Journal of Ethnopharmacology 151 (2014) 287–291

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Pharmacokinetic comparisons of berberine and palmatine in normal and metabolic syndrome rats

Qing-Feng Liu, Xiao-Jin Shi, Zhong-Dong Li, Ming-kang Zhong *, Zheng Jiao, Bin Wang

Clinical Pharmacy Laboratory, Huashan Hospital, Fudan University, 12 Wu Lu Mu Qi M Road, Shanghai 200040, PR China

ARTICLE INFO

Article history:
Received 29 August 2013
Received in revised form
1 October 2013
Accepted 22 October 2013
Available online 22 November 2013

Keywords:
Berberine
Palmatine
San-Huang formula extract
Pharmacokinetics
Metabolic syndrome

ABSTRACT

Ethnopharmacological relevance: San-Huang formula is a popular traditional Chinese medicine (TCM) preparation to replenish Qi, resolve phlegm, dissipate blood stasis, and therapy metabolic syndrome in China. Metabolic syndrome, which is accompanied by Qi and blood stasis, mainly arises from spleen deficiency in essence. There is limited information available for differences of pharmacokinetic properties of San-Huang formula between normal and metabolic syndrome rats. The present study was conducted to compare the pharmacokinetics of berberine as well as palmatine in normal and metabolic syndrome rats following oral administration of San-Huang formula extract.

Materials and methods: The animals were orally administered with San-Huang formula extract with the equivalent dose of 60.4 and 12.5 mg/kg for berberine and palmatine, respectively. The blood samples were collected according to the time schedule. The concentrations of berberine and palmatine in rat plasma were determined by LC–ESI/MS. Various pharmacokinetic parameters were estimated from the plasma concentration versus time data using non-compartmental methods.

Results: It was found that AUC_{0-t}, C_{max}, Vd and CL of berberine and palmatine in metabolic syndrome rats were significantly different ($P < 0.05$) from normal rats.

Conclusions: The results indicated that berberine and palmatine have higher uptake and slower elimination in the rats with metabolic syndrome, which suggests that the rate and extent of drug metabolism were altered in metabolic syndrome rats.

1. Introduction

San-Huang formula, which consists of *Astragalus membranaceus* (Fisch.) Bunge, *Coptis chinensis* Franch., *Artemisia capillaris* Thunb., *Typha angustifolia* L. and *Alisma orientale* (Sam.) Juz., is widely used in traditional Chinese medicine to prevent and treat metabolic syndrome (Wang, 2007). It is mainly administrated as decoction in traditional Chinese medicinal prescription. Berberine and palmatine, having similar molecular structure, are important ingredients that responsible for the curative effects of San-Huang formula, and have chosen as active markers for controlling the quality of *Coptis chinensis* Franch. in Chinese Pharmacopoeia (The State Pharmacopoeia Commission of China, 2010) and San-Huang Formula (Xu et al., 2011). It has been demonstrated that berberine [Fig. 1(A)] has the activities of anticancer (Anis et al., 2001), antiatherosclerosis (Huang et al., 2011) and treating infectious diarrhea effects (Stermitz et al., 2000), and that palmatine [Fig. 1(B)] has the activities of antitumor (Kuo et al., 1995), liver-protective (Lee et al., 2010) and cardiovascular protective effects (Kim et al., 2009), respectively.

Metabolic syndrome is a combination of medical disorders that increase the risk of cardiovascular disease and diabetes, and is prevalent in up to 25% of the US population (Ford et al., 2002) and up to 16.5% of the Chinese population (Gu et al., 2005), and has been described as a cluster of multiple, partially or fully expressed, metabolic abnormalities within the single individual. These metabolic abnormalities consist of hypertension, dyslipidemia, obesity, and impaired glucose tolerance (Skilton Michael et al., 2007). Moreover, metabolic syndrome, which mainly arised from spleen deficiency and disorders of Qi and blood transportation in traditional Chinese medicine theory, was also known as syndrome X, cardiometabolic syndrome, insulin resistance syndrome, Reaven's syndrome, CHAOS (an abbreviation for coronary artery disease, hypertension, atherosclerosis, obesity, and stroke), and the Deadly Quartet (Kaplan, 1989; Haffner et al., 1992; Schindler, 2007). With metabolic syndrome developing, the Qi and blood circulation will further be affected and thus lead to new pathological changes. Pharmacokinetic characteristics could be affected by disease condition (Ren et al., 2006; Wen et al., 2001; Hardwick et al., 2012). Therefore, it is very important to investigate the pharmacokinetics

