Pharmacokinetics of Paeoniflorin After Oral Administration of Shao-yao Gan-chao Tang in Mice

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ABSTRACT—Paeoniflorin, a monoterpene glycoside, is the principal bioactive component of Paeoniae Radix. The traditional prescription Shao-yao Gan-chao Tang (SGT; Kampo: Shakuyaku-Kanzo-To), which is composed of Paeoniae Radix and Glycyrrhizae Radix, has been widely used in China and Japan. Quantification of paeoniflorin in mouse plasma after oral administration of SGT (at a dose containing 10 mg/kg paeoniflorin) was achieved using a simple and rapid high-performance liquid chromatography method. The plasma concentration-time curves were fitted with mean terminal half-lives (t₁/₂) of 116.17 min. The maximum plasma concentration (Cmax) of paeoniflorin was 111.56 ng/ml, time to reach maximum concentration (tmax) was 17.00 min, the area under the plasma concentration-time curve (AUC)₀₋₉ was 12293.42 ng · min/ml, clearance / bioavailability (CL/F) value was 644.74 ml/min · kg, apparent volume of distribution / bioavailability (Vd/F) value was 103.05 l/kg, and the mean residence time (MRT) was 169.64 min. These results, together with the previously reported kinetic data of paeoniflorin after oral administration of Paeoniae Radix extract alone, indicated that absorption of paeoniflorin after oral administration of SGT was significantly greater than that after oral administration of Paeoniae Radix alone.

Keywords: Paeoniflorin, Pharmacokinetics, Shao-yao Gan-chao Tang

Paeoniflorin (structure shown as Fig. 1), a characteristic monoterpene glucoside isolated from the root of Paeonia lactiflora in 1963 (1), is one of the bioactive components in Paeoniae Radix and has been reported to exhibit anticoagulant (2), neuromuscular blocking (3–9), cognition-enhancing (10–14), immunoregulating (15) and antihyperglycemic effects (16). Since paeoniflorin has been demonstrated to exhibit these pharmacological effects, the pharmacokinetics of paeoniflorin are useful for designing an ideal dosing regimen in pharmacological studies. Furthermore, the pharmacokinetic profile contributes to the safety and efficacy of paeoniflorin in clinical applications. Recent studies showed that paeoniflorin has a low bioavailability (F) (17, 18). The low F value, about 3% (18), may be attributed to the first-pass metabolism in the gut wall or liver, metabolism or decomposition in the intestine by bacterial microflora, and/or poor absorption from gastrointestinal tract (18, 19). To improve the clinical efficacy, therefore, it is important to efficiently increase the F value of paeoniflorin and enhance its concentration in blood. It was suggested that paeoniflorin absorbed is mainly excreted in the urine because approximately 50% of the dose was excreted in urine after intravenous administration (18). A large variability in half-life (t₁/₂) values was observed, which might be caused by the enterohepatic circulation (18). Furthermore, we have previously reported

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Fig. 1. Structure of paeoniflorin.
the pharmacokinetics of paeoniflorin after oral administration of Paeoniae Radix extract (20). Compared with the results from oral administration of purified paeoniflorin by Takeda et al. (18), our previous results indicated a later time to reach maximum concentration \( t_{\text{max}} \) and longer \( t_{1/2} \) after oral administration of Paeoniae Radix extracts (20).

In clinical application, most traditional Chinese medicines (TCMs) are prescribed by more than two herbal crude drugs to obtain the additive effects or to diminish the possible adverse responses. Therefore, to evaluate the effect of Chinese medicinal prescriptions on the pharmacokinetics of paeoniflorin is an important topic for further studies. The traditional Chinese prescription Shao-yao Gan-chao Tang (SGT; Kampo: Shatsuyaku-Kanzo-To), composed of Paeoniae Radix and Glycyrrhiza Radix, is one of the famous Chinese prescriptions and is widely used in China and Japan for acute abdominal pain and muscles stiffness (21). Paeoniflorin, the major component of Paeoniae Radix, is one of the indicated ingredients for SGT. To provide a firm basis for the design of dosing regimens in clinical applications and pharmacological experiments, the present study was designed to describe the plasma profiles and pharmacokinetics of paeoniflorin in mice after oral administration of SGT.

MATERIALS AND METHODS

Chemicals and reagents

The reference standard of paeoniflorin was supplied by Nacalai Tesque (Kyoto). The internal standard, pentoxifylline, was purchased from Sigma (St. Louis, MO, USA). Acetonitrile and ether (HPLC grade) were obtained from Merck (Darmstadt, Germany).

