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Short communication

Studies of the pharmacokinetics of paeoniflorin in two Jing–Zhi–Guan–Xin formulations after oral administration to beagle dogs

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Abstract

Paeoniflorin is the principal bioactive component of Paeoniae Radix. The traditional chinese medicine compound recipe (TCMCR) tablets of Jing–Zhi–Guan–Xin (JZGX), which is composed of *Radix Salviae Miltiorrhizae, Radix Paeoniae Rubrae, Rhizoma Chuan-xiong, Flos Carthami* and *Lignum Dalbergiae Odorafera,* have been widely used in China and Japan. The plasma concentrations of paeoniflorin in beagle dogs after oral administration of two Jing–Zhi–Guan–Xin formulations (the dose used in the two formulations were both 200 mg paeoniflorin) were measured using a simple and rapid HPLC method. The mean terminal half-lives ($t_{1/2}$) of JZGX tablet and JZGX elementary osmotic pump tablet (EOPT) formulations of paeoniflorin were 147.52 ± 28.98 and 276.60 ± 24.24 min, the maximum plasma concentrations (C_{max}) of paeoniflorin were 210.49 ± 23.89 and 94.36 ± 14.01 ng/ml, times to reach maximum concentrations (t_{max}) were 130.00 ± 30.98 and 280.00 ± 48.99 min, the area under the plasma concentration–time curves ($AUC_{0-\infty}$) were 43066.50 ± 10119.51 and 42266.87 ± 2654.90 ng min/ml, the mean residence times (MRT) were 212.87 ± 41.82 and 399.14 ± 34.98 min, respectively, and the relative bioavailability (Fr) of JZGX EOPT compared with JZGX tablet was $101.8 \pm 18.8\%$. These results, compared with the pharmacokinetic parameters of paeoniflorin after oral administration of Paeoniae Radix extract alone, indicated that the absorption of paeoniflorin after oral administration of the two JZGX formulations was significantly greater than that after oral administration of Paeoniae Radix extract alone.

Keywords: Paeoniflorin; Pharmacokinetics; Jing–Zhi–Guan–Xin; Elementary osmotic pump tablet

1. Introduction

Traditional chinese medicine (TCM) involves the use of natural therapeutic agents in accordance with the theory of traditional Chinese medical science and has been successfully used by TCM practitioners for thousands of years. It mainly involves the use of combinations, in which the composite formulae will produce a synergistic effect or antagonistic action. This medical approach has played an important role in the prevention and treatment of many diseases.

Jing–Zhi–Guan–Xin (JZGX) prescription is a traditional chinese medicine compound recipe (TCMCR) which is effective in improving the blood circulation and removing waste material from the body. The main action of Jing–Zhi–Guan–Xin prescription is to treat coronary disease and angina arising from the silts of blood in the heart. It can also protect cardiac muscle. The formula consists of five crude drugs including *Radix Salviae Miltiorrhizae, Radix Paeoniae Rubrae, Rhizoma Chuan-xiong, Flos Carthami* and *Lignum Dalbergiae Odorafera* in a ratio of 6:3:3:3:2 on the dry weight basis. Paeoniflorin, a water-soluble compound first isolated from the root of *Paeonia lactiflora* in 1963 [1], has been reported to exhibit anticoagulant [2], neuromuscular blocking [3–9], cognition-enhancing [10–14] and antinociceptive properties [15]. Thus, paeoniflorin can be used as a marker compound to characterize JZGX. Owing to the complexity of the composite formulae, few investigations have been carried out on the pharmacokinetics of paeoniflorin [16–21], especially in beagle dogs. Since most Chinese medicines are administered orally as powder extracts, pills or tablets in clin-

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ics, the self-made purified paeoniflorin and *Paeonia* extract in the literature could not be used as a suitable reference for clinical applications. In this paper, we have developed a simple and rapid HPLC method to determine paeoniflorin in plasma of beagle dogs after oral administration of JZGX elementary osmotic pump tablets (EOPT) and JZGX tablets and to investigate their pharmacokinetics.

2. Materials and methods

2.1. Chemicals and reagents

The five crude drugs were purchased from Tailun Co. Ltd., (Liaoning, China). The reference formulation: JZGX tablet was purchased from Fenglin Pharmaceutical Co. Ltd., (Jilin, China). The test formulation: JZGX EOPT was prepared in our laboratory. Paeoniflorin was purchased from Shanghai TCM Standard Tech Co. Ltd., (Shanghai, China). The internal standard, pentoxifylline, was purchased from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade) was purchased from Shandong Yuwangshiye Co. Ltd., (Shandong, China). Phosphate (analytical grade) was purchased from Shenyang Reagent Factory (Liaoning, China). Distilled water was used and all other reagents were of analytical grade.

