

Pharmacokinetic comparison of puerarin after oral administration of Jiawei-Xiaoyao-San to healthy volunteers and patients with functional dyspepsia: influence of disease state

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Abstract

Objectives An investigation has been designed and conducted to compare the pharmacokinetics of puerarin after oral administration of Jiawei-Xiaoyao-San to healthy volunteers and to patients with functional dyspepsia.

Methods Quantification of puerarin in serum was achieved using a simple and rapid HPLC method for pharmacokinetic study.

Key findings After oral administration of decoctions of Jiawei-Xiaoyao-San to healthy volunteers and patients with functional dyspepsia, puerarin was absorbed and reached a maximum concentration of 56.47 ± 15.60 and 29.40 ± 8.89 ng/ml at 51.00 ± 8.22 and 50.00 ± 12.25 min, respectively. Compared with the value of AUC_{0-360} (5.55 ± 1.47 μ g/ml min) in healthy volunteers, a smaller value of AUC_{0-360} (3.35 ± 1.17 μ g/ml min) was observed in patients with functional dyspepsia.

Conclusions There were statistically significant differences in the pharmacokinetic parameters of puerarin including the values for C_{max} , AUC_{0-360} , Cl/F and MRT_{0-360} between healthy volunteers and patients with functional dyspepsia. The pharmacokinetic parameters showed that functional dyspepsia reduced the absorption of puerarin after oral administration of Jiawei-Xiaoyao-San.

Keywords functional dyspepsia; Jiawei-Xiaoyao-San; pharmacokinetics; puerarin

Introduction

Functional dyspepsia is a syndrome defined by chronic or recurrent upper abdominal symptoms without any organic or biochemical abnormality.^[1] As many as 40% of the population have upper digestive tract symptoms regularly, and up to 63% report having had dyspepsia at some time in their life.^[2,3] Recent reviews have indicated that disease states can modify the pharmacokinetics of drugs; functional dyspepsia was one example.^[4]

Jiawei-Xiaoyao-San is a traditional Chinese medicine which has been used successfully to manage functional dyspepsia and depression in clinical practice for thousands of years.^[5–7] The decoction is prepared by boiling 14 kinds of herbs.^[6] The main medicinal component of this decoction is Radix Puerariae, the dried root of *Pueraria lobata* (Willd.) Ohwi, with puerarin as its major effective constituent.^[8] It has been reported that puerarin exerted a protective effect against gastric mucosal injury and intestinal ischaemia–reperfusion injury.^[9,10] Recently there have been many reports on the pharmacokinetics of puerarin.^[11–15] Kamerling *et al.*^[16] reported the pharmacokinetics of motilin in patients with functional dyspepsia, but there has been no report on the pharmacokinetics of this traditional Chinese medicine in functional dyspepsia patients.

We have explored the influence of functional dyspepsia by comparing the pharmacokinetics of puerarin after oral administration of Jiawei-Xiaoyao-San in healthy volunteers and patients with this condition. Information obtained might be useful for the clinical applications of Jiawei-Xiaoyao-San in patients with functional dyspepsia.

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Materials and Methods

Crude drugs

The Jiawei-Xiaoyao-San formula consisted of 14 crude drugs: Radix Puerariae, Radix Angelicae Sinensis, Radix Paeoniae Alba, bupleurum root, Poria, Rhizoma Atractylodis Macrocephalae, Rhizoma Zingiberis Recens, Fructus Jujubae, Herba Menthae, Fructus Gardeniae, Radix Glycythizae, Cortex Moutan, magnoliae cortex, and Fructus Aurantii in a ratio of 8 : 5 : 5 : 5 : 5 : 3 : 2 : 3 : 3 : 3 : 3 : 3 : 6 : 6 on a dry weight basis. All were purchased from the dispensary store for traditional Chinese medicine at the West China Hospital (Chengdu, China). The herbal materials were extracted twice by refluxing in water (1 : 12, g/ml) for 1 h and the water extract was concentrated and lyophilised. The dried powder was stored at 4°C before use.

