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Pharmacokinetic comparisons of rutaecarpine and evodiamine after oral administration of Wu-Chu-Yu extracts with different purities to rats

Shixi Xu[a,b], Jun Peng[a], Yuanjian Li[a], Lingyun He[a], Fei Chen[a], Jie Zhang[a], Jinsong Ding[a,*]

[a] School of pharmaceutical Sciences, Central South University, Changsha 410013, Hunan Province, PR China
[b] Chenzhou No.1 People's Hospital, Chenzhou 423000, Hunan Province, PR China

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ABSTRACT

Ethnopharmacological relevance: Wu-Chu-Yu is a well-known herbal drug used for hypertension. Rutaecarpine and evodiamine are main bioactive components of the medicine.
Materials and methods: A sensitive and specific HPLC method was developed to analyze rutaecarpine (Rut) and evodiamine (Evo) in rat whole blood. The pharmacokinetics of Rut and Evo after oral administration of Wu-Chu-Yu extracts with different purities to rats was compared to evaluate the effect of purity of Wu-Chu-Yu extracts on the absorption of Rut and Evo. Male Sprague–Dawley rats were given Wu-Chu-Yu extracts with different purities (high, medium and low) approximately the same doses of equivalent to Rut (40 mg/kg) and Evo (31 mg/kg). The contents of Rut and Evo were 45 and 35%, 28 and 21%, 9 and 7% in high, medium and low purity extracts, respectively. At different time points (0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3 and 4 h) after administration, the concentrations of Rut and Evo in rat whole blood were determined by HPLC, and main pharmacokinetic parameters were calculated.
Results: The results indicated that the absorption of Rut and Evo in Wu-Chu-Yu extracts was improved when compared with the pure Rut and Evo and there were significant differences among different groups.
Conclusions: The bioavailability of Rut and Evo was increased along with the increasing of purity (16%–80%) in Wu-Chu-Yu extracts.

1. Introduction

Wu-Chu-Yu, the dried unripe fruit of *Evodia rutaecarpa* (Juss) Benth (*Tetradium ruticarpum*), is a well-known Chinese herbal drug used widely to treat hypertension, angina pectoris, gastrointestinal disorder and headache (Yuan, 2000). Rutaecarpine (Rut) and evodiamine (Evo) (Fig. 1), the bioactive alkaloids existing in Wu-Chu-Yu, have been used as phytochemical marker for the quality control of Wu-Chu-Yu in Chinese pharmacopoeia. It has been reported that Rut is able to enforce contraction of atrium, increase the frequency of contraction and protect the myocardium against ischemia-reperfusion injury. Rut also has hypotensive effect, which is mediated by stimulation of calcitonin gene-related peptide (CGRP) synthesis and release via activation of vanilloid receptor subtype 1 (VR1) (Bell and McDermott, 1996; Kobayashi et al., 2001; Rang et al., 2003). Similarly, Evo is able to exert protective effect on cardiac anaphylaxis and vasodilatation effect by decreasing Ca^{2+} influx of vascular smooth muscle cells (VSMC) (Hu and Li, 2003).

Although Rut and Evo are highly permeable, they are poorly absorbed and bioavailabilities are low due to the poor solubility. C_{max} of Evo was 49.0 ± 19.0 ng/ml after oral administration to Sprague–Dawley rats (SD rats) (500 mg/kg), and the bioavailability was about 0.1% (Shyr et al., 2006). Our previous study has demonstrated that C_{max} of Rut was below the Lower Limit of Quantification (LLOQ) of our assay method after oral administration to SD rats with Rut crude drug (40 mg/kg). The plasma concentration of Rut was 2.4 ± 3.0 ng/ml 30 min after administering intragastrically to the spontaneously hypertensive rats (SHR) with Rut crude drug (40 mg/kg) for 18 days, whereas it reached 5.2 ± 1.0, 8.7 ± 0.4 or 18.1 ± 1.8 ng/ml 30 min after oral administration with the solid dispersion of Rut at a dosage of 10, 20 or 40 mg/kg for 18 days (Ding et al., 2008). C_{max} of Rut in Beagle dogs was below LLOQ when given with Rut crude drug (75 mg/kg) orally, whereas it respectively reached 11.7 ± 0.3 and 51.0 ± 10.4 ng/ml after oral administration with the solid dispersion and nanosuspension of Rut at a dosage of 25 mg/kg (Wu and Jiang, 2007). These results showed that the solid dispersion and nanosuspension increased the bioavailability of Rut, but it would be difficult to develop these two dosage forms due to their complicated pharmaceutical techniques, high manufacturing cost, and poor stability of Rut as well.

The purity of herbal extracts plays very important role in the solubility and absorption in gastrointestinal tract for the natural medicine. It has been reported that for hesperidin, berberine and puerarin, the low purity of extract displayed higher absorption

* Corresponding author. Tel.: +86 731 82650367; fax: +86 731 82650442.
E-mail address: Dingjs0221@163.com (J. Ding).