Animals

Healthy male ICR mice (20 – 30 g; from the Laboratory Animal Center at the National Taiwan University, Taipei, Taiwan) were used. A total of 45 mice were used for this study, which were distributed into 9 groups with 5 mice in each group (\( n = 5 \) for each data point). Animals were kept in an environmentally controlled breeding room (temperature: 24 ± 1°C, humidity: 60 ± 5%, 12 h dark-light cycle) for 1 week before the start of the experiments. They were fed standard laboratory chow with water ad libitum and fasted overnight before the experiments.

Preparation of SGT freeze-dried extract powder

SGT, which is composed of Paeoniae Radix and Glycyrrhizae Radix (1:1, w/w), was prepared from the roots of Paeonia lactiflora Pall. and Glycyrrhiza uralensis Fisch. purchased commercially (Sun-yun Herbal Shop, Taipei, Taiwan). The herb materials were extracted twice by refluxing with boiling water (1:10, w/v) for 1 h, and the solution obtained was concentrated and then made into freeze-dried powder. A 29.6-g amount of SGT freeze-dried extract powder was obtained from 200 g raw material (the yield was 14.8%). The freeze-dried powders were stored at 4°C until use.

Content of paeoniflorin in SGT freeze-dried extract powder

Experimental mice were orally administered with aqueous solutions of SGT freeze-dried extracts in this study. To calculate the administered dose, the content of paeoniflorin in SGT freeze-dried extract powder had been quantitatively analyzed according to the HPLC method we previously reported (20). The content of paeoniflorin in the freeze-dried powder of SGT was determined (9.31%) from the peak height ratios by using the equation for linear regression obtained from the calibration curve.

Drug administration and blood sampling

Aqueous solutions of SGT freeze-dried extracts (2 mg/ml) were orally administered to mice (by gavage with 20 gauge syringe) at a dose containing 10 mg/kg paeoniflorin (0.1 ml per 20 g body weight). Under anesthesia with ether in a glass chamber, blood samples (0.8 – 1 ml) were collected from each mouse by cardiac puncture according to the specific schedule (at times of 5, 10, 15, 20, 40, 60, 120, 180 and 240 min after dosing) with \( n = 5 \) for each time point. Data from these samples were used to construct pharmacokinetic profiles by plotting drug concentration vs time.

Preparation of plasma samples and determination of paeoniflorin in plasma

Preparation of plasma samples for analysis was described before (20). The same sample handling process was used for recovery and precision determination in plasma. Plasma concentrations of paeoniflorin were assayed according to the HPLC method we previously reported (20).

Validations of analytical method

Validations of the analytical method (including the calibration curves, recovery and reproducibility) were performed by the procedure described in our previous paper (20).

Pharmacokinetic analyses

All data were subsequently processed by the computer program WINNONLIN (SCI, Lexington, KY, USA). The non-compartmental pharmacokinetic parameters of \( t_{1/2} \), mean residence time (MRT), area under the plasma concentration-time curve (AUC), clearance / bioavailability (Cl/F) and apparent volume of distribution / bioavailability (\( V_{d}/F \)) were calculated based on the moment theory. All data were expressed as the mean ± S.D.
RESULTS

HPLC chromatograms
The HPLC chromatograms indicated the retention times of paeoniflorin and pentoxifylline (internal standard) were approximately 9.46 and 16.59 min, respectively. No interfering peaks were observed within the time frame in which paeoniflorin and pentoxifylline were detected.

Calibration curves
The calibration curve for paeoniflorin was linear ($r^2 = 0.998$) over the concentration range of 10–200 ng/ml. With the least-squares method, a regression equation of $y = 0.0119x + 0.0865$ (y: peak height ratio of paeoniflorin to pentoxifylline, x: concentration of paeoniflorin in plasma) was obtained.

Reproducibility and recovery tests
The reproducibility of the method was defined by examining both intra- and inter-day variance. The coefficient of variance (CV) values of intra-day assay were 14.82%, 7.30%, 6.15%, 6.55% and 6.62% at concentrations of 10, 60, 100, 160 and 200 ng/ml, respectively. The CV values of inter-day assay were 12.86%, 6.11%, 3.60%, 5.51% and 4.52% at concentrations of 10, 60, 100, 160 and 200 ng/ml, respectively. The recoveries of paeoniflorin from mouse plasma were 74.51%, 76.83%, 80.38% and 80.57% for the concentrations of 30, 80, 120 and 160 ng/ml, respectively.

Determination of paeoniflorin in plasma
The plasma concentration vs time profile of paeoniflorin in mice is shown in Fig. 2. There was a rapid absorption, followed by a flat slope of elimination. The concentration was lower than the quantitative limit (10 ng/ml) after 4 h.

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Fig. 2. Plasma concentration-time curve of paeoniflorin in mice after oral administration of Shao-yao Gan-chao Tang (at a dose containing 10 mg/kg paeoniflorin). Each point and bar represent the mean ± S.D. (n = 5).

