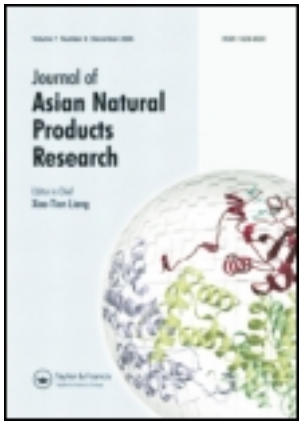

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Pharmacokinetic properties of albiflorin and paeoniflorin after oral administration of pure compound, Radix *Paeoniae alba* extract and Danggui-Shaoyao-San extract to rats

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Pharmacokinetic properties of albiflorin and paeoniflorin after oral administration of pure compound, Radix *Paeoniae alba* extract and Danggui-Shaoyao-San extract to rats

Ying-Fei Li[a], Meng Wang[a], Xiao-Ying Wang[a], He-Shui Yu[b], Li-Ping Kang[b], Bai-Ping Ma[b], Jin-Xiu Ruan[a] and Zhen-Qing Zhang[a]*

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This study compared the pharmacokinetics of albiflorin (ALB) and paeoniflorin (PAE), respectively, after oral administration of ALB, PAE, Radix *Paeoniae alba* (RPA) extract, and Danggui-Shaoyao-San (DSS) extract to rats on separate occasions. Analytes were detected simultaneously with liquid chromatography-tandem mass spectrometry. Noncompartmental pharmacokinetic parameters were calculated. After oral administration of RPA and DSS extract to rats, ALB reached maximum concentrations of 4637 ± 2774 ng/ml (0.40 ± 0.14 h) and 226 ± 122 ng/ml (0.35 ± 0.14 h) and PAE reached maximum concentrations of 2132 ± 560 ng/ml (0.40 ± 0.14 h) and 143 ± 65 ng/ml (0.45 ± 0.11 h), respectively. Compared to the AUC_{0-t} value (1122 ± 351 and 722 ± 158 ng h/ml for ALB and PAE, respectively) after administration of monomers, larger AUC_{0-t} value of ALB (4755 ± 2560 ng h/ml) and PAE (2259 ± 910 ng h/ml) after administration of RPA extract and smaller AUC_{0-t} value of ALB (411 ± 118 ng h/ml) and PAE (242 ± 126 ng h/ml) after administration of DSS extract were obtained. The C_{max}, AUC, and K_{el} of ALB and PAE were remarkably increased ($P < 0.05$, 0.01 or 0.005) during oral administration of RPA extract in comparison to that of DSS extract.

Keywords: albiflorin; paeoniflorin; pharmacokinetics; Radix *Paeoniae alba*; Danggui-Shaoyao-San

1. Introduction

Compound recipe is the major clinical application mode of traditional Chinese medicine (TCM). Different combinations of TCM lead to different compound recipes, which leads to different pharmacokinetics of compounds, to treat different diseases. Due to the complexity and/or trace of chemical ingredients in compound prescriptions, it is difficult to investigate pharmacokinetics of all the compounds in prescription simultaneously. In addition, studies on compatibility of complex prescriptions are important and pharmacokinetics is usually considered as one of the useful ways in this process. Therefore, many of the reports focused on possible representative ingredients to investigate their pharmacokinetic properties in prescription [1]. Only few compared results of pharmacokinetics about pure compound, herb, and prescription were reported [2], which will help us to know not only the real pharmacokinetics properties of compound in complex prescriptions, but also the compatibility of complex prescriptions further.

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As one of widely used formula in clinic, Danggui-Shaoyao-San (DSS) which is also called 'Toki-shakuyaku-san' in Japanese contains Radix *Paeoniae alba* (RPA), Radix *Angelica sinensis*, *Rhizoma Chuanxiong*, *Poria cocos*, Rhizoma *Atractylodis macrocephalae*, and *Rhizoma Alismatis*. The prescription was initially used as antiabortifacient, and nowadays has been widely used to treat gynecological diseases including anemia of pregnancy, hypoovarianism, infertilitas feminis, and melancholia [3–5]. In addition, it has been used for some diseases such as Alzheimer's disease, Parkinson's disease, and cerebrovascular accident residual with its further study [6–10].

Recently, some active ingredients related to pharmacological functions of DSS have been gradually revealed [11]. Among them, monoterpene glycosides, mainly albiflorin (ALB) and paeoniflorin (PAE), were considered as the most representative components of DSS as far as both the contents and biological activities [12] are concerned.

The aim of this study was to compare the pharmacokinetics of ALB and PAE, respectively, after oral administration of the ALB, PAE, RPA extract, and DSS extract to rats on separate occasions (Figure 1). The information obtained might be useful for understanding pharmacological mechanisms, different clinical applications, as well as the rules of compatibility of the compound recipe of DSS.

