action of enzymes. The mixed solution was ultrasonicated for 1 min and then diluted with 400 μL of water. The mixture was loaded onto a HLB SPE tube pre-conditioned with 1 mL of methanol and 1 mL of water successively. After washing with 1 mL of 2% acetonitrile in water, 1.6 mL of 100% methanol was added and collected during the elution step. The elution was dried under nitrogen gas at room temperature and re-dissolved in 150 μL of 50% methanol. The elution was centrifuged at 13 000 r·min^{-1} for 10 min at 4 °C and the supernatant was injected for LC-Q-TOF/MS analysis. The SPE method was also applied for the pretreatment of urine and bile samples. An aliquot of each sample (1 mL) without addition of phosphoric acid or water was directly loaded to a pre-conditioned HLB SPE tube, which was eluted through the same procedures as described above for the treatment of the plasma samples.

Chromatography and mass spectrometry

Chromatographic separation was performed on an Agilent 1290 series UHPLC system (Agilent Technologies, CA, USA) with Waters XSELECT HSS T3 column (150 mm × 4.6 mm, 3.5 μm) protected by a low dispersion in-line filter with 2-μm frits (Agilent Technologies). The mobile phase was consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution was carried out as follows: 0−25 min, 10%−25% B; 25−30 min, 25%−40% B; 30−45 min, 40%−70% B; 45−50 min, 70%−95% B; and 50−55 min, 95% B. The flow rate was set at 0.8 mL·min^{-1}, the injection volume was set at 2 μL, and the column temperature was main-