Retractions

Retraction: Pharmacokinetic Interaction of Paeoniflorin and Sinomenine: Pharmacokinetic Parameters and Tissue Distribution Characteristics in Rats and Protein Binding Ability In Vitro

Zhong Qiu Liu, Zhi Hong Jiang, Kelvin Chan, Hua Zhou, Yuen Fan Wong, Zhao Xiang Bian, Hong Xi Xu, and Liang Liu

Journal of Pharmacological Sciences, Vol. 99, pp. 381–391 (2005)

doi: 10.1254/jphs.FP0050687

Retraction: Erratum to the above article

Journal of Pharmacological Sciences, Vol. 103, p. 438 (2007)

This article along with its erratum has been retracted at the request of the Editorial Board of the *Journal of Pharmacological Sciences* as of June 4, 2007 because a part of data in this article has been published in the previous publications shown below.

- 1) “Influence of co-administrated sinomenine on pharmacokinetic fate of paeoniflorin in unrestrained conscious rats”
Zhong Qui Liu, Hua Zhou, Liang Liu, Zhi Hong Jiang, Yuen Fan Wong, Ying Xie, Xiong Cai, Hong Xi Xu, Kelvin Chan
Journal of Ethnopharmacology, Vol. 99, pp. 61–67 (2005)
- 2) “The pharmacokinetics and tissue distribution of sinomenine in rats and its protein binding ability in vitro”
Zhong-Qui Liu, Kelvin Chan, Hua Zhou, Zhi-Hong Jiang, Yuen-Fan Wong, Hong-Xi Xu, Liang Liu
Life Sciences, Vol. 77, pp. 3197–3209 (2005)

The Japanese Pharmacological Society, the publisher

Full Paper

Pharmacokinetic Interaction of Paeoniflorin and Sinomenine: Pharmacokinetic Parameters and Tissue Distribution Characteristics in Rats and Protein Binding Ability In Vitro

Zhong Qiu Liu¹, Zhi Hong Jiang¹, Kelvin Chan¹, Hua Zhou¹, Yuen Fan Wong¹, Zhao Xiang Bian¹, Hong Xi Xu², and Liang Liu^{1,*}

¹School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

²Hong Kong Jockey Club Institute of Chinese Medicine Limited, Hong Kong, China

Received August 12, 2005; Accepted October 19, 2005

Abstract. The root of *Paeonia lactiflora* and the stem of *Sinomenium acutum* are two herbs widely used in Chinese herbal medicine for the treatment of inflammatory and arthritic diseases. Studies on the interaction of the active constituents of these herbs, i.e., paeoniflorin and sinomenine, in pharmacokinetic parameters, tissues distribution, and protein binding ability could provide empirical data to support their clinical application. Following oral administration to rats, the pharmacokinetic alterations were compared. The results showed that the pharmacokinetic parameters ($C_{\max}$, $T_{\max}$, AUC, MRT, CL , and V_d) of paeoniflorin were markedly enhanced when co-administrated with sinomenine. At 45 min after oral administration, the concentrations of paeoniflorin in the main internal organs were significantly increased when co-administrated with sinomenine. These phenomena were not ascribable to the alteration of the protein binding ability of paeoniflorin by sinomenine because obvious interactions of paeoniflorin and sinomenine in protein binding abilities in vitro to rat and rabbit plasma, human albumin, and α -1-acid-glycoprotein were not observed. However, with respect to the in vivo influence of paeoniflorin on sinomenine, the results showed that co-administration of paeoniflorin did not affect the pharmacokinetic parameters and tissue distribution of sinomenine.

Keywords: paeoniflorin, sinomenine, pharmacokinetics, bioavailability, tissue distribution

Introduction

Paeoniflorin is a bioactive monoterpene glucoside (Fig. 1A) present in the root of *Paeonia lactiflora* PALL. (family Ranunculaceae) that has been widely used to treat inflammatory 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 (1, 2). Paeoniflorin is characterized as a neutral

glucoside (molecular weight, MW: 428.47). It has a good solubility with a log P value of 2.88, which indicates it possesses a low lipophilicity (<http://www.chemnetbase.com>). In Mainland China, the total glucosides of *P. lactiflora* PALL., which comprise more than 70% of paeoniflorin, have been approved by the State Food and Drugs Administration of China for clinical application in treating rheumatic and arthritic diseases as a botanical drug (<http://www.sfda.org.cn>).

The alkaloid sinomenine (7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methyl-9 α ,13 α ,14 α -morphinan-6-one, Fig. 1B) is a bioactive component derived from the Chinese medicinal plant, *Sinomenium acutum* REHDDER

*Corresponding author. FAX: +852 34112461

E-mail: liuliang@hkbu.edu.hk

Abbreviations used are (alphabetical order):

AUC, area under concentration-time curve; $AUC_{0-\infty}$, area under concentration-time curve from zero up to infinite time; AUC_{0-t} , area under concentration-time curve from zero up to a definite time t ; CL , total clearance; $C_{\max}$, maximum plasma concentration; F_{rel} , relative bioavailability; HPLC, high-performance liquid chromatography; MRT, mean residence time; RSD, relative standard deviation; $t_{1/2}$, half-life; $T_{\max}$, time to reach maximum plasma concentration; V_d , volume of distribution.

Fig. 1. Chemical structure of paeoniflorin (A) and sinomenine (B).

