Influence of co-administrated sinomenine on pharmacokinetic fate of paeoniflorin in unrestrained conscious rats

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Abstract

Paeonia lactiflora Pall. (Ranunculaceae) root and Sinomenium acutum Rehder and Wilson (Menispermaceae) stem are two herbs widely used in Chinese medicine to treat rheumatoid arthritis. While, in theory, either herb could be used alone, in practice, Chinese medicine practitioners prescribe them together. Studies on pharmacokinetic interaction between the active constituents of these two herbs (paeoniflorin and sinomenine, respectively) provide empirical evidence to support their clinical practice. A single dose of paeoniflorin (150 mg/kg) alone and with sinomenine hydrochloride (90 mg/kg) was administered by gastric gavage to unrestrained conscious male Sprague-Dawley rats (n = 6, 250–300 g). Blood samples were collected periodically via a jugular vein before and after dosing from 10 min to 12 h. A high-performance liquid chromatographic (HPLC) assay was developed to determine the plasma concentrations of paeoniflorin. Non-compartmental pharmacokinetic profiles were constructed by using the software PK Solutions 2.0. The pharmacokinetic parameters were compared using unpaired Student t-test. After co-administration of sinomenine, the peak plasma concentration of paeoniflorin was elevated (P < 0.01), the peak time was delayed (P < 0.01), the AUC 0–t was increased (P < 0.001), the mean residence time (MRT) was prolonged (P < 0.01), the CL was decreased (P < 0.01) and the Vd was reduced (P < 0.05). These results indicate that sinomenine hydrochloride at 90 mg/kg significantly improved the bioavailability of paeoniflorin in rats.

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1. Introduction

Paeoniflorin is a bioactive monoterpene glucoside (Fig. 1) presenting in the root of Paeonia lactiflora Pall. (family Ranunculaceae), which has been widely used to treat inflammatory and arthritic conditions according to the traditional Chinese medical system. The therapeutic effects of the herb and its active component, paeoniflorin, have been confirmed by experimental pharmacological investigations (Takagi and Harada, 1969a,b). However, previous pharmacokinetic studies have shown that paeoniflorin has a poor absorption rate, and thus a very low bioavailability (3–4%) when adminis-
stem of *Sinomenium acutum* Rehder and Wilson (family Menispermaceae) in their medical prescriptions. This clinical practice suggests that there might be synergic effects between the herbs (Wong and Wu, 1936). Previous work on the Chinese medicinal herb *Sinomenium acutum* demonstrated that the alkaloid sinomenine is one of the main active components of the plant (Tai and Hopkins, 1998). Sinomenine (7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methylmorphinan-6-one; Fig. 2) has been demonstrated to significantly inhibit inflammatory reactions caused by various phlogistic agents (Irino, 1958; Cheng et al., 1964; Huo and Che, 1989). The therapeutic efficacy of sinomenine was also confirmed in patients with rheumatoid arthritis (Ke and Xiu, 1986; Shi et al., 1986). Studies in our previous work have revealed that the alkaloid has marked effects in inhibiting the proliferation of mouse spleen cells, human peripheral blood mononuclear cells as well as rat synovial fibroblasts, the synthesis of proinflammatory mediators of prostaglandin E2 and leukotriene C4 and the production of nitric oxide by macrophages (Liu et al., 1994a,b, 1999). It has also been shown to significantly ameliorate arthritic pathologies in adjuvant arthritis rats (Liu et al., 1999). Studies on the pharmacokinetics of sinomenine using technologies of microdialysis and a hepato-duodenal shunt probe in rats demonstrated that sinomenine undergoes active hepatobiliary elimination; this process might be regulated by P-glycoprotein and probably involves P450 (Tsai and Wu, 2003). Therefore, in the present study we chose to investigate the possible influences of sinomenine on paeoniflorin by pharmacokinetics to see if interaction between these two constituents was responsible for the enhancement observed in using the two herbs together in clinical practice.