Chemicals and reagents

Puerarin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and methanol were of HPLC grade from Tedia Company, Inc., Fairfield, Ohio (US). House triple-distilled water from silica glass equipment was always used.

Instrumentation

The HPLC system consisted of a Waters 717 pump, a Waters 2487 dual λ absorbance detector and a Waters 2515 pump, which were from Waters Assoc., Milford, Massachusetts (US). An LC workstation for data collection was also from Waters Assoc. A Diamonsil C₁₈ reversed-phase column (5 μ m, 250 \times 4.6 mm), which was protected by an RP18 (5 μ m) guard column were both from Dikma (China). The mobile phase was water–acetonitrile (81 : 19, v/v) with a UV detector setting at 254 nm. The flow rate was 1 ml/min.

Volunteers

Six patients with functional dyspepsia (three females, three males; mean age, 27.67 years; range 23–30 years) and five healthy volunteers (two females, three males; mean age, 24.4 years; range 23–26 years) participated in this study. The Medical Ethics Committee of West China Hospital, Sichuan University, China approved the study protocols. The study was performed according to Good Clinical Practice and International Conference on Harmonization guidelines.

Volunteers were eligible to participate in the study if they were healthy, as assessed by medical screening. The main exclusion criteria were a history of gastrointestinal symptoms, abdominal surgery and the use of medication. Patients underwent medical screening and were eligible if they had functional dyspepsia, defined as persistent or recurrent upper abdominal pain or discomfort for at least 12 weeks within the last 12 months, which did not need to be consecutive, according to the Rome II criteria.^[1] All patients suffered from symptoms of epigastric fullness or distension after a meal. They were excluded if there was evidence of organic disease or a history of gastrointestinal disease (verified by recent endoscopy) or surgery that was likely to explain the symptoms. All subjects gave informed consent.

Preparation of assay standard samples

Standard stock solution was prepared by dissolving puerarin in methanol to yield a nominal concentration of 81 μ g/ml and was kept at 4°C before use. Standard samples (12.15, 24.3, 48.6, 121.5, 243 and 486 ng/ml) were prepared by mixing blank serum with appropriate amounts of standard stock solution prepared as above. Quality control samples to determine the recovery, accuracy and precision of the method were independently prepared at low (24.3 ng/ml), medium (121.5 ng/ml) and high (486 ng/ml) concentrations. All samples were stored at –20°C until analysis.

Blood sample preparation

The content of puerarin was calculated first, and then Jiawei-Xiaoyao-San (containing 443.4 mg/kg puerarin) at a dose of 5 g/kg was orally administered to patients and volunteers. The patients and volunteers had been fasting for 12 h before drug administration but they had free access to water. Blood samples (10 ml) were collected at 0, 5, 10, 15, 30, 45, 60, 120, 180, 240 and 360 min after administration, placed for 30 min at room temperature, and then centrifuged at 3000g for 10 min to obtain serum. Methanol (60 μ l: including the internal standard 2,4-dihydroxy benzaldehyde 64 ng) was added to 1-ml serum samples, vortex-mixed for 30 s, and then for deproteinisation and extraction the samples were put in a boiling water bath (> 95°C) for 10 min. The samples then underwent agitation with a mini-electric agitator under cooling to room temperature, followed by centrifugation at 8500 rev/min for 10 min. The supernatant was collected and filtered through a 0.45- μ m filter, and 80 μ l sample solution was injected into the HPLC system for analysis. The same sample preparation was applied for validation of the analytical method.

Statistical analysis

All data were expressed as mean \pm SD. The database was set up with the SPSS 15.0 software package from SPSS Inc., Chicago, Illinois (US). Differences between two groups were analysed by one-way analysis of variance. A probability of less than 0.05 was considered to be statistically significant.