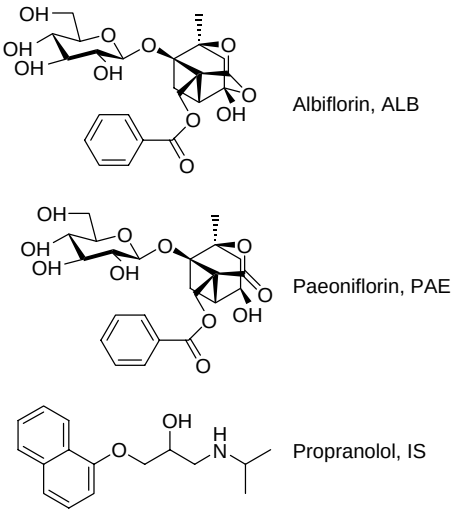

Figure 1. Chemical structures of PAE, ALB, and IS.

2. Results

During reversed-phase HPLC, the retention times for ALB, PAE, and IS were 2.51, 3.52, and 5.58 min, respectively (Figure 2). No interfering peaks were detected at these times in blank plasma samples. The overall chromatographic run time was finished within 8 min. The assay time is short and suitable for the analysis

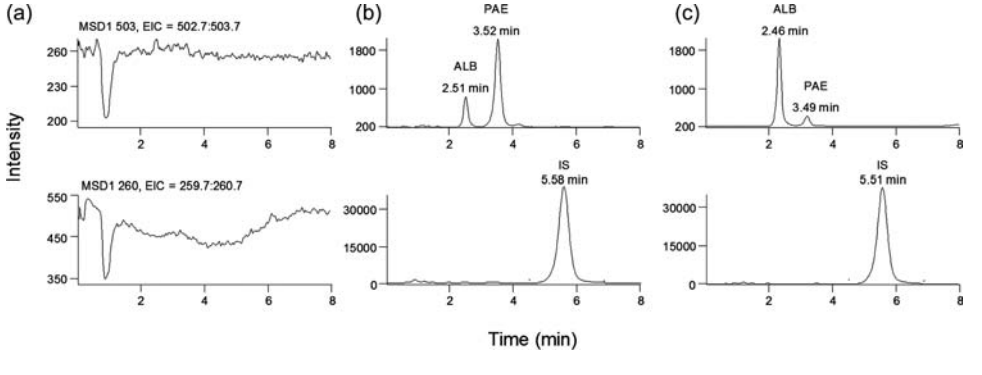

Figure 2. The representative liquid chromatography-tandem mass spectrometry chromatograms of ALB, PAE, and IS in rat plasma. (A) Blank plasma; (B) blank plasma sample spiked with ALB and PAE; (C) plasma sample after p.o. administration of RPA extraction.

of high-number samples in pharmacokinetic study.

Matrix-matched calibration curves were constructed for the analytes (3–3000 ng/ml for ALB and PAE) using weighted linear regressions of the area ratio of analyte to IS against the corresponding nominal concentrations of the analyte (ng/ml). Linear regression equations of ALB and PAE were $Y = 0.00834121X + 0.0048$ ($n = 3$, $r = 0.998$) and $Y = 0.01152389X + 0.0224$ ($n = 3$, $r = 0.993$), respectively, with a weighting factor of $1/X$. Y was the peak area ratio of analytes to IS, and X is equal to the analytes concentration in ng/ml. Lower limit of quantifications of ALB and PAE was 3 ng/ml.

The accuracies of *intra*-day and *inter*-day of analytes were within 110% of nominal value of three quality control (QC) levels and the precision of intra-day and inter-day of analytes calculated as coefficient of variation across all QC levels were lower than 14.7%. The results demonstrated that the accuracy and precision of this method were acceptable.

The recoveries of the ALB and PAE from rat plasma were 73.7–79.4 and 81.3–86.6% at all QC concentrations, respectively. For ALB and PAE, matrix effects were 85.4–88.1 and 90.3–92.8%, respectively, which indicated that the endogenous substances suppressed ionization slightly.

The plasma concentration of ALB and PAE and the logarithmic plasma concentration vs. time profile after the intravenous injection of ALB or PAE solution in rats were shown in Table 1 and Figure 3, respectively. The pharmacokinetic parameters of ALB and PAE after the intravenous injection in rats were presented in Table 2. After the intravenous injection of ALB and PAE solution, the plasma concentration vs. time profile showed an exponential trend (Figure 3). After the intravenous injection of ALB or PAE, the plasma concentration–time

Table 1. The plasma concentration of ALB and PAE at a different time after intravenous administration of ALB and PAE at a dose of 3 mg/kg (mean ± SD, $n = 5$).

	Plasma concentration (ng/ml)	
Time (h)	ALB	PAE
0.083	6001 ± 4123	13,605 ± 11,652
0.25	2509 ± 350	5367 ± 1881
0.5	718 ± 94.8	859 ± 221
1	177 ± 41.9	176 ± 55.8
2	34.0 ± 7.77	32.1 ± 6.28
4	17.0 ± 7.33	16.3 ± 3.59
8	7.68 ± 1.85	7.69 ± 1.89
12	5.26 ± 1.28	4.31[a]

Note: [a] $n = 1$.