2.2. Animals

Healthy beagle dogs (9.3 ± 1.0 kg, from the Lab Animal Center of Shenyang Pharmaceutical University, Shenyang, China, Grade I) were used. Six dogs which were used in this study and split into two groups of three. The animals were kept in an environmentally controlled breeding room for 1 week before the start of the experiments. They were fed standard laboratory chow with water and fasted overnight before the experiments.

2.3. Preparation of JZGX EOPT

The five herb materials *Radix Salviae Miltiorrhizae, Radix Paeoniae Rubrae, Rhizoma Chuan-xiong, Flos Carthami* and *Lignum Dalbergiae Odorafera* were extracted and the solutions obtained were concentrated and made into freeze-dried power. Then, the freeze-dried power was used to prepare the EOPT. The composition of the five crude drugs in EOPT and the reference formulation were equivalent.

2.4. Drug administration and blood sampling

During the first period, the dogs were given a single dose of 3025 mg (containing 200 mg paeoniflorin) tablets of either formulation (reference or test tablet) in a randomized fashion with 200 ml water. The dogs were fed at 4 h post-dosing and water was available ad libitum from 2 h post-dosing onwards. Approximately 4 ml blood samples were collected in heparinized tubes using an indwelling cannula at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 24 h after administration of the reference tablets. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 24, 36 h after administration of the test tablets. The blood samples were centrifuged at $1600 \times g$ for 10 min, and plasma was separated and kept frozen at $-20\,^{\circ}C$. After a washout period of 7 days, the study was repeated in the same manner to complete the crossover design.

2.5. Preparation of plasma samples

The resulting plasma 0.5 ml was mixed with 1.0 ml acetonitrile and 80 μl internal standard (pentoxifylline 50 ng/ml). The denatured protein precipitate was separated by centrifugation at $1600 \times g$ for 10 min. The supernatant was then mixed with 5 ml ether by ultrasonic vortexing for 1 min. Then the mixture was centrifuged at $1600 \times g$ for 10 min. The ether layer, containing non-polar interfering impurities, was discarded. The water layer was evaporated to dryness below $40\,^{\circ}C$ in a vacuum and then dissolved in 50 μl of the mobile phase and 20 μl of this sample solution was injected onto the HPLC for analysis. The same procedure was used to determine the recovery and precision in plasma.

2.6. HPLC chromatographic conditions

The HPLC system consisted of a pump (LC-10ATVP HPLC pump, Shimadzu, Japan), and a UV detector (SPD-10AVP Spectrometer, Shimadzu, Japan). Chromatography was carried out on an ODS column (Diamonsil C18 200 mm × 4.6 mm, 5 μm, Dikma, Beijing). The mobile phase consisted of acetonitrile–0.1% phosphate (18:83, v:v) and the flow rate was 1.0 ml/min. Detection was performed at a wavelength of 230 nm under a constant temperature of $30\,^{\circ}C$.

2.7. Calibration curve

A calibration curve was constructed based on the HPLC analysis of different concentrations (5, 10, 20, 50, 100, 200, 250 ng/ml) of paeoniflorin added to dog plasma. The concentrations of paeoniflorin in plasma were determined from the peak area ratios using the linear regression equation obtained from the calibration curve.

2.8. Recovery and precision

Plasma samples were spiked with three different concentrations (10, 50, 250 ng/ml) of paeoniflorin. After preparation of the plasma samples fixed amounts of internal standard were added to the plasma for normalization. The resulting peak area ratios (ratios of paeoniflorin:internal standard) were compared with those of standard in distilled water to provide recovery values. The precision over the entire working dose range was determined by triplicate analyses of plasma samples ($n = 3$) spiked with three different concentrations (10, 50, 250 ng/ml) of paeoniflorin. To determine the intra-day variance, the assays were carried out on the same samples at different times during the day. The inter-day variance was determined by assaying the spiked samples over five consecutive days. Coefficients of variation (CV) were calculated from these values.