Table 1. Pharmacokinetic parameters of paeoniflorin in mice (n = 5 for each data point) after oral administration of Shao-yao Gan-chao Tang and Paeoniae Radix extracts$^a$ (at a dose containing 10 mg/kg paeoniflorin)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Shao-yao Gan-chao Tang</th>
<th>Paeoniae Radix extracts$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{max}$ (min)</td>
<td>17.00 ± 4.47</td>
<td>14.00 ± 4.18</td>
</tr>
<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>111.56 ± 20.83*</td>
<td>86.34 ± 18.67</td>
</tr>
<tr>
<td>$AUC_{0-t}$ (ng·min/ml)</td>
<td>12293.42 ± 1945.96*</td>
<td>8126.97 ± 2004.62</td>
</tr>
<tr>
<td>$AUC_{0-5h}$ (ng·min/ml)</td>
<td>16335.28 ± 3641.46*</td>
<td>9746.10 ± 2554.62</td>
</tr>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>116.17 ± 35.02</td>
<td>94.16 ± 12.35</td>
</tr>
<tr>
<td>CL/F (ml/min·kg)</td>
<td>644.74 ± 84.45*</td>
<td>1113.35 ± 420.63</td>
</tr>
<tr>
<td>$V_d$/F (l/kg)</td>
<td>103.05 ± 20.45*</td>
<td>147.44 ± 40.78</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>169.64 ± 50.12</td>
<td>135.64 ± 18.51</td>
</tr>
</tbody>
</table>

$^a$ The data were derived from our previous report (ref. 20). Each value represents the mean ± S.D. *P<0.05.
Kinetic analysis

The pharmacokinetic parameters of paeoniflorin are presented in Table 1. After oral administration of SGT, paeoniflorin was absorbed in a short time and reached a maximum concentration within 20 min (mean observed \( t_{\text{max}} \) 17 min). The plasma concentration was very low, with a mean observed maximum plasma concentration \( (C_{\text{max}}) \) value of 111.56 ng/ml. The plasma level of paeoniflorin declined with a \( t_{1/2} \) of 116.17 min.

DISCUSSION

TCMs are widely used in China and Japan. Nowadays, the clinical efficacy of TCMs must be elucidated by related scientific results. In addition to pharmacological studies, the pharmacokinetic study of TCMs is an important and useful approach. Pharmacokinetics is the study and characterization of the time course of drug absorption, distribution, metabolism, and excretion, and it is used in the clinical setting to enhance the safe and effective therapeutic management. Knowledge of the pharmacokinetics can help to explain and predict a variety of events related to the efficacy and toxicity of herbal preparations (22, 23). The results derived from the pharmacokinetic studies of TCMs can provide a firm basis for the design of reasonable dosing regimens in clinical applications and pharmacological experiments.

Paeoniae Radix is one of the most extensively studied Chinese herbal medicines. Paeoniflorin, one of the bioactive components in Paeoniae Radix, has been reported to exhibit many pharmacological effects. However, recent studies showed that paeoniflorin has a low bioavailability (17, 18). The low F value, about 3% (18), may be attributed to the first-pass metabolism in the gut wall or liver, metabolism or decomposition in the intestine by bacterial microflora, and/or poor absorption from gastrointestinal tract (18, 19). It’s important to evaluate the significance of paeoniflorin and the role of its metabolites in the pharmacological effects of Paeoniae Radix.

Clinically, most of the TCMs are prescribed by more than two herbal crude drugs to obtain the additive effects or to diminish the possible adverse responses. SGT, composed of Paeoniae Radix and Glycyrrhizae Radix in an even dose, is the popular Chinese prescription and is widely used in China and Japan for acute abdominal pain and muscles stiffness (21). Paeoniflorin and glycyrrhizin are principal components of Paeoniae Radix and Glycyrrhizae Radix, respectively (21, 24). The early studies demonstrated that paeoniflorin and glycyrrhizin decrease intracellular Ca\(^{2+}\)-aequorin luminescence transients (5). Furthermore, the combination of paeoniflorin and glycyrrhizin was indicated to depolarize the muscle membrane and to exhibit anticholinergic effect (6).

To evaluate the effect of Glycyrrhizae Radix on the pharmacokinetics of paeoniflorin, the present study was designed to describe the plasma profiles and pharmacokinetics of paeoniflorin in mice after oral administration of SGT. In the present study, the F value of paeoniflorin could not be obtained because at this time, the multi-ingredient herbal prescription is not approved and not applicable for intravenous injection. Therefore, CL/F (= dose/AUC) was described instead of CL (= dose*F/AUC) and \( V_d/F \) was described instead of \( V_d \).

An ideal analytic method is necessary for the pharmacokinetic evaluation of TCMs. A simple and rapid HPLC method (20) was used in this study to quantitatively analyze the concentrations of paeoniflorin. The accuracy and precision of this calibration curve, except the CV value of the low concentration point (10 ng/ml), were <10%. CVs of intra- and inter-day assay were also <10%, except at 10 ng/ml. The HPLC method has good reproducibility, accuracy and precision, and it could be applied for the quantitative assay of paeoniflorin in blood samples.