2. Materials and methods

2.1. High-performance liquid chromatography analysis

The HPLC system consisted of an Agilent quaternary HPLC model HP 1100 series (Hewlett-Packard, Palo Alto, CA, USA), fitted with an Altima C18 column (250 mm × 4.5 mm, 5 μm). The mobile phase used was water-acetonitrile (86:14), filtered through a 0.45 μm Millipore filter (Millipore, Hong Kong). The flow-rate was maintained at 1 ml/min, and the detection was performed at a wavelength of 231 nm under constant temperature (25 ± 0.1 °C).

2.2. Chemicals

The reference standards of paeoniflorin and sinomenine hydrochloride (>98%) were purchased from the State Institute for the Control of Pharmaceutical and Biological Products of China. Pentoxifylline, the internal standard for paeoniflorin, was purchased from Sigma (St. Louis, MO, USA). Acetonitrile and perchloric acid were obtained from Merck (Darmstadt, Germany).

2.3. Animals

Male Sprague–Dawley rats weighing 250–300 g were purchased from the Laboratory Animal Services Center of the Chinese University of Hong Kong. The animals were housed...
in different cages (five animals per cage) and acclimated in the laboratory for at least one-week prior to testing. Before experiments the animals were fasted for 12 h with water ad libitum, and maintained at 21°C temperature with 60% relative humidity and a 12 h light/12 h dark cycle. After surgery, rats were housed individually in metabolite cages for one-week recovery and underwent pharmacokinetic treatment according to a jugular-catheterized rat model (Thrivikraman et al., 2002). All procedures involving animals and their care were carried out according to the regulations of the Committee on the Use of Human & Animal Subjects in Teaching and Research of Hong Kong Baptist University and Health Department of Hong Kong Special Administrative Region.

2.4. Surgery

Silicone medical grade tubing 100 mm long (Silastic Cat. No. 602-155; Dow Corning, Midland, MI, USA) with two stoppers made of medical adhesive silicone (Silastic Cat. No. 801; Dow Corning) was used. For insertion of the catheter in the sinus venosus, the length of the tip of the catheter to the first stopper was set at 26 mm (Harms and Ojeda, 1974).

Rats were anesthetized by intraperitoneal administration of a mixture of 1.0 ml acepromazine maleate (10 mg/ml), 4 ml sterile water, 2.5 ml ketamine hydrochloride and 2.5 ml xylazine hydrochloride in a sealed vial at a dosage of 125 µl/100 g of body weight. A longitudinal skin incision was made over the area where the right external jugular vein passed dorsal to the pectoralis major muscle. The catheter, filled with 20 units/ml heparinized physiologic saline, was put into the right jugular vein and then advanced into the sinus venosus. The catheter was inserted up to the first silicone stopper and anchored in place by suturing the stopper to muscle. The free end of the catheter was passed under the skin of the dorsum of the neck just caudal to the ears and attached to the skin, together with a metal spring, which was covered with PVC tubing for protection of the outer part of the catheter. Finally, the catheter was filled with 500 units/ml heparinized saline, and a plug was inserted in the free end of the catheter (Cocchetto and Bjornsson, 1983; Yoburn et al., 1984; NRC, 1996; Kohn et al., 1997; Remie, 2000; Thrivikraman et al., 2000).

2.5. Drug administration and blood sampling

Catheterized rats were left to recover from surgery in individual metabolite cages for at least 7 days. Before experiments, three types of 1 ml syringes namely, flush syringe, collection syringe and heparinized syringe were prepared. After administration of the test compounds, blood samples were, firstly, drawn from the catheter using a flush syringe fitted with 27 gauge needle until a small amount of blood appeared in the needle bulb, and then the heparin solution was removed from the catheter together with the first sampling of 30–50 µl blood. Next, the flushing syringe was removed and replaced by a collection syringe the desired amount (0.2 ml) of blood was taken. Next, the collection syringe was removed and replaced with a heparinized syringe. Blood was gently rinsed from the catheter by flushing with the same volume of heparinized saline (20 units/ml) to replace the loss volume of blood a plug was inserted in the catheter. Finally, the blood was expelled from the collection syringe into a heparinized 1.5 ml micro-centrifuge tube.