2.9. Pharmacokinetics analyses

All data were subsequently processed by the computer program WINNONLIN (SCI, Lexington, KY, USA) The non-compartmental pharmacokinetic parameters of half-life ($t_{1/2}$), mean residence time (MRT), and area under the plasma concentration–time curve (AUC) were calculated based on the moment theory. All data were expressed as the mean ± S.D. The relative bioavailability (Fr) of JZGX EOPT (test tablet) compared with JZGX tablet (reference tablet) was calculated using Eq. (1)

$$\mathrm{Fr}\,(\%) = \frac{(\mathrm{AUC}_{\mathrm{test}}/D_{\mathrm{test}})}{(\mathrm{AUC}_{\mathrm{ref}}/D_{\mathrm{ref}})} \times 100 \quad (1)$$

where D is the dose.

3. Results

3.1. HPLC chromatograms

Under the conditions described above, the HPLC chromatograms of blank plasma, plasma spiked with paeoniflorin (200 ng/ml) and plasma obtained 240 min after oral administration of JZGX EOPT are shown in Fig. 1. The retention times of paeoniflorin and pentoxifylline (internal standard) were approximately 11.20 and 20.78 min, respectively. No interfering peaks were observed within the time frame during which paeoniflorin and pentoxifylline were eluted.

3.2. Calibration curve

The linear relation was good ($r^2 = 0.9964$) over the concentration range from 5 to 250 ng/ml. Using the least-squares method, a regression equation of $y = 0.013x - 0.0574$ [y: peak area ratio of paeoniflorin to pentoxifylline, x: concentration of paeoniflorin in plasma (ng/ml)] was obtained. The limit of quantification was 5 ng/ml based on the signal-to-noise ratio.

3.3. Recovery tests and reproducibility

The recoveries of paeoniflorin from dogs plasma were 75.48%, 78.25% and 80.31% at concentrations of 10, 50, 250 ng/ml, respectively. The reproducibility of the method was defined by examining both intra- and inter-day variance. The CV values of intra-day assay were 12.81%, 7.85% and 6.34% at concentrations of 10, 50, 250 ng/ml, respectively. The CV values of inter-day assay were 13.94%, 8.99% and 6.57% at concentrations of 10, 50, 250 ng/ml, respectively. These results indicate that the method is suitable for the aims of the present study.