& WILSON (family Menispermaceae), which has been utilized by Chinese doctors to treat inflammatory and arthritic diseases for over one thousand years (3). Sinomenine (MW: 329.40) is characterized as a weak base ($pK_a = 6.5$) with a log P value of 0.46, which indicates it possesses a good lipophilic property (<http://www.chemnetbase.com>). Clinical trials done in China have demonstrated that the pure alkaloid sinomenine has significant therapeutic effect for patients who suffer from rheumatoid arthritis (4, 5). Previous pharmacological studies showed that sinomenine has a wide range of actions including anti-inflammation (6, 7), immunosuppression (8), arthritis amelioration (9), and protection against hepatitis induced by lipopolysaccharides (10). However, in treating arthritis, Chinese medicine practitioners usually include both the root of *P. lactiflora* and the stem of *S. acutum* in their medical prescriptions, which suggests that there might be synergic effects between these two herbs (3).

In pharmacokinetic studies, it was found that paeoniflorin has a very poor absorption rate and thus a very low bioavailability (3%–4%) when administered orally. This was explained to be due to the limited transportation of paeoniflorin across the gastrointestinal mucosa (11, 12), and has been confirmed by our previous pharmacokinetic studies on paeoniflorin (13). Paeoniflorin could be metabolized into metabolites, named paeonimetabolins I and II, by human intestinal bacteria in vitro (14, 15). After oral administration, the blood concentration of paeonimetabolin I was found to be even higher than the plasma concentration of paeoniflorin (16, 17). Hsiu et al. further reported that paeoniflorin was not readily absorbed *per se*, whereas its aglycone paeoniflorigenin was absorbable and circulated in the bloodstream (18). Study on the excretion of paeoniflorin showed that the absorbed paeoniflorin was mainly excreted in urine because approximately 50% of the dose was found in urine at 30 min after intravenous administration, with little being excreted in feces and bile (11).

In our previous studies of pharmacokinetics of

sinomenine, when Lewis rats were i.p. injected with a single dose of 150 mg/kg sinomenine, the peak concentration of the drug in plasma was reached at 30 min after the injection, with $t_{1/2}$ of 4.24 ± 0.99 h (mean $\pm$ S.E.M.), while it was undetectable after 24 h (9). When healthy human male volunteers were orally given a single dose of 80-mg tablets, the serum concentration-time curve of the drug was consistent with a two-compartmental open pharmacokinetic model (19). A recent study on the regulation of hepatobiliary excretion of sinomenine by using microdialysis technology in rats demonstrated that sinomenine underwent active hepatobiliary elimination, which might be regulated by P-glycoprotein, and that P-450 was likely involved in its metabolism (20). The disposition of sinomenine, particularly in metabolism, might undergo phase I biotransformation and active hepatobiliary excretion in which P-glycoprotein might play an important role (20). Demethyl-sinomenine, a metabolite of sinomenine by the phase I metabolic pathway was found in urine (21). However, the bioavailability of orally-administered sinomenine, as well as its distribution in tissue and its protein binding ability remain unknown.

Based on the clinical practice of using both *P. lactiflora* and *S. acutum* for treating arthritis, we investigated the interactive effects of paeoniflorin and sinomenine on each other in the pharmacokinetics and tissue distribution in vivo and protein binding ability in vitro, so as to demonstrate the pharmacokinetic characteristics of the two herbal chemicals when co-administered.

Materials and Methods

Chemicals

The reference standards of paeoniflorin and sinomenine hydrochloride ($\geq 98\%$) were purchased from the Institute for the Control of Pharmaceutical and Biological Products of China, Beijing, China. Pentoxifylline, the internal standard, was purchased from Sigma (St. Louis, MO, USA). Human albumin and human α -1-acid-glycoprotein were obtained from Sigma. Rat and rabbit's plasma were from the Laboratory Animal Services Center of the Chinese University of Hong Kong. The SpectraPor dialysis membranes (molecular weight cutoff 10,000) were obtained from Spectrum Laboratories, Inc. (Los Angeles, CA, USA). Acetonitrile and perchloric acid were obtained from Merck (Darmstadt, Germany). *N*-Butyl alcohol, chloroform, triethylamine, glacial acetic acid, sodium hydroxide, and hydrochloric acid were from Fisher Scientific (St. Louis, MO, USA). Phosphate-buffered solution (pH = 7.4) powder was from Aldrich (Milwaukee, WI, USA), All

mobile phases solvents were HPLC grade and all reagents were analytical reagent grade.

HPLC determination for paeoniflorin and sinomenine and the validation of the assay

HPLC analyses were conducted by using an HP1100 series HPLC system and Altima C₁₈ (250 mm × 4.5 mm, 5 μm) column. The mobile phases were water : acetonitrile (86:14) for paeoniflorin and acetonitrile : 0.5% triethylamine water solution : 0.2% glacial acetic acid water solution (15:42.5:42.5) for sinomenine. All of the flow-rates of mobile phases were maintained at 1 ml/min, and the detection was performed at the wavelength of 231 nm for paeoniflorin and of 262 nm for sinomenine, respectively. The validation for the paeoniflorin assay has been described in our previous paper (13). The method for the determination of sinomenine was previously described (19). The validation results showed that RSDs of the accuracy and precision of determination for paeoniflorin and sinomenine were less than 10%, all RSDs of the intra- and inter-daily assay were also less than 10%, and the recovery rates were more than 80% for both the determinations of paeoniflorin and sinomenine.

Animals

Male Sprague-Dawley rats weighing 250–300 g (Laboratory Animal Services Center, the Chinese University of Hong Kong) were used. The rats were fasted overnight before the day of the experiments.

Animal surgery

The surgical procedures were the same as those described in our previous paper (13). After surgery, rats were housed individually in metabolite cages for one-week recovery and underwent pharmacokinetic treatment according to the method of the jugular-catheterized rat model (22). All procedures involving animals and their care were carried out according to the guidelines of the Committee on the Use of Human & Animal Subjects in Teaching & Research of Hong Kong Baptist University and the Health Department of Hong Kong Special Administrative Region.