In the present study, paeoniflorin plasma levels were assayed after oral administration of aqueous solutions of SGT freeze-dried extract. The pharmacokinetic parameters of paeoniflorin derived from the plasma profiles were presented in Table 1. These results, together with the previously reported kinetic data of paeoniflorin after oral administration of Paeoniae Radix extract alone, indicated the longer \( t_{\text{max}} \) (17 vs 14 min), higher \( C_{\text{max}} \) (111.56 vs 86.34 ng/ml, \( P<0.05 \)), larger \( \text{AUC}_{0-24} \) (12293.42 vs 8126.97 ng·min/ml, \( P<0.01 \)), smaller \( V_d/F \) (103.05 vs 147.44 l/kg, \( P<0.05 \)), smaller CL/F (644.74 vs 1113.35 ml/min · kg, \( P<0.05 \)) and the longer \( t_{1/2} \) (116.17 vs 94.16 min), relatively.

From these observations, comparatively, a significant increase in both \( C_{\text{max}} \) and AUC were found. Since the F value could not be obtained from the present study, AUC is an important parameter used in the determination of the relative F value of dosage forms. The relative extent of absorption can be estimated by comparing the AUC only if the CL remains practically unchanged. The significant increase in AUC might suggest that a relatively greater extent of paeoniflorin was absorbed after oral administration of SGT. However, it is necessary to consider the smaller CL/F (or CL) value. In addition, a relatively long \( t_{\text{max}} \) and elimination half-life \( t_{1/2} \) were obtained, implying a delayed absorption and slow excretion of paeoniflorin after oral administration of SGT. Since the significant decreases in both \( V_d/F \) and CL/F were found, the tissue distribution and excretion of paeoniflorin would be an important topic for further studies. However, it should be noted that the change of CL/F (and \( V_d/F \)) should not be interpreted as the change of CL (and \( V_d \)) alone; the change of F value must also be considered.

Kimura et al. revealed the neuromuscular blocking ef-
fects of paeoniflorin and glycyrrhizin on isolated muscles and concluded that paeoniflorin combined with glycyrrhizin (SGT) was found to have the pharmacological synergistic effect (7). Glycyrrhizin, one of the main components of Glycyrrhizae Radix, is a triterpenoid saponin (which is a diglucuronide of glycyrrhetic acid). It has been shown that saponins (especially bidesmosides) have the ability to increase solubilities of water-insoluble compounds. Like all other saponins, glycyrrhizin is highly surface-active, but in contrast to them it is nonhemolytic and practically nontoxic (25). Since our present study indicated that a relatively greater extent of paeoniflorin was absorbed after oral administration of SGT, it was assumed that glycyrrhizin exhibited surfactant activity to increase the solubility and enhance the absorption of paeoniflorin. However, the extent of the increase in solubility and its relationship to the dose used need to be further studied.

Recent studies indicated the low F value of paeoniflorin and suggested that the active moiety was not paeoniflorin but its metabolite (18); however, there is no definite evidence to support that the metabolites of paeoniflorin may be involved in all of the pharmacological actions of Paeoniae Radix. Although paeoniflorin has a low F, it was found to exhibit some pharmacological effects. For example, recent findings indicated that paeoniflorin significantly improved aging-induced learning impairment and scopolamine-induced performance deficits at quite low dosages (11 – 14). Furthermore, increase in absorption of paeoniflorin is also suggested to increase the amount of its metabolites. Therefore, it is still important to reveal that a relatively greater extent of paeoniflorin is absorbed after oral administration of SGT.

In conclusion, the present study described the pharmacokinetics of paeoniflorin in mouse plasma after oral administration of SGT. These results, together with the previously reported kinetic data of paeoniflorin after oral administration of Paeoniae Radix extract alone, indicated that absorption of paeoniflorin after oral administration of SGT was significantly greater than that after oral administration of Paeoniae Radix alone. A significant increase in AUC was observed, suggesting that a relatively greater extent of paeoniflorin is absorbed after oral administration of SGT. The bioavailability for oral administration of paeoniflorin is low, but Glycyrrhizae Radix can efficiently increase the absorption of paeoniflorin and enhance its concentration in blood. Since the absorption of paeoniflorin is increased following the use of SGT (Paeoniae Radix combined with Glycyrrhizae Radix), we can expect that a great benefit for clinical efficacy of paeoniflorin will be obtained. The pharmacokinetic results were suggested to explain the pharmacological synergetic effect of SGT in clinical use. Another interesting finding was that significant decreases in both Vd/F and CL/F were found in this study. Further studies on the tissue distribution and excretion of paeoniflorin are in progress.

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