In each of the experiments, one rat was administered with paeoniflorin alone, while another was administered with paeoniflorin plus sinomenine hydrochloride. Both compounds were prepared as an aqueous solution and administered orally to the fasted rats. For the co-administration protocol, paeoniflorin was first administered at the dosage of 150 mg/kg body weight, and then, immediately following, sinomenine was administered at the dosage of 90 mg/kg body weight.

After administration, jugular vein blood samples were collected (0.2 ml) from the rats into heparinized 1.5 ml micro-centrifuge tubes at the following time intervals: 0, 10, 20, 30, 45, 60, 80, 100, 120, 150, 180, 240, 300, 360, 540 and 720 min. Data from these samples were used to construct pharmacokinetic profiles by plotting drug concentrations versus times.

2.6. Preparation of plasma samples

Each collected blood sample was immediately transferred into a heparinized 1.5 ml micro-centrifuge tube and centrifuged at 8000 rpm for 6 min at room temperature. The resulting plasma (50 µl) was then mixed with 50 µl 4.5% perchloric acid aqueous solution and 50 µl internal standard (pentoxifylline in water 4.5 µg/ml) by vortex for 5 min. The denatured protein precipitate was separated by centrifugation at 13,000 rpm for 10 min at room temperature. An 80 µl volume supernatant was injected into the HPLC system for analysis. For recovery and precision determination of paeoniflorin in plasma, the same procedures for handling the blood samples were used.

2.7. Calibration curve

A calibration curve was constructed based on the HPLC analysis of blank rat plasma spiked with various concentrations (0.22, 0.45, 0.89, 1.78, 3.55, 7.10 and 10.65 µg/ml) of paeoniflorin, together with a fixed amount of pentoxifylline (4.5 µg/ml). The various concentrations of paeoniflorin in the plasmas were calculated from the values of peak areas by using the equation for linear regression obtained from the calibration curve.

2.8. Recovery

Blank rat plasma samples were spiked with four different concentrations (0.15, 0.59, 4.73 and 7.10 µg/ml) of paeoniflorin. After preparation of the plasma samples, fixed amounts
of internal standard (pentoxifylline) were added to the plasma for normalization. The resulting peak areas were compared with those standards carried in distilled water so as to calculate the recovery values.

2.9. Precision assay

The precision of the test was determined by triplicate analyses of plasma samples (n = 3) spiked with four different concentrations (0.15, 0.59, 4.73 and 7.10 µg/ml) of paeoniflorin. To determine intra-daily variances, the assays were carried out on the same samples at three time intervals during one day. Inter-daily variances were also determined by assaying the spiked samples over three consecutive days. Relative standard deviations (R.S.D.) were calculated from these sampled values of paeoniflorin.

2.10. Pharmacokinetic analysis

All data were subsequently processed by the pharmacokinetic software, PK Solutions 2.0 (Summit Co., USA). The non-compartmental pharmacokinetic parameters of half-life (t1/2), mean residence time (MRT), area under the plasma concentration-time curve (AUC), clearance (CL) and volume of distribution (Vd) were calculated based on moment methods. Relative bioavailability (Fref) of paeoniflorin was calculated using the following formula:

\[ F(\%) = \frac{\text{AUC}_{0-\infty}(A)}{\text{AUC}_{0-\infty}(B)} \times 100 \]

where AUC0–∞(A) represents the area under the concentration-time curve from zero up to infinite time when paeoniflorin and sinomenine were given jointly and AUC0–∞(B) represents the area when paeoniflorin was given alone.

3. Results

3.1. HPLC chromatograms

The HPLC chromatograms of blank plasma, plasma spiked with paeoniflorin (4.73 µg/ml) and the plasma obtained at 45 min after oral co-administration of paeoniflorin and sinomenine together, derived as explained above, are shown in Fig. 3. The retention time of paeoniflorin was found to be around 19.1 min. No interfering peaks were observed within the time frame in which paeoniflorin was detected.