Pharmacokinetic experiments in the jugular-catheterized rat model

Experiments were performed on 25 jugular-catheterized rats that were randomly divided into five groups for five protocols: oral administration of sinomenine alone (90 mg/kg), oral administration of paeoniflorin alone (150 mg/kg), and oral administration of paeoniflorin (150 mg/kg) plus sinomenine (20, 45, or 90 mg/kg). Blood samples (0.2 ml/time point) were collected

via a jugular vein catheter before administration and at different time intervals after administration. The blood sampling was scheduled from 10 min to 9 h after administration. Each collected blood sample was immediately transferred into a heparinized 1.5 ml microcentrifuge tube and centrifuged at 10,000 rpm for 6 min at room temperature. The resulting plasma (50 μl) was mixed with 50 μl 4.5% perchloric acid aqueous solution by vortexing for 5 min, and then the denatured protein precipitate was separated by centrifugation at 13,000 rpm for 10 min at room temperature. An 80-μl sample of the supernatant was injected into the HPLC system for analysis.

Tissue distribution experiments in rats

Twenty non-surgical rats were randomly divided into four groups for the oral administration using four test protocols: paeoniflorin alone (150 mg/kg), paeoniflorin (150 mg/kg) plus sinomenine (90 mg/kg), sinomenine alone (90 mg/kg), and sinomenine (90 mg/kg) plus paeoniflorin (150 mg/kg). Tissue samples were taken from the internal organs of the rats 45 min after drug administration. Each tissue sample, that is, approximately 0.5 g wet weight of heart, liver, spleen, lungs, kidneys, brain, and testis, was rinsed with 0.9% NaCl, accurately weighed, added with 1 ml ice-cold saline, and homogenized for 10 min in a bath of ice water, and then the mixture was centrifuged at 13,000 rpm. A 0.5-ml aliquot of the supernatant of each sample was then mixed with 50 μl of 4.5% perchloric acid aqueous solution by vortexing for 5 min; the denatured protein precipitate was further separated by centrifugation at 13,000 rpm for 10 min at room temperature. Finally, 80 μl of the supernatant of each sample for determinations of paeoniflorin and sinomenine concentrations in organ tissues was analyzed by HPLC. Furthermore, the tissue to plasma concentration ratios (K_p values) of paeoniflorin or sinomenine were calculated.

Equilibrium dialysis assay for protein binding characteristics in vivo

To assess the protein binding characteristics, a spectrum equilibrium dialyzer system (Spectrum Laboratories, Inc.) was employed. Test solutions (sinomenine at 4 μg/ml, paeoniflorin at 6 μg/ml, and two compounds together) were injected into each of four base solutions (rat or rabbit blood plasma, human albumin, and α-1-acid glycoprotein). A 1.0-ml aliquot of the resulting samples was dialyzed against drug-free PBS solution (25 ml) in Teflon dialysis chambers separated by a Spectrapor dialysis membrane. All above samples were dialyzed at 37°C with a rotation speed of 50 rpm for 4 h. For the PBS buffer (25 ml) solution obtained by

dialyzing sinomenine alone, its pH value was adjusted to 9.0 with 1 ml 0.2 N NaOH solution, and then it was extracted three times with chloroform (20 ml each time). The chloroform layers were pooled, and then the chloroform was removed in vacuo. The residue was dissolved in 2 ml 0.1 N HCl solution. For the PBS buffer (25 ml) solution obtained by dialyzing paeoniflorin alone, it was extracted three times with *n*-butyl alcohol (20 ml each time). The *n*-butyl alcohol layers were pooled, and then the *n*-butyl alcohol was removed in vacuo. The residue was dissolved in 2 ml distilled water. For the PBS buffer (25 ml) solution obtained by dialyzing sinomenine and sinomenine together, its pH value was adjusted to 9.0 as described above, and then the solution was extracted with *n*-butyl alcohol and the resulting *n*-butyl alcohol extracts were processed as described above. The residue was dissolved in 2 ml 0.1 N HCl solution. Subsequently, each water or HCl solution was filtered through a 0.45- μ M filter, and 80 μ l of the filtrate was analyzed by HPLC.

Data analyses

All data in pharmacokinetic experiments were processed with the pharmacokinetics software, PK Solutions 2.0 (Summit Research Services, Ashland, OH, USA). The non-compartmental pharmacokinetic parameters of $t_{1/2}$, MRT, AUC, C_L , and V_d were calculated based on moment methods. F_{rel} values for paeoniflorin and sinomenine were calculated as follows:

$$F(\%) = [AUC_{0-\infty}(A) / AUC_{0-\infty}(B)] \times 100\%$$

Where $AUC_{0-\infty}(A)$ represents the area under the concentration-time curve from zero up to infinite time when paeoniflorin and sinomenine were given together and $AUC_{0-\infty}(B)$ represents the area when paeoniflorin or sinomenine was given alone.

The distribution of paeoniflorin or sinomenine (D) in the internal organs of rats was calculated as follows:

$$D = [\text{Compound amount } (\mu\text{g}) \text{ in tissue} / \text{tissue weight by testing (g)}] \times 100\%$$

The ratio of tissue concentration vs plasma concentration of paeoniflorin or sinomenine (K_p value) was calculated as follows:

$$K_p = [\text{Compound concentration } (\mu\text{g/g}) \text{ in tissue after administration 45 min} / \text{Compound concentration } (\mu\text{g/ml}) \text{ in plasma after administration 45 min}] \times 100\%$$

The protein binding rates (P) of paeoniflorin or sinomenine in the plasma of rats or rabbits, in the solutions of albumin, or α -1-acid-glycoprotein were calculated as follows:

$$P = [(\text{Total amount of compound added} - \text{free amount of compound determined}) / \text{Total amount of compound added}] \times 100\%$$

All values are expressed as the mean $\pm$ S.D. Statistical

significances of the difference among pharmacokinetic parameters were assessed with ANOVA followed by *post hoc* test with the LSD test and significances of the data in tissue distribution and protein binding tests were assessed with Student's *t*-test. *P* values lower than 0.05 were considered significant.