* Corresponding author. Tel.: +86 21 52889381; fax: +86 21 32120059.
E-mail address: wooo_08@163.com (M.-k. Zhong).

0378-8741/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2013.10.031

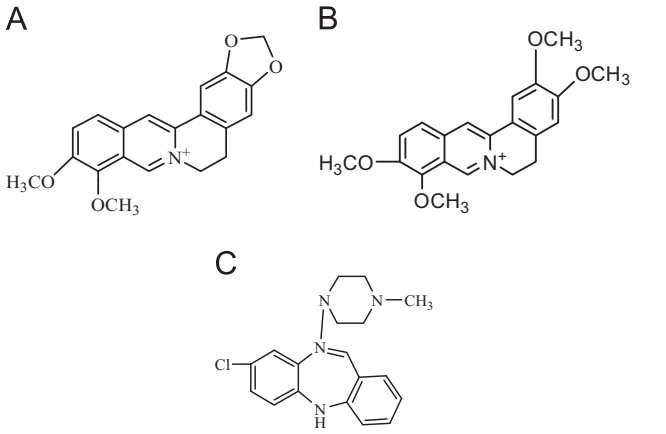

Fig. 1. Chemical structures of two compounds and IS: berberine (A), palmatine (B), and internal standard and clozapine (C).

of drugs in animals with metabolic syndrome, which may influence absorption, metabolism and elimination of drugs in blood.

In recent years, some pharmacokinetic studies on berberine and palmatine have been performed on healthy Chinese volunteers (Hua et al., 2007; Pan et al., 2002; Zeng and Zeng, 1999) and normal animals (Deng et al., 2008; Lu et al., 2006; Wu et al., 2009). However, the pharmacokinetics of berberine and palmatine has not been investigated in detail. So far, most studies were focused on the pharmacokinetic characteristics of berberine and palmatine in healthy human beings or normal animals. And the pharmacokinetic properties of berberine and palmatine in animal with metabolic syndrome and the differences between normal animals and metabolic syndrome animals were seldom reported. The primary objective of this study was to investigate the possible pharmacokinetic differences of the compounds after oral administration of San-Huang formula extract in normal rats and metabolic syndrome rats.

2. Materials and methods

2.1. Chemicals and reagents

Raw materials, including *Astragalus membranaceus* (Fisch.) Bunge, root, thick section; *Coptis chinensis* Franch. rhizome; *Artemisia capillaris* Thunb., aerial part; *Typha angustifolia* L., pollen and *Alisma orientale* (Sam.) Juz., tuber, thick section, all recorded in Chinese Pharmacopoeia (The State Pharmacopoeia Commission of China, 2010), were purchased from Chinese Herbal Pieces, Shanghai Hongqiao Co. Ltd., Shanghai, China, and were identified by Dr. Ying Wang (Shanghai University of Traditional Chinese Medicine, Shanghai, China). The materials, consisting of physiological traits, microscopic identification of Chinese drug powder and the contents of water, total ash, ethanol-soluble extractives, water-soluble extractives, heavy metalst, harmful elements, organochlorine pesticide residues, active compounds, were all subject to quality control according to Chinese Pharmacopoeia (The State Pharmacopoeia Commission of China, 2010). Especially, active compounds including astragaloside IV, calycosin-7-O-beta-D-glucopyranoside, berberine, epiberberine, coptisine, palmatine, chlorogenic acid, isorhamnetin-3-O-neohesperidoside and alisol B-23-acetate in these materials were strictly determinded by reversed-phase high-performance liquid chromatography (HPLC). The contents of astragaloside IV and calycosin-7-O-beta-D-glucopyranoside in the decoction pieces of *Astragalus membranaceus* (Fisch.) Bunge were 1.22 ± 0.25 and 0.42 ± 0.11 mg/g; berberine, epiberberine, coptisine and palmatine in *Coptis chinensis* Franch. were 83.31 ± 17.23, 12.72 ± 3.81, 24.17 ± 4.31 and 23.03 ± 4.91 mg/g; chlorogenic acid in *Artemisia capillaris* Thunb. was 5.93 ± 0.57 mg/g; isorhamnetin-3-O-neohesperidoside in *Typha angustifolia* L. was 5.85 ± 1.13 mg/g; alisol B-23-acetate in the decoction pieces of *Alisma orientale* (Sam.) Juz. was 5.95 ± 0.87 mg/g, respectively.