3.2. Calibration curve

The calibration curve for area ratio of paeoniflorin to pentoxifylline was linear (r2 = 0.99997) over the range of concentrations of 0.15–7.10 µg/ml. With the least-squares method, a regression equation of \[ Y = 1.12643 X + 0.00135 \] (X is the amount ratio of paeoniflorin to pentoxifylline in plasma and Y is the area ratio of paeoniflorin to pentoxifylline) was obtained.

3.3. Recovery test and reproducibility

The data for validating the HPLC assay are shown in Tables 1–3. The recovery was higher than 84% (Table 1), the intra-daily variation was lower than 4.22% (Table 2) and the inter-daily variation lower than 7.69% (Table 3).

3.4. Determination of paeoniflorin blood plasma of rats

Fig. 4 shows that, after oral co-administration of paeoniflorin and sinomenine hydrochloride, plasma levels of paeoniflorin were significantly increased with a Cmax of 13.7 µg/ml.

3.5. Kinetic analysis

As calculated from plasma concentrations of paeoniflorin following oral administration of paeoniflorin alone.
Table 1
Recovery of the paeoniflorin assay

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Spiked concentration (μg/ml)</th>
<th>Measured value (μg/ml)</th>
<th>Recovery (%)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.10</td>
<td>6.81 ± 0.14</td>
<td>95.92</td>
<td>2.11</td>
</tr>
<tr>
<td>2</td>
<td>4.73</td>
<td>4.62 ± 0.09</td>
<td>97.67</td>
<td>2.01</td>
</tr>
<tr>
<td>3</td>
<td>0.59</td>
<td>0.50 ± 0.03</td>
<td>84.75</td>
<td>6.06</td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>0.14 ± 0.01</td>
<td>97.77</td>
<td>10.41</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± S.D. (n = 3).

b Recovery denotes the ratio of measured value over spiked concentration.

Table 2
Precision of the intra-daily assay

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Spiked concentration (μg/ml)</th>
<th>Calculated concentration (μg/ml)</th>
<th>Measured value (μg/ml)</th>
<th>Precision (%)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.10</td>
<td>6.78</td>
<td>6.91 ± 0.12</td>
<td>97.32</td>
<td>1.69</td>
</tr>
<tr>
<td>2</td>
<td>4.73</td>
<td>4.65</td>
<td>4.68 ± 0.06</td>
<td>97.89</td>
<td>1.35</td>
</tr>
<tr>
<td>3</td>
<td>0.59</td>
<td>0.56</td>
<td>0.55 ± 0.02</td>
<td>91.53</td>
<td>3.83</td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>0.14</td>
<td>0.14 ± 0.01</td>
<td>93.33</td>
<td>4.22</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± S.D. (n = 3).

b Precision denotes the ratio of measured value over spiked concentration.

Table 3
Precision of the inter-daily assay

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Spiked concentration (μg/ml)</th>
<th>Treated concentration (μg/ml)</th>
<th>Measured value (μg/ml)</th>
<th>Precision (%)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.10</td>
<td>6.78</td>
<td>6.91 ± 0.14</td>
<td>97.28</td>
<td>1.97</td>
</tr>
<tr>
<td>2</td>
<td>4.73</td>
<td>4.65</td>
<td>4.68 ± 0.04</td>
<td>98.97</td>
<td>0.81</td>
</tr>
<tr>
<td>3</td>
<td>0.59</td>
<td>0.56</td>
<td>0.57 ± 0.02</td>
<td>96.05</td>
<td>3.67</td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>0.14</td>
<td>0.13 ± 0.01</td>
<td>86.67</td>
<td>7.69</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± S.D. (n = 3).

b Precision denotes the ratio of measured value over spiked concentration.

or co-administration with sinomenine hydrochloride to the rats (n = 6, each group), the pharmacokinetic parameters of paeoniflorin are presented in Table 4. Computation of pharmacokinetic parameters using PK solutions 2.0 software (www.summitpk.com) showed that many parameters (i.e., t_{max}, C_{max}, AUC, t_{1/2}, C_L, and V_d) were dramatically altered by co-administration of paeoniflorin plus sinomenine in comparison with administration of paeoniflorin alone. These results suggest that the pharmacokinetics of the interactions between paeoniflorin and sinomenine might be quite complex.