Results

HPLC analyses of paeoniflorin and sinomenine in rat plasma

The HPLC chromatograms of blank plasma, plasmas spiked with paeoniflorin or sinomenine, and plasma obtained at 45 min after oral administration of paeoniflorin plus sinomenine are shown in Fig. 2. The retention times of paeoniflorin and sinomenine were found to be around 19.1 and 8.9 min, respectively. No interfering peaks were observed within the time frame in which paeoniflorin and sinomenine were detected.

Paeoniflorin pharmacokinetics influenced by co-administration of sinomenine at different doses

The time courses of the representative plasma concentrations of paeoniflorin after oral administration at the dose of 150 mg/kg without or with co-administration of sinomenine at the doses of 20, 45, and 90 mg/kg body weight in the unrestrained conscious rats are shown in Fig. 3A. Table 1 summarizes the pharmacokinetic parameters of paeoniflorin calculated from plasma concentrations of paeoniflorin following oral administration of paeoniflorin alone or with sinomenine at different doses to rats ($n=5$). In comparison with paeoniflorin given alone, many pharmacokinetic parameters, including T_{max} , C_{max} , AUC, $t_{1/2}$, C_L , and V_d , of paeoniflorin co-administrated with sinomenine in the groups of 45 and 90 mg/kg were dramatically improved in a dose-dependent manner. Although there was no significant difference between the group of paeoniflorin alone and paeoniflorin with sinomenine at 20 mg/kg, we found the obvious tendency that those parameters of paeoniflorin could be improved by sinomenine at 20 mg/kg. These results indicated that sinomenine significantly enhances the pharmacokinetic behavior of paeoniflorin when they are co-administered.

Sinomenine pharmacokinetics influenced by co-administration of paeoniflorin

The time courses of the representative plasma concentrations of sinomenine after oral administration at the dose of 90 mg/kg without or with co-administration of paeoniflorin at the doses of 150 mg/kg body weight in the unrestrained conscious rats are shown in Fig. 3B. Table 2 summarizes the pharmacokinetic parameters of

Fig. 2. The HPLC chromatograms of paeoniflorin and sinomenine in rat plasma. A: plasma sample obtained from rat at 45 min after oral co-administration of paeoniflorin and sinomenine and spiked with pentoxifylline ($4.50 \mu\text{g/ml}$ as internal standard), B: blank plasma spiked with paeoniflorin ($4.73 \mu\text{g/ml}$) and pentoxifylline ($4.50 \mu\text{g/ml}$), C: blank plasma spiked with pentoxifylline ($4.50 \mu\text{g/ml}$), D: plasma sample obtained from rat at 45 min after oral co-administration of paeoniflorin and sinomenine, E: blank plasma spiked with sinomenine ($2.55 \mu\text{g/ml}$) alone, F: blank plasma.

sinomenine after oral administration alone or with paeoniflorin. Significant difference between each pharmacokinetic parameter was not observed, including $T_{\max}$, $C_{\max}$, AUC, $t_{1/2}$, C_L , and V_d , obtained when sinomenine was given alone and co-administrated with paeoniflorin.

Paeoniflorin concentration in tissues of rat internal organs affected by co-administration of sinomenine

The concentrations of paeoniflorin in the internal organ tissues of the rats treated by paeoniflorin alone or with sinomenine at 45 min after oral administration are showed in Fig. 4A. The concentrations of paeoniflorin were significantly higher ($P < 0.01$) in all organs of the rats that received a combinative treatment of paeoni-

Fig. 3. The representative curves of the compounds concentrations vs time profiles in rat plasma. A: Paeoniflorin concentrations vs time curves of oral administration of paeoniflorin (150 mg/kg) alone (filled triangles) and co-administration of paeoniflorin (150 mg/kg) and sinomenine (20 mg/kg, blank triangles; 45 mg/kg, blank squares; 90 mg/kg, blank circles), B: Sinomenine concentrations vs time curves of oral administration of sinomenine (90 mg/kg) alone (filled triangles) and co-administration (blank triangles) of sinomenine (90 mg/kg) and paeoniflorin (150 mg/kg).

Table 1. Pharmacokinetic parameters of paeoniflorin after oral administration (150 mg/kg) alone or co-administration with sinomenine in rats (n = 5)