The reference standards of berberine (86.7% purity), palmatine (86.2% purity), and clozapine [IS, 99.8% purity, Fig. 1(C)] were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany); and AR-grade formic acid was purchased from Sinopharm Group Chemical Reagent (Shanghai, China). Water was distilled and purified using a Milli-Q Water Purification System (Millipore, Bedford, MA, USA). Drug-free rat plasma was gained from healthy rat provided by the Department of Animal Science of Fudan University (Shanghai, China).

2.2. Preparations of San-Huang formula extract

Five crude herbs including *Astragalus membranaceus* (Fisch.) Bunge, *Coptis chinensis* Franch., *Artemisia capillaris* Thunb., *Typha angustifolia* L. and *Alisma orientale* (Sam.) Juz. (30, 9, 15, 15 and 15 g) were thoroughly soaked in water for 30 min and decocted twice with water (1:10, w/v). The first and second decoctions were respectively boiled using high heat for 60 min and 45 min, then they were put together and condensed under 60 °C. After five times of ethanol (v/v) was added, the solution was stayed overnight, filtered, condensed to 8.0 ml and stored at 4 °C before use. The quantitative analysis for San-Huang formula extract has been studied and published in our previous article (Liu et al., 2010; Xu et al., 2011), and that the contents of berberine and palmatine in the San-Huang formula extract has been determined by LC–UV to be 8.39 and 1.74 mg/ml, respectively (Xu et al., 2011). So 1.44 ml/kg dose of this decoction (according to human dosage in clinical practice and human-rat coefficient of skin surface area) was equivalent to 60.40 mg/kg dose of berberine and 12.52 mg/kg dose of palmatine.

2.3. Animals

The investigation was conducted in accordance with the ethical principles of animal use and care (Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes, 1986). A total of 24 male rats weighing 180 ± 20 g were used for this study. The rats were supplied by the Department of Animal Science of Fudan University (Shanghai, China). The rats were maintained in an air-conditioned animal quarter at a temperature of 22 ± 2 °C and a relative humidity of $50 \pm 10\%$.

Rats were randomly divided into the following two groups ($n=12$): normal control group and metabolic syndrome model group. Water with 20% sucrose and food with high sugar, fat and salt (laboratory rodent chow, Shanghai, China) were allowed ad libitum to metabolic syndrome model group for 3 months; however, conventional water and food (laboratory rodent chow, Shanghai, China) were allowed ad libitum to normal control group. The animals were acclimatized to the facilities for 5 days, and then fasted with free access to water for 12 h prior to each experiment.

2.4. In vivo study

Each of the four rats was in an individual cage, and were fasted overnight (16 h) prior to oral administration of 1.44 ml/kg San-Huang formula extract. 0.25 ml Blood samples were collected in heparinized Eppendorf tube via the tail nick before dosing and subsequently at 0.25, 0.5, 0.75, 1, 2, 3, 5, 7 and 12 h following oral administration. Following centrifugation (1441*g* for 10 min), the