Results

The retention time of puerarin was 6.2 min. No interfering peaks were detected at this retention time (Figure 1). The curve of peak area vs puerarin concentration was linear over the range 12.15–486 ng/ml, and the detection limit was 6.1 ng/ml. Using the least-squares method a regression equation was obtained:

$$C \text{ (ng/ml)} = 57.44A + 0.42 \quad (1)$$

($n = 6$, $r = 0.9976$)

where A refers to peak area ratio (analyte/internal standard), and C is the concentration of puerarin. The coefficients of variation values of intra-day assay were 1.05%, 3.17% and 4.93% at low, medium and high concentrations of puerarin, respectively ($n = 5$). The coefficients of variation values of inter-day assay were 1.13%, 4.93% and 4.51% at low, medium and high concentrations of puerarin, respectively ($n = 5$, Table 1). The recovery rates of the puerarin from serum were $97.20 \pm 4.74\%$, $106.03 \pm 3.84\%$ and $95.33 \pm 7.05\%$ at low,

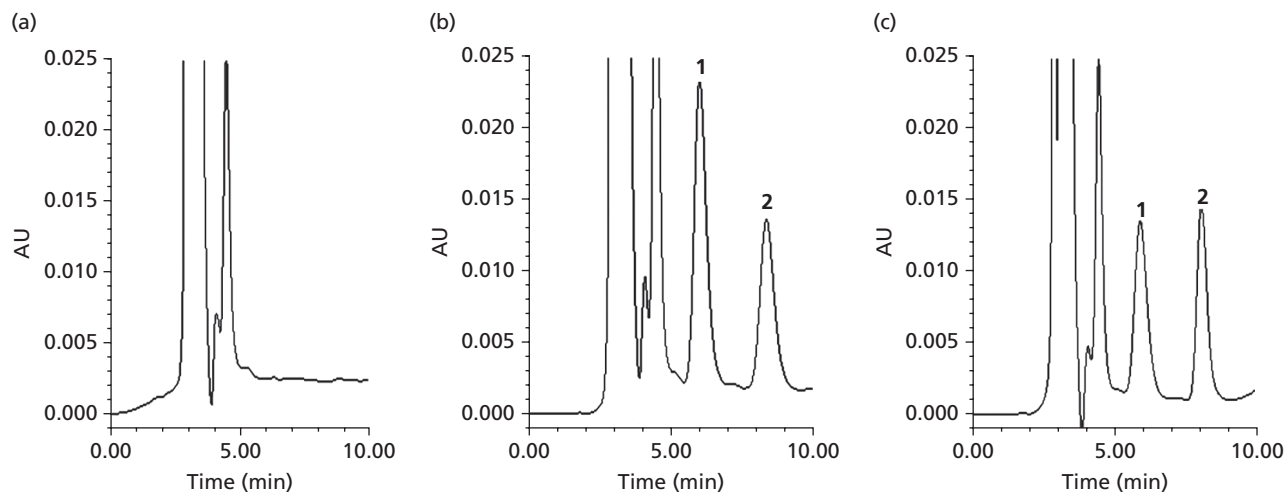


Figure 1 Chromatogram of puerarin. (a) Blank patient serum chromatogram; (b) blank plasma mixed with (1) puerarin and (2) internal standard; (c) serum sample at 60 min after oral administration of Jiawei-Xiaoyao-San at a dose of 5 g/kg to healthy volunteers.

Table 1 The precision data of the proposed HPLC method for puerarin in rat plasma samples

Nominal concn (ng/ml)	Precision			
	Intra-day ($n = 5$)		Inter-day ($n = 5$)	
	Mean \pm SD (ng/ml)	RSD (%)	Mean \pm SD (ng/ml)	RSD (%)
24.3	23.26 \pm 2.46	1.05	23.40 \pm 2.65	1.13
121.5	129.68 \pm 4.12	3.17	128.92 \pm 6.35	4.93
486	486.82 \pm 24.01	4.93	460.09 \pm 20.77	4.51

RSD, relative standard deviation.