Pharmacokinetic parameters	Paeoniflorin alone	Paeoniflorin and sinomenine (90 mg/kg)	Paeoniflorin and sinomenine (45 mg/kg)	Paeoniflorin and sinomenine (20 mg/kg)
T_{max} (min)	45.00 ± 5.00	77.30 ± 17.50 ^b	65.00 ± 14.14 ^b	51.00 ± 13.42
C_{max} (µg/ml)	1.26 ± 0.23	6.03 ± 2.45 ^b	5.78 ± 2.37 ^b	3.18 ± 1.98
AUC_{0-t} (µg·min/ml)	116.68 ± 37.54	1528.27 ± 555.07 ^c	1327.56 ± 503.71 ^c	383.18 ± 240.97
$AUC_{0-\infty}$ (µg·min/ml)	124.62 ± 36.91	1540.43 ± 548.96 ^c	1332.96 ± 502.25 ^c	391.10 ± 236.23
$T_{1/2 E}$ phase (min)	55.07 ± 24.91	84.14 ± 53.11	80.76 ± 19.38	101.80 ± 82.12
$T_{1/2 D/A}$ phase (min)	19.58 ± 9.01	53.78 ± 22.17 ^a	60.87 ± 23.75 ^a	41.12 ± 20.37
$T_{1/2 A}$ phase (min)	17.63 ± 11.62	44.70 ± 10.26 ^a	28.87 ± 12.23	38.96 ± 21.37
CL (ml/min per kg)	1301.83 ± 429.03	110.44 ± 45.61 ^b	197.06 ± 97.00 ^b	717.75 ± 306.23
V_d (ml/kg)	102044.14 ± 46608.43	16064.25 ± 17189.33 ^a	15742.10 ± 9811.57 ^a	58188.68 ± 37855.76
MRT (min)	133.12 ± 38.63	224.07 ± 26.62 ^b	237.12 ± 56.76 ^b	270.00 ± 97.54
F_{rel} (%)	N/A	12.36	11.34	3.28

Data are expressed as the mean ± S.D. C_{max} : Maximum plasma concentration, t_{max} : time to reach maximum plasma concentration, AUC_{0-t} : area under the concentration-time curve from zero up to a definite time t , $AUC_{0-\infty}$: area under the concentration-time curve from zero up to infinite time, $t_{1/2 E}$ phase: half-life of elimination phase, $t_{1/2 D/A}$ phase: half-life of distribution phase, $t_{1/2 A}$ phase: half-life of absorption phase, V_d : volume of distribution, CL : total clearance, MRT: mean residence time, F_{rel} : relative bioavailability. ^{a,b,c}: Values are significantly different from that of the paeoniflorin alone group by Student's t -test at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

florin and sinomenine, as compared to the organs of rats that received only paeoniflorin. The tissue and plasma ratio (K_p value) of paeoniflorin in the animals treated with or without sinomenine together was calculated as shown in Table 3, in which it can be seen that the K_p s treated by co-administration of paeoniflorin and sinomenine in all of the organs detected were higher than those treated by paeoniflorin alone. These results indicate that sinomenine could increase paeoniflorin in internal organs along with the increase of drug in plasma.

Sinomenine concentration in tissues of rat internal organs affected by co-administration of paeoniflorin

The concentrations of sinomenine in the internal organ tissues of the rats treated by sinomenine alone or with paeoniflorin at 45 min after oral administration are shown in Fig. 4B. The concentration of sinomenine was similar in all organs of the rats that received a combinative treatment of sinomenine and paeoniflorin, as compared to organs of rats that received sinomenine only. Furthermore, the tissue and plasma ratio (K_p value) of sinomenine in the animals treated with or without

Table 2. Pharmacokinetic parameters of sinomenine after oral administration (90 mg/kg) alone or co-administration with paeoniflorin (150 mg/kg) to rats (n = 8)

Pharmacokinetic parameters	Sinomenine alone	Sinomenine and paeoniflorin
T _{max} (min)	39.5 ± 8.49	44.4 ± 11.19
C _{max} (μg/ml)	13.89 ± 4.29	13.70 ± 3.65
AUC _{0-t} (μg·min/ml)	2331.53 ± 1172.77	2112.9 ± 539.52
AUC _{0-∞} (μg·min/ml)	2832.06 ± 1295.80	2521.2 ± 676.22
T _{1/2 E phase} (min)	332.56 ± 246.47	263.50 ± 153.10
T _{1/2 D/A phase} (min)	55.80 ± 41.69	59.40 ± 59.59
T _{1/2 A phase} (min)	61.28 ± 53.62	50.60 ± 27.56
C _L (ml/min per kg)	42.95 ± 14.40	44.40 ± 8.49
V _d (ml/kg)	17067.29 ± 12826.49	13373.2 ± 6848.32
MRT (min)	361.33 ± 175.96	293.20 ± 132.10

Data are expressed as the mean ± S.D. Abbreviations are the same as those in Table 1.

Fig. 4. The concentrations of paeoniflorin and sinomenine in rat internal organs at 45 min after administration. A: The concentrations of paeoniflorin after a single oral administration of paeoniflorin at 150 mg/kg (blank bars) and a single oral co-administration (filled bars) of paeoniflorin (150 mg/kg) plus sinomenine (90 mg/kg). ** $P < 0.01$, compared with the same organs in the other group (n = 5). B: The concentrations of sinomenine after a single oral administration of sinomenine at 90 mg/kg (blank bars) and a single oral co-administration (filled bars) of sinomenine (90 mg/kg) plus paeoniflorin (150 mg/kg) (n = 5).

paeoniflorin together was calculated as shown in Table 4, in which it can be seen that the K_{ps} treated by co-administration of sinomenine and paeoniflorin in all of the organs detected were similar to those treated with sinomenine alone. These results indicate that paeoniflorin could not significantly affect tissue distribution of sinomenine.

In vitro interaction of sinomenine and paeoniflorin on protein binding characteristics

In our pervious studies (23), we optimized the working concentrations of sinomenine and paeoniflorin for the protein binding experiments in rat plasma and determined that the concentrations of sinomenine from 1 to 8 μg/ml and of paeoniflorin from 2 to 10 μg/ml were optimal for the protein binding experiments. Hence, 4

and 6 μg/ml concentrations for sinomenine and paeoniflorin, respectively, were chosen for further experiments with rabbit plasma, albumin, and α-1-acid glycoprotein. Moreover, we also optimized the concentrations of albumin and α-1-acid glycoprotein in the testing solutions, that is, for binding rates of sinomenine and paeoniflorin at 30 and 0.25 μg/ml, respectively. Under this condition, the interactive effects of sinomenine and paeoniflorin in the protein binding ability either in the plasma of rats and rabbits or in the solutions of human albumin and α-1-acid glycoprotein were examined. The results shown in Fig. 5 indicated that sinomenine and paeoniflorin did not influence each other's protein binding ability when they are present simultaneously.