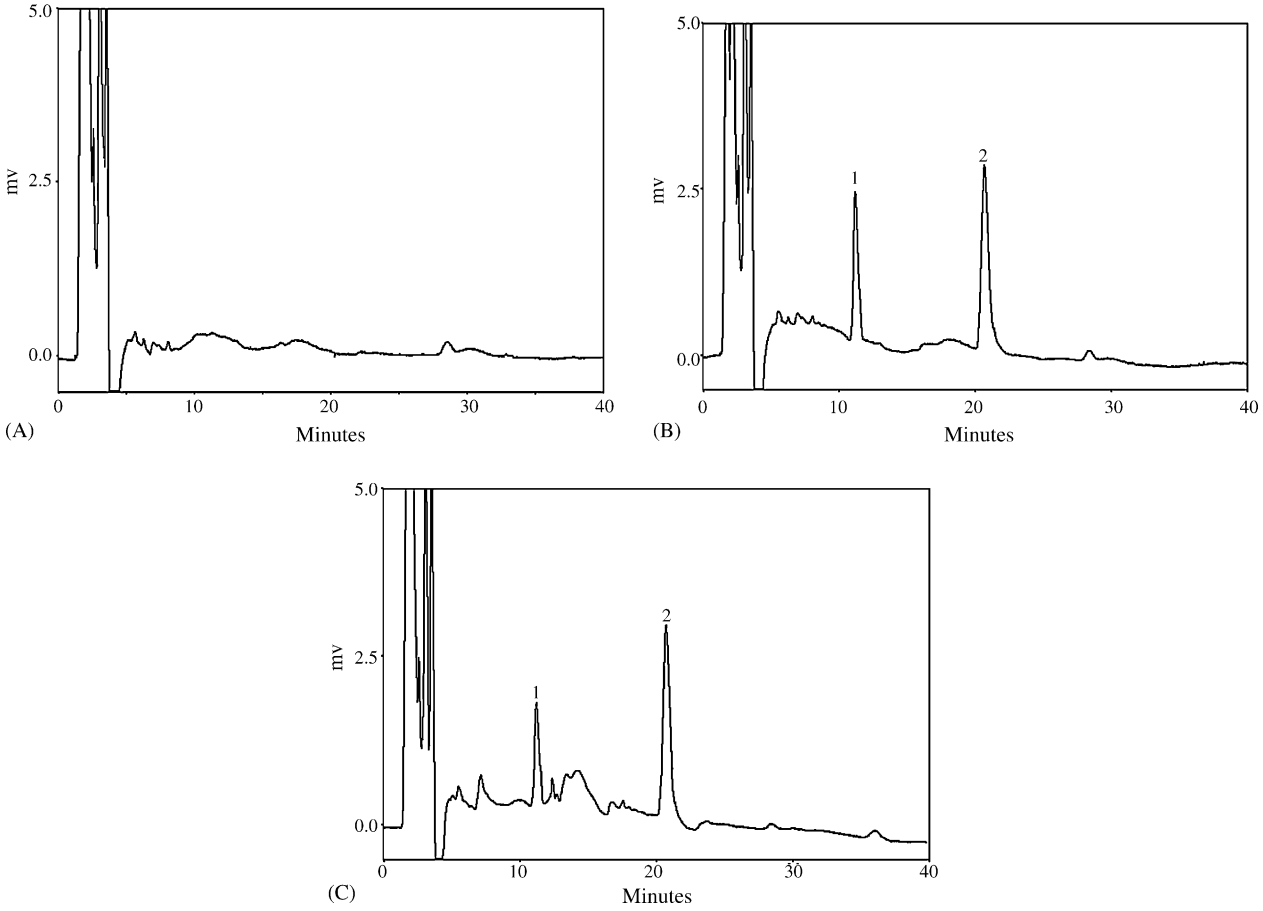

Fig. 1. Chromatograms of paeoniflorin in beagle dogs plasma: (A) blank plasma; (B) blank plasma spiked with paeoniflorin (200 ng/ml) and internal standard (pentoxifylline); (C) plasma blank obtained 240 min after oral administration of JZGX EOPT. (1) Paeoniflorin, (2) pentoxifylline.

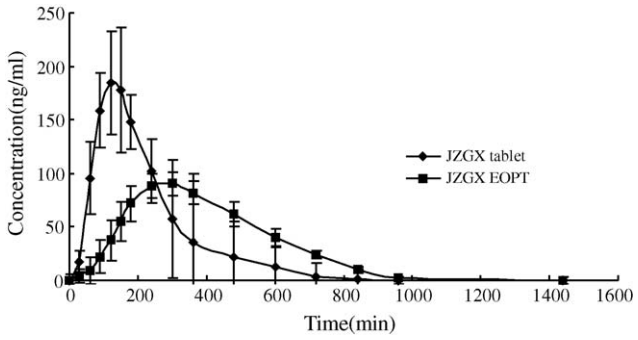

Fig. 2. Plasma concentration–time profile of paeoniflorin in beagle dogs after oral administration of two JZGX formulations (each dose containing 200 mg paeoniflorin). Each point represent the mean ± S.D. ($n = 6$).

Table 1
Pharmacokinetic parameters of paeoniflorin in beagle dogs ($n = 6$) after oral administration of two JZGX formulations (each dose containing 200 mg paeoniflorin)

Parameters	Jing–Zhi–Guan–Xin tablet	Jing–Zhi–Guan–Xin EOPT
t_{max} (min)	130.00 ± 30.98	280.00 ± 48.99**
C_{max} (ng/ml)	210.49 ± 23.89	94.36 ± 14.01**
$AUC_{0-\infty}$ (ng min/ml)	43066.50 ± 10119.51	42266.87 ± 2654.90
$t_{1/2}$ (min)	147.52 ± 28.98	276.60 ± 24.24**
MRT (min)	212.87 ± 41.82	399.14 ± 34.98**
Fr	101.8 ± 18.8%	

$AUC_{0-\infty}$: the area under curve concentration-time; C_{max}: maximum concentration at t_{max}; $t_{1/2}$: elimination half-life time; MRT: mean residence time; Fr: AUC ratio of JZGX EOPT to JZGX tablet.
** $P < 0.01$.

3.4. Determination of paeoniflorin in plasma

The plasma concentration versus time profile of paeoniflorin in beagle dogs is shown in Fig. 2. After oral administration of Jing–Zhi–Guan–Xin tablets and EOPT (each dose containing 200 mg paeoniflorin), the plasma levels of paeoniflorin declined with a half-life of 147.52 and 276.60 min, respectively. The concentrations were lower than the quantitative limit (5 ng/ml) after 12 and 16 h, respectively.

3.5. Kinetic analysis

The pharmacokinetic parameters of paeoniflorin, calculated from the plasma concentrations of paeoniflorin following oral administration of two JZGX formulations, are listed in Table 1.