Table 2 Recovery of puerarin from human serum

Standard sample concn (ng/ml)	Recovery (%) (mean \pm SD)	RSD (%)
24.3	97.20 \pm 4.74	4.88
121.5	106.03 \pm 3.86	3.64
486	95.33 \pm 7.05	7.48

$n = 3$. The standard sample concentrations represented low (24.3 ng/ml), medium (121.5 ng/ml) and high (486 ng/ml) concentrations of puerarin. RSD, relative standard deviation.

medium and high concentrations of puerarin, respectively (Table 2). The results of precision and recovery rates conformed to the principle of bio-sample analysis.

The serum puerarin concentration–time curves were analysed using a DAS program (the Chinese Society of Mathematical Pharmacology) on a personal computer to determine the compartment model, and the pharmacokinetic parameters were calculated. The serum puerarin concentration–time curve conformed to the one-compartment with the first absorption model. The profiles of puerarin concentration vs time in patients and healthy volunteer sera after oral administration of

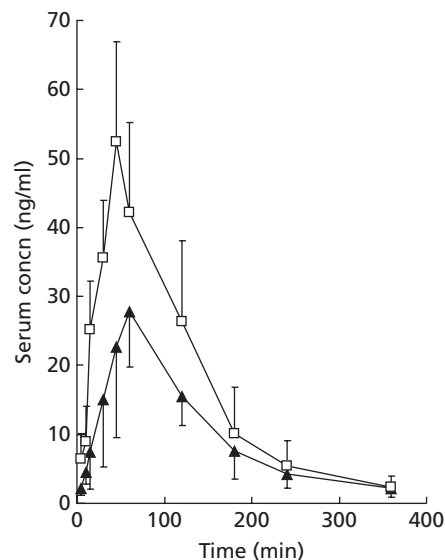


Figure 2 Serum concentration vs time profile of puerarin in patients with functional dyspepsia and healthy volunteers following oral administration of Jiawei-Xiaoyao-San. Patients and volunteers were administered 5 g/kg Jiawei-Xiaoyao-San. Values are mean \pm SD, $n = 6$ patients with functional dyspepsia (\blacktriangle) and $n = 5$ healthy volunteers (\square).

Jiawei-Xiaoyao-San are presented in Figure 2. Table 3 shows the pharmacokinetic parameters of puerarin derived from the serum after oral administration of the decoctions of Jiawei-Xiaoyao-San.

Discussion

After oral administration of Jiawei-Xiaoyao-San in healthy volunteers, puerarin was absorbed at a fast absorption rate and reached a maximum plasma concentration (C_{max}) value (56.47 \pm 15.60 ng/ml) within 51.00 \pm 8.22 min. The serum concentration of puerarin declined with a half-life ($t_{1/2}$) value of

Table 3 Pharmacokinetic parameters of puerarin in serum after oral administration of Jiawei-Xiaoyao-San to patients with functional dyspepsia and healthy volunteers

Parameter	Patients with functional dyspepsia	Healthy volunteers
T _{max} (min)	50.00 ± 12.25	51.00 ± 8.22
C _{max} (ng/ml)	29.40 ± 8.898	56.47 ± 15.60**
AUC ₀₋₃₆₀ (μg/ml min)	3.35 ± 1.17	5.55 ± 1.47*
t _{1/2} (min)	17.44 ± 20.85	11.91 ± 7.14
k _a (1/min)	0.113 ± 0.107	0.081 ± 0.057
Cl/F (L/min per kg)	38.44 ± 11.12	22.12 ± 6.78*
MRT ₀₋₃₆₀ (min)	115.2 ± 16.38	83.06 ± 11.25**

Values are mean ± SD, *n* = six patients with functional dyspepsia, *n* = five health volunteers. **P* < 0.05, ***P* < 0.01. T_{max}, time to C_{max}; C_{max}, maximum plasma concentration; AUC, area under the curve; t_{1/2}, half life; k_a, absorption constant; Cl/F, apparent clearance; MRT, mean residence time.