Table 3. Tissue to plasma concentration ratio (K_p value) of paeoniflorin at 45 min after administration of paeoniflorin alone or with sinomenine

Sample source	Paeoniflorin (150 mg/kg) administered alone		Paeoniflorin (150 mg/kg) administered with sinomenine (90 mg/kg)	
	Conc. of paeoniflorin ($\mu\text{g/ml}$)	K_p value (%)	Conc. of paeoniflorin ($\mu\text{g/ml}$)	K_p value (%)
Blood	0.86 ± 0.31	N/A	8.84 ± 2.29	N/A
Heart	0.56 ± 0.50	65.12	16.72 ± 15.35	189.14
Liver	0.65 ± 0.34	75.58	14.59 ± 3.34	165.05
Spleen	0.90 ± 0.89	104.65	40.58 ± 4.29	459.05
Lung	1.03 ± 0.59	119.77	45.27 ± 18.18	512.10
Kidney	1.07 ± 0.65	124.42	33.66 ± 5.73	380.77
Brain	0.10 ± 0.10	11.63	18.25 ± 5.42	206.45
Testicle	0.12 ± 0.09	13.95	11.77 ± 1.56	133.14

Table 4. Tissue to plasma concentration ratio (K_p value) of sinomenine at 45 min after administration of sinomenine alone or with paeoniflorin

Sample source	Sinomenine (90 mg/kg) administered alone		Sinomenine (90 mg/kg) administered with paeoniflorin (150 mg/kg)	
	Conc. of paeoniflorin ($\mu\text{g/ml}$)	K_p value (%)	Conc. of paeoniflorin ($\mu\text{g/ml}$)	K_p value (%)
Blood	12.10 ± 4.65	N/A	12.60 ± 3.91	N/A
Heart	60.50 ± 14.94	500.03	56.48 ± 18.31	448.28
Liver	141.68 ± 67.49	1170.88	139.44 ± 83.92	1106.70
Spleen	111.96 ± 19.93	925.32	103.55 ± 45.59	821.84
Lung	128.33 ± 41.49	1060.60	121.98 ± 30.16	968.08
Kidney	176.49 ± 40.38	1458.61	158.87 ± 68.78	1260.86
Brain	10.14 ± 0.61	83.76	11.70 ± 3.17	92.88
Testicle	9.28 ± 1.50	76.67	9.33 ± 3.62	74.02

Fig. 5. The in vitro protein binding ability of paeoniflorin and sinomenine. A: Comparison between the group of paeoniflorin alone (blank bars) and the group of paeoniflorin plus sinomenine (filled bars, $n = 5$), B: Comparison between the group of sinomenine alone (blank bars) and the group of sinomenine plus paeoniflorin (filled bars, $n = 5$).

Discussion

Traditional medicines, including Chinese medicines, are increasingly used by patients who typically advise them not to use them concomitantly (24). Because the interactions between herbs may increase or decrease the pharmacological or toxicological effects of each other, the herb-herb interactions should be documented. Pharmacokinetic study is one of the most important investigations for elucidating the clinical efficacy or/and explaining and predicting a variety of events related to the efficacy and toxicity of Chinese herbs (25, 26). The root of *P. lactiflora* PALL. and the stem of *S. acutum* are commonly used together for the treatment of arthritic diseases by Chinese medicine practitioners (3), while paeoniflorin and sinomenine are two representative bioactive components of these two herbs, respectively (1, 2, 4). In the present study, we have, therefore, evaluated the potential pharmacokinetic interactions of paeoniflorin and sinomenine in terms of the pharmacokinetic parameters and tissue distribution characteristics in vivo and the protein binding ability in vitro.

The pharmacokinetic parameters of paeoniflorin summarized in Table 1 show that there were statistically significant differences in all parameters including the $T_{\max}$, $C_{\max}$, AUC, $t_{1/2}$, C_L , V_d , and MRT between the animals that had received paeoniflorin (150 mg/kg) alone and the animals that received a combined treatment of paeoniflorin (150 mg/kg) plus sinomenine (20, 45, or 90 mg/kg). In particular, in the animals that received both paeoniflorin and sinomenine, the peak plasma concentration of paeoniflorin was markedly elevated ($P<0.01$), the peak time was delayed ($P<0.01$), the AUC was increased ($P<0.001$), the mean residence time was prolonged ($P<0.01$), the C_L was decreased ($P<0.01$), and the V_d was reduced ($P<0.05$), in comparison to the animals that had received paeoniflorin alone. The F_{rel} value of paeoniflorin was also markedly improved. These enhancements on pharmacokinetics of paeoniflorin induced by co-administration of sinomenine were dose-dependent. Results of the drug distribution in the internal organs of rats showed that co-administration of sinomenine could markedly increase the tissue concentrations of paeoniflorin at 45 min after administration; the highest concentration of paeoniflorin was found in the lungs and then (in descending order of concentration) spleen, kidneys, brain, heart, liver, and testicles. Furthermore, paeoniflorin ratios of tissue concentration vs plasma concentration in all the internal organs of rats treated with paeoniflorin and sinomenine together were higher than those in the animals treated with paeoniflorin alone. The results implied that sinomenine could not only increase the plasma level of

paeoniflorin, but also could promote the distribution of paeoniflorin from plasma to the internal organs like liver, kidney, spleen, and lung (Table 3). These enhancements of the drug distribution in the internal organs may result from the significant improvement of the bioavailability of paeoniflorin induced by sinomenine. However, the distribution and K_p value of sinomenine were not affected by co-administration of paeoniflorin.