4. Discussion

TCMs are widely used in China and Japan and in most cases they are prescribed in combination in clinics. TCM combinations are used to treat many diseases as a whole entity. Each herb that forms part of the formula is necessary in order to obtain an integral effect. As the chemical constituents of the formula are complex, pharmacokinetic studies usually focus on the main active constituents. According to previous investigations [3,2,10–12,15], paeoniflorin can be used as a marker compound to characterize JZGX.

Most of the recent reports about the pharmacokinetics of paeoniflorin have involved mice and rats [16–21]. The present study describes for the first time the pharmacokinetics of paeoniflorin in two JZGX formulations in beagle dogs.

Jing–Zhi–Guan–Xin tablets described in Ch.P (2005) [22] have several disadvantages, such as a higher dosage (six to eight tablets given three times a day), unsophisticated preparation technology, and no quantifiable target. After being prepared as an osmotic pump tablet, it can be given at a lower dosage (three to four tablets given two times a day). Furthermore, patient compliance is greatly improved by reducing the dosing frequency and side effects because the drug release process from the tablet into the body continues over a long period of time.

In the present study, paeoniflorin plasma levels were assayed after oral administration of two JZGX formulations. The pharmacokinetic parameters of paeoniflorin derived from the plasma profiles are presented in Table 1. These results indicate that JZGX EOPT exhibits a satisfactory sustained-release effect, with less fluctuation in the plasma concentrations.

The pharmacokinetic parameters of paeoniflorin after oral administration of JZGX tablets compared with that of Paeoniae Radix extract alone [17], provide evidence of a longer t_{max} (130.00 min versus 14.00 min, $P < 0.01$), a higher C_{max} (210.49 ng/ml versus 86.34 ng/ml, $P < 0.01$), a higher $AUC_{0-\infty}$ (430660.50 ng min/ml versus 19492.20 ng min/ml, $P < 0.01$, AUC of Paeoniae Radix extract was revised according to the dosage), a longer MRT (212.87 min versus 135.65 min, $P < 0.05$) and a longer $t_{1/2}$ (147.52 min versus 94.16 min, $P < 0.05$). These results confirm a significant improvement in all these parameters. The significant increase in AUC suggests that more paeoniflorin was absorbed after oral administration of JZGX tablets. In addition, a relatively long t_{max} and $t_{1/2}$ were obtained, implying a delayed absorption and slow excretion of paeoniflorin after oral administration of JZGX tablets. Therefore, it is important to investigate their pharmacokinetic properties to explain the pharmacological effects of the two JZGX formulations in clinical situations.

The reason why the pharmacokinetic parameters of paeoniflorin in JZGX tablets differs from those of the extract involves the following: firstly, because the JZGX tablet is a compound recipe while the Paeoniae Radix extract is a crude drug, some ingredients might improve the absorption of paeoniflorin; secondly, different animals were used, beagles in our research and mice in the research by Chen et al. [17] and, so, the difference in animal species might lead to differences in the pharmacokinetics; thirdly, the dosage forms used were different, in the case of JZGX tablets, the drug cannot be absorbed until the tablets disintegratey and then form a solution after oral administration while the paeoniflorin extract was given as a solution; hence, the differences in dosage forms are responsible for the result that the MRT of JZGX tablets was greater than that of the paeoniflorin extract; finally, the dosage used was different, the dosage in our research was approximately 20 mg/kg while the dosage used in the investigation by Chen et al. [17] was 10 mg/kg, and,

so, differences in dosage could also contribute to the observed differences in pharmacokinetic parameters.

5. Conclusion

This paper describes a simple and rapid HPLC method to determine paeoniflorin in beagle dog plasma after oral administration of JZGX elementary osmotic pump tablets (EOPT) and JZGX tablets. The pharmacokinetics of paeoniflorin in beagle dogs after oral administration of the two formulations was studied. The results obtained show that JZGX EOPT has a good sustained-release effect, which reduces fluctuations in plasma concentrations due to a controlled-release. The pharmacokinetic parameters of paeoniflorin after oral administration of JZGX tablets compared with that of Paeoniae Radix extract alone, indicate that the absorption of paeoniflorin after oral administration of JZGX tablets is significantly greater than that of Paeoniae Radix extract alone. A significant increase in AUC was observed, suggesting that a relatively more paeoniflorin is absorbed after oral administration of JZGX tablets. The pharmacokinetic results are important for explaining the pharmacological effects of JZGX in clinical situations.

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