11.91 ± 7.14 min. However, after oral administration of Jiawei-Xiaoyao-San to functional dyspepsia patients the C_{max} value of puerarin was 29.40 ± 8.89 ng/ml within 50.00 ± 12.25 min, and the serum concentration of puerarin declined with a value of t_{1/2} of 17.44 ± 20.85 min. Compared with the AUC₀₋₃₆₀ (area under the curve) value (5.55 ± 1.47 μg/ml min) after oral administration of Jiawei-Xiaoyao-San in healthy volunteers, a smaller AUC₀₋₃₆₀ value (3.35 ± 1.17 μg/ml min, *P* < 0.05) of puerarin in functional dyspepsia patients was obtained.

The analysis of puerarin in animal and human plasma by HPLC based on liquid–liquid extraction has been reported, but unfortunately, those methods could not provide satisfactory sensitivity in the human study after oral administration of Jiawei-Xiaoyao-San.^[11–14] We have reported the development of a reversed-phase HPLC method in combination with a boiling-water-bath extraction, which showed sufficient specificity, sensitivity and simplicity for the measurement of puerarin in human serum.^[6] After oral administration of Jiawei-Xiaoyao-San, the T_{max} (time to C_{max}) of puerarin was advanced, compared with oral administration of *Pueraria lobata* Ohwi.^[15] The value of t_{1/2} of puerarin was 11.91 ± 7.14 min after oral administration of Jiawei-Xiaoyao-San in healthy volunteers, which was diminished, compared with the reports of Ma *et al.*^[11] and Penetar *et al.*^[12]. The published studies mainly focused on a single component, a vegetable drug (*Pueraria lobata*), and not on complicated prescriptions based on the theory of traditional Chinese medicine and its traditional use.^[11,12] Jiawei-Xiaoyao-San is a complicated prescription, which can improve intestinal motility.^[5,6] The compatibility principle of traditional Chinese medicine could affect the pharmacokinetics of the prescription.^[17] The presence of the other 13 drugs in the Jiawei-Xiaoyao-San prescription could affect the pharmacokinetics of puerarin. Furthermore, different doses of puerarin were administered in the study by Penetar *et al.*^[12] compared with this study.

Although the dose of Jiawei-Xiaoyao-San administered to both groups in our study was 5 g/kg, the estimated pharmacokinetic parameters of puerarin between patients with functional dyspepsia and healthy volunteers were very different. Previous studies had shown that the plasma

concentration–time profiles for motilin were similar between healthy volunteers and patients with functional dyspepsia.^[16] However, in our study Jiawei-Xiaoyao-San was administered orally whilst in the motilin study intravenous administration was used, which could have led to the difference in the pharmacokinetics. This study involving patients with functional dyspepsia and healthy volunteers suggested that this disease state could modify the pharmacokinetics of puerarin.

Conclusions

This is the first study to explore the relationship between functional dyspepsia and traditional Chinese medicine by comparing the pharmacokinetics of puerarin after oral administration of Jiawei-Xiaoyao-San in healthy volunteers and patients with functional dyspepsia. The pharmacokinetic parameters showed that functional dyspepsia reduced the absorption of puerarin after oral administration of Jiawei-Xiaoyao-San. The pharmacokinetic parameters could guide the clinical use of Jiawei-Xiaoyao-San.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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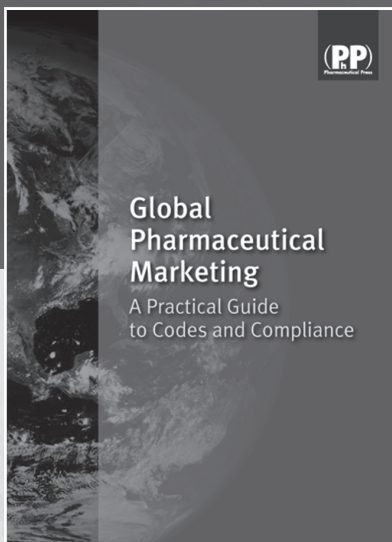
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