Nevertheless, the mechanism by which sinomenine is able to enhance the bioavailability of paeoniflorin in the treated-rats remains unclear. It has been well known that the protein binding ability of a drug in blood plasma is a very important factor influencing its concentration in blood as well as its distribution in tissues. So, we have also investigated the interaction between paeoniflorin and sinomenine on protein binding ability in plasma, albumin, and α -1-acid glycoprotein, so as to investigate the possible influence of sinomenine on the plasma concentration of paeoniflorin. In vitro experiments using blood plasma of rats and rabbits, human albumin, and α -1-acid glycoprotein showed that co-administration of sinomenine did not influence the protein binding rates of paeoniflorin in vitro. This implied that the enhancement of the free blood concentrations of paeoniflorin induced by co-administration of sinomenine might not result from the alteration of protein binding ability of the chemicals.

There are many reports supporting the hypothesis that P-glycoprotein inhibitors can enhance drug absorption from small intestine, elevating the bioavailability of the drugs (27, 28). Sinomenine was reported to be a potent inhibitor of immunity (8), and its excretion was partially regulated by P-glycoprotein (20). Hence, it is estimated that sinomenine can alter the disposition, absorption, and bioavailability of paeoniflorin. Our experimental data using a model of rat everted intestinal sacs (28) showed that sinomenine could act as a P-glycoprotein inhibitor and therefore promote the intestinal transportation of drug from the mucosa side to the serosa side (29). This implies that the enhanced bioavailability of paeoniflorin when co-administered with sinomenine might be closely related to the inhibition of P-glycoprotein. On the other hand, it was previously found that paeoniflorin could be hydrolyzed into its aglycone, paeoniflorigenin, which could be detected in rats' serum (17). The $C_{\max}$, $T_{\max}$, and AUC_{0-t} of paeoniflorigenin were 8.0 $\mu\text{g}/\text{ml}$, 10 min, and 487.0 $\mu\text{g}/\text{ml}$, respectively (18). In order to expound the mechanism of the enhanced bioavailability of paeoniflorin by sinomenine, using the Caco-2 cultured cell model and perfused rat intestinal model, it was found that paeoniflorin could be hydrolyzed by β -glucosidase in small intestine; while sinomenine could not affect the hydrolysis. However, sinomenine could influence the

absorption and transport of paeoniflorin that are modulated by P-glycoprotein efflux transporter (data were submitted elsewhere for publication).

The pharmacokinetic parameters of sinomenine summarized in Table 2 demonstrated that there was no significant difference between the animals that received sinomenine at 90 mg/kg and those received a combinative treatment of sinomenine at 90 mg/kg plus paeoniflorin at 150 mg/kg. When sinomenine was orally administered to the rats either alone or co-administrated with paeoniflorin, it was absorbed moderately, reached a peak concentration at about 40 min after administration (Fig. 3B). The pharmacokinetic parameters of sinomenine, such as T_{max} , C_{max} , $t_{1/2}$, and AUC, in the animals administered either with one or two drugs were quite similar. So, paeoniflorin did not significantly affect any pharmacokinetic parameters of sinomenine. Moreover, there were no significant alterations of protein binding rate in vitro or of the concentrations of sinomenine in the internal organ tissues of rats when it was co-administered with paeoniflorin. In short, the pharmacokinetic parameters, protein binding ability, and tissue distribution of sinomenine were not influenced by the co-administration of paeoniflorin.

The pharmacokinetic interactions of paeoniflorin and sinomenine in pharmacokinetic parameters and tissue distribution in rats and the protein binding ability in vitro were investigated. A jugular-catheterized rat model and a non-compartmental pharmacokinetic model for construction of pharmacokinetic profiles, an equilibrium dialysis assay for evaluating protein binding in vitro, and HPLC assays for determining sinomenine and paeoniflorin were effectively employed. The results showed that sinomenine could markedly elevate the plasma concentration and extend the disposition time of paeoniflorin, resulting in significant improvement of the bioavailability of paeoniflorin in rats. It could also increase the paeoniflorin concentrations in various internal organs of rats, although it could not influence the protein binding rates of paeoniflorin in vitro. In contrast, paeoniflorin could not enhance any of the pharmacokinetic parameters, tissue distribution, or protein binding ability of sinomenine by co-administration. This study justifies and, in some sense, explains the traditional use of the two herbs together in herbal prescriptions.

Acknowledgment

This work is funded by the Hong Kong Jockey Club Charities Trust.