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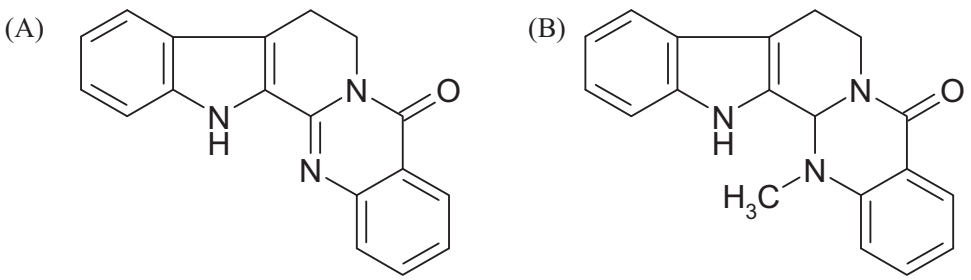

Fig. 1. Chemical structures of Rut (A) and Evo (B).

and accumulated absorption rate (Huang and Yang, 2008; Zhang et al., 2008; Zhou and Yang, 2008), whereas it was a different story for asperosaponin VI (Wu et al., 2008). Saponins of *Panax notoginseng* aqueous solution could improve bioavailability of ginsenoside Rg1 compared with ginsenoside Rg1 monomer (Xu et al., 2003). Extract of *Cortex Moutan* could delay the elimination of paeoniflorin which is one of the main active ingredients and thus enhance its bioavailability (Wu et al., 2009). The pharmacokinetic parameters of baicalin significantly changed after oral administration of Shuang-Huang-Lian with the different combinations of its constitutional herbs (Di et al., 2006). Based on these reports, we hypothesize that some ingredients in Wu-Chu-Yu extracts may improve the absorption of Rut and Evo in gastrointestinal tract, and in turn to enhance their bioavailability. Therefore, in present study we investigated the effect of the purity of Wu-Chu-Yu extract on the absorption of Rut and Evo in rats. An HPLC method was developed and used to measure the whole blood concentrations of Rut and Evo. The pharmacokinetic parameters of Rut and Evo were determined and analyzed. The results of this study could be utilized to improve the clinical therapeutic efficacy and further pharmacological studies for Rut and Evo.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 200–250 g, were purchased from Slac Laboratory Animal Co., (Hunan, China) and kept in an environmentally controlled breeding room (temperature: 20 ± 2 °C, humidity: $60 \pm 5\%$) for 1 week before the experiments and fed standard laboratory food and tap water. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 85-23, revised 1986).

2.2. Reagents and materials

Wu-Chu-Yu was purchased from Shaoyang Prefecture of Hunan Province, China, in 2009, and was authenticated by Dr. Yingjun Zhou (Department of pharmacognosy, Central South University). Standard Rut (98.9% purity) was prepared by the Department of Pharmaceutical Chemistry (School of Pharmaceutical Science, Central South University). Standard Evo was purchased from Sigma Co. (USA). Internal standard (IS) of diazepam was purchased from National Institute for the Control of Pharmaceutical and Biological Products (China). Methanol and acetonitrile (chromatographic grade) were purchased from Tedia Co., Inc. (USA). Water is ultra pure grade, and all other chemicals used in the study were of analytical grade at least.

2.3. Preparation of Wu-Chu-Yu extract

Wu-Chu-Yu was extracted by refluxing with 12 volumes of 60% ethanol for 1.5 h, repeated for 3 times, and then concentrated by Rotary Evaporator until no ethanol smell from the solution. Precipitation was obtained by centrifuging at 3000 rpm for 10 min, and then dissolved in 15 volumes of 90% ethanol. Water was slowly added to this extracted solution step by step until ethanol final concentrations reached 51.4%, 46.8% and 42.5%. Recrystallized in methanol, precipitations of three different purities were collected, respectively. The concentrations of Rut and Evo were measured by HPLC (Chuang et al., 1996), which were 45 and 35% (high purity); 28 and 21% (medium purity); 9% and 7% (low purity) in these three different samples.

2.4. Experimental protocol

18 rats were divided randomly into 3 groups ($n = 6$): Wu-Chu-Yu (high purity, H), Wu-Chu-Yu (medium purity, M) and Wu-Chu-Yu (low purity, L). Animals were deprived of food but given free access to water for 12 h and then orally received Wu-Chu-Yu extracts of different purities (suspended in 0.5% carboxymethyl cellulose sodium salt aqueous solution) at doses of equivalent to 40 mg/kg (Rut) and 31 mg/kg (Evo). Blood samples (0.3 ml) were collected in heparinized tube from the retroorbital sinus (Xu et al., 2008) at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3 and 4 h after Wu-Chu-Yu extracts administration.

2.5. Analysis of Rut and Evo in whole blood samples

To disrupt the red cells, the whole blood sample (250 μl) was sonicated for 15 min. 50 μl ammonia water and 50 μl of IS (20 ng/ml diazepam in methanol) were added and mixed well. Then 1 ml of ethyl acetate was added and vortexed for 3 min. To enhance the chemicals dissolving, the sample was sonicated for 15 min followed by centrifuging at 3000 rpm for 10 min. The organic extract (upper layers) was transferred into another tube and evaporated by N_2 at 40 °C. The residues were reconstituted in 50 μl of mobile phase (methanol–water 67:33, v/v), vortexed for 30 s and then centrifuged at 12,000 rpm for 10 min. The sample of upper layers (20 μl) was injected into the HPLC system for analysis. All the procedures were performed at room temperature.

2.6. HPLC analysis

The HPLC analysis was carried out using the LC-2010C HPLC system (Shimadzu, Japan) with a Weldwom-C_{18} (250 mm × 4.6 mm, 5 μm) column. The mobile phase consisted of methanol and water (67:33, v/v) at a flow rate of 1.0 ml/min. The column maintained at ambient temperature. The UV-vis detector was set at 343 nm (Rut) and 225 nm (Evo) to determine the pharmacokinetic parameters for Rut and Evo simultaneously. The mobile phase was filtered