4. Discussion
Pharmacokinetics aims to characterize drug adsorption, distribution, metabolism and excretion, and it is used in the clinical setting to design safe and effective protocols for drug administration. Data and information obtained from pharmacokinetic studies on active compounds of medicinal herbs could help us to understand the complex action of Chinese herbal medicines and to predict a variety of events related to efficacy and toxicity of herbs and herbal preparations (De Smet and Rivier, 1989; De Smet and Brouwers, 1997). Moreover, pharmacokinetic studies of new herbal products could be extremely important as a means to design the most effective therapeutic dosing regimes.

A simple and rapid analytical method is needed for the pharmacokinetic evaluation of paeoniflorin. Our approach to this problem was to use the HPLC method to analyze the concentrations of paeoniflorin in rat’s plasma. The validation results showed the R.S.D. of accuracy and precision of the
plasma concentration; AUC 0–V phase; convenient. The recoveries, precision and reproducibility, indicate that the ous solution was used to denature the plasma protein (Keung et al., 1996). The results of method validation, particularly Guan et al., 2003). In our method, 4.5% perchloric acid aque- and involving the use of toxic solvents (Chen et al., 1999; to remove non-polar interfering impurities. This is a cumber- sipitating protein with acetonitrile and extracting with ether in rat blood samples.

In previous reports, the biosamples were obtained by pre- capitaing protein with acetonitrile and extracting with ether to remove non-polar interfering impurities. This is a cumber- some, complex process resulting in rather lower recoveries and involving the use of toxic solvents (Chen et al., 1999; Guan et al., 2003). In our method, 4.5% perchloric acid aque- eous solution was used to denature the plasma protein (Keung et al., 1996). The results of method validation, particularly the recoveries, precision and reproducibility, indicate that the processes for denaturing plasma protein are simple, quick and convenient. The results of AUC0–V of pharmacokinetics obtained in the study demonstrate that oral bioavailability of paeoniflorin was elevated by more than 12 times in rats treated with co-administration of sinomenine at the tested doses that are comparable to the doses of these two herbs used in clinical for- mulas for the treatment of rheumatoid arthritis.

Compared with those of paeoniflorin given alone, the phar- macokinetic parameters of paeoniflorin can be dramatically improved by the concomitant administration with sinome- nine; AUC, Cmax, Vd and MRT, especially, showed significant differences. The results indicate that sinomenine hydrochloro- ride at a dosage of 90 mg/kg can markedly elevate the plasma concentration, delay the peak time and consequently increase the bioavailability of paeoniflorin in rats. However, the mech- anism of sinomenine on enhancement of the bioavailability of paeoniflorin in rats remains unclear. There are many reports in the literature that support the hypothesis that P-glycoprotein inhibitors enhance drug absorption from the intestine, elevating the bioavailability of the drug (Barthe et al., 1999; Bouer et al., 1999). Previous studies show that sinomenine is a po- tent inhibitor of immunity (Vieregg et al., 1999), and that its excretion is regulated by P-glycoprotein (Tsai and Wu, 2003); hence, it is possible that sinomenine can alter the disposition, absorption and bioavailability of paeoniflorin. Our prelimi- nary experimental data using a model of rat everted intestinal sacs (Bouer et al., 1999) showed that sinomenine could act as a P-glycoprotein inhibitor, and therefore promote the in- testinal transportation of drug from mucosa to serosa, this implied that the enhanced bioavailability of paeoniflorin by the co-administration of sinomenine might be closely related to P-glycoprotein (data not shown).

5. Conclusion

In the present study, an HPLC method has been devel- oped and validated for the characterization of plasma, and a jugular-catheterized rat model has been established to inves- tigate the influences of co-administration of sinomenine on the pharmacokinetics of paeoniflorin in freely moving rats. The results show that sinomenine hydrochloride enhanced the plasma concentration and extended the disposition time for paeoniflorin resulting in a marked increase of bioavailability in rats.

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References