References

- 1 Yamahara J, Yamada T, Kimura H, Sawada T, Fujimura H. Biologically active principles of crude drugs. II. Anti-allergic principles in "Shoseiryu-To" anti-inflammatory properties of paeoniflorin and its derivatives. *J Pharmacobiodyn.* 1982;5:921–929.
- 2 Kimura M, Kimura I, Takahashi K, Muroi M, Yoshizaki M, Kanaoka M, et al. Blocking effects of blended paeoniflorin or its related compounds with glycyrrhizin on neuromuscular junctions in frog and mouse. *Jpn J Pharmacol.* 1984;36:275–282.
- 3 Wong LC, Wu LT. [History of Chinese medicine]. 2nd ed. Shanghai: National Quarantine Service; 1936. (in Chinese)
- 4 Wang Y, Zhou L, Li R. [Study progress in *Sinomenium acutum* (Thunb.) Rehd. et Wils]. *Zhong Yao Cai.* 2002;25:209–211. (text in Chinese with English abstract).
- 5 Yamasaki H. Pharmacology of sinomenine, an anti-rheumatic alkaloid from *Sinomenium acutum*. *Acta Med Okayama.* 1976; 30:1–20.
- 6 Tai Z, Hopkins SJ. Sinomenine: antiarthritic antiinflammatory. *Drugs of the Future.* 1998;23:45–49.
- 7 Kok TW, Yue PY, Mak NK, Fan TP, Liu L, Wong RN. The anti-angiogenic effect of sinomenine. *Angiogenesis.* 2005;8:3–12.
- 8 Vieregge B, Resch K, Kaever V. Synergistic effects of the alkaloid sinomenine in combination with the immunosuppressive drugs tacrolimus and mycophenolic acid. *Planta Med.* 1999;65:80–82.
- 9 Liu L, Resch K, Kaever V. Inhibition of lymphocyte proliferation by the anti-arthritis drug sinomenine. *Int J Immunopharmacol.* 1994;16:685–691.
- 10 Kondo Y, Takano F, Yoshida K, Hojo H. Protection by sinomenine against endotoxin-induced fulminant hepatitis in galactosamine-sensitized mice. *Biochem Pharmacol.* 1994;48: 1050–1052.
- 11 Takeda S, Isono T, Wakui Y, Matsuzaki Y, Sasaki H, Amagaya S, et al. Absorption and excretion of paeoniflorin in rats. *J Pharm Pharmacol.* 1995;47:1036–1040.
- 12 Takeda S, Isono T, Wakui Y, Mizuhara Y, Amagaya S, Maruno M, et al. In-vivo assessment of extrahepatic metabolism of paeoniflorin in rats: relevance to intestinal floral metabolism. *J Pharm Pharmacol.* 1997;49:35–39.
- 13 Liu ZQ, Zhou H, Liu L, Jiang ZH, Wong YF, Xie Y, et al. Influence of co-administrated sinomenine on pharmacokinetic fate of paeoniflorin in unrestrained conscious rats. *J Ethnopharmacol.* 2005;99:61–67.
- 14 Hattori M, Shu YZ, Shimizu M, Hayashi T, Morita N, Kobashi K, et al. Metabolism of paeoniflorin and related compounds by human intestinal bacteria. *Chem Pharm Bull.* 1985;33:3838–3846.
- 15 Shu YZ, Hattori M, Akao T, Kobashi K, Kagi K, Fukuyama K, et al. Metabolism of paeoniflorin and related compounds by human intestinal bacteria. II. Structures of 7S- and 7R-paeonimetabolites I and II formed by *Bacteroides fragillis* and *Lactobacillus brevis*. *Chem Pharm Bull.* 1987;35:3726–3733.
- 16 Heikal OA, Miyashiro H, Akao T, Hattori M. Quantitative determination of paeoniflorin and its major metabolites, paeonimetabolin I, in the rat plasma by enzyme immunoassay. *J Trad Med.* 1997;14:34–40.
- 17 Heikal OA, Akao T, Takeda S, Hattori M. Pharmacokinetic study of paeonimetabolin I, a major metabolite of paeoniflorin

- from paeony roots. *Biol Pharm Bull.* 1997;20:517–521.
- 18 Hsiu SL, Lin YT, Wen KC, Hou YC, Chao PD. A deglycosylated metabolite of paeoniflorin of the root of *Paeonia lactiflora* and its pharmacokinetics in rats. *Planta Med.* 2003;69:1113–1118.
- 19 Yan XH, Li HD, Peng WX, Liu FQ, Shao Y, He YQ. [Determination of sinomenine HCl in serum and urine by HPLC and its pharmacokinetics in normal volunteers]. *Yao Xue Xue Bao.* 1997;32:620–624. (text in Chinese with English abstract)
- 20 Tsai TH, Wu JW. Regulation of hepatobiliary excretion of sinomenine by P-glycoprotein in Sprague-Dawley rats. *Life Sci.* 2003;72:2413–2426.
- 21 Christians U, Kaever VW. Epimorphian compound and its use. US patent 6,372,756. 1999; April 16.
- 22 Thiruvikraman KV, Huot RL, Plotsky PM. Jugular vein catheterization for repeated blood sampling in the unrestrained conscious rat. *Brain Res Brain Res Protoc.* 2002;10:84–94.
- 23 Liu ZQ, Chan K, Zhou H, Jiang ZH, Wong YF, Xu HX, et al. The pharmacokinetics and tissue distribution of sinomenine in rats and its protein binding ability in vitro. *Life Sci.* 2005;77:3197–3209.
- 24 Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL. Unconventional medicine in the United States. Prevalence, costs, and patterns of use. *N Engl J Med.* 1993;328:246–252.
- 25 De Smet PA, Rivier L. A general outlook on ethnopharmacology. *J Ethnopharmacol.* 1989;25:127–138.
- 26 De Smet PA, Brouwers JR. Pharmacokinetic evaluation of herbal remedies. Basic introduction, applicability, current status and regulatory needs. *Clin Pharmacokinet.* 1997;32:427–436.
- 27 Barthe L, Woodley J, Houin G. Gastrointestinal absorption of drugs: methods and studies. *Fundam Clin Pharmacol.* 1999;13:154–168.
- 28 Bouer R, Barthe L, Philibert C, Tournaire C, Woodley J, Houin G. The roles of P-glycoprotein and intracellular metabolism in the intestinal absorption of methadone: in vitro studies using the rat everted intestinal sac. *Fundam Clin Pharmacol.* 1999;13:494–500.
- 29 Chan K, Liu ZQ, Jiang ZH, Zhou H, Wong YF, Xu HX, et al. The effects of sinomenine on intestinal absorption of paeoniflorin by everted rat gut sac model. *J Ethnopharmacol.* In press.