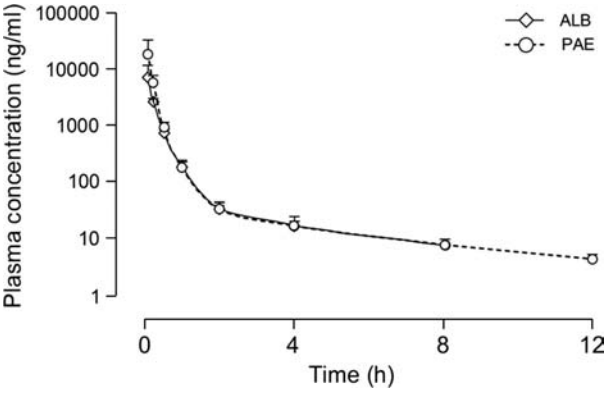

Figure 3. Semi-logarithmic plasma concentration–time profiles of ALB and PAE in rats after intravenous administration of pure compound alone with dose of 3 mg/kg/ml.

Table 2. The pharmacokinetic parameters of ALB and PAE in rat plasma after intravenous administration of ALB or PAE to rats calculated at the dose of 3 mg/kg (mean ± SD, $n = 5$).

PK parameters	ALB	PAE
$C_{5\,min}$ (ng/ml)	6001 ± 4123	13.605 ± 11,652
T_{max} (h)	0.083	0.083
$AUC_{0-\infty}$ (ng h/ml)	2085 ± 753	4309 ± 2146
K_{el} (1/h)	0.23 ± 0.05	0.23 ± 0.03
$AUMC_{tot}$ (ng h^2/ml)	1411 ± 362	1533 ± 193
$t_{1/2}$ (h)	3.21 ± 0.86	3.02 ± 0.34
MRT (h)	0.72 ± 0.23	0.41 ± 0.16
CL (l/h/kg)	1.57 ± 0.48	0.81 ± 0.32
V_{SS} (l/kg)	1.60 ± 2.62	0.37 ± 0.24

curve both produced a sharp decline in plasma concentration followed by a slower phase of decrease with a $t_{1/2}$ of 3.24 ± 0.86 and 3.02 ± 0.34 h for ALB and PAE, respectively, until the levels fell below the quantification limits, within 12 h after administration. The $AUC_{0-\infty}$ value after the intravenous injection of ALB solution and PAE solution at a dose of 3 mg/kg was 2085 ± 753 and 4309 ± 2146 ng h/ml, respectively.

The logarithmic plasma concentrations of ALB and PAE vs. time profile after oral administration of extract of DSS, extract of RPA, ALB, or PAE are shown in Figures 4 and 5, respectively, with corresponding data shown in Tables 3 and 4, respectively.

The pharmacokinetic parameters of ALB and PAE, calculated at the dose of 35 and 20 mg/kg monomers, are summarized in Tables 5 and 6, respectively. After oral administration of ALB or PAE alone, ALB and PAE were absorbed fast and reached C_{max} (812 ± 370 ng/ml) at 0.25 h and (659 ± 147 ng/ml) within 0.35 ± 0.14 h, respectively. The plasma concentration of ALB and PAE declined with $t_{1/2}$ of 3.30 ± 1.50 and 0.41 ± 0.09 h, respectively. After oral administration of the extracts of RPA and DSS to rats, ALB reached a maximum concentration of 4637 ± 2774 ng/ml (0.40 ± 0.14 h) and 226 ± 122 ng/ml (0.35 ± 0.14 h) and PAE reached a maximum concentration of 2132 ± 560 ng/ml (0.40 ± 0.14 h) and

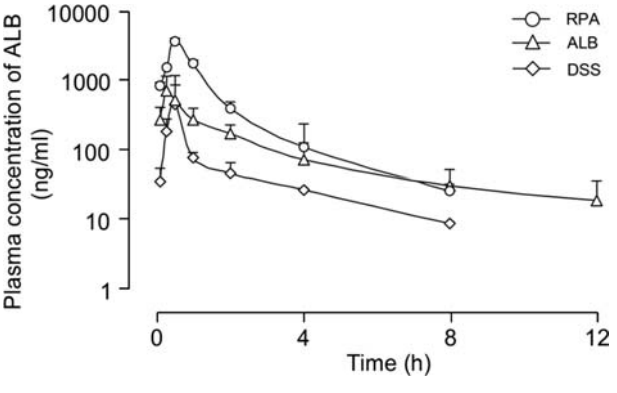

Figure 4. Semi-logarithmic plasma concentration–time profiles of ALB in rats after oral administration of ALB, RPA extract, and DSS prescription extract with doses of 35 mg/kg, 10 g/kg (33.5 mg/kg for ALB), and 10 g/kg (32.6 mg/kg for ALB), respectively, and the administration volume was 10 ml/kg for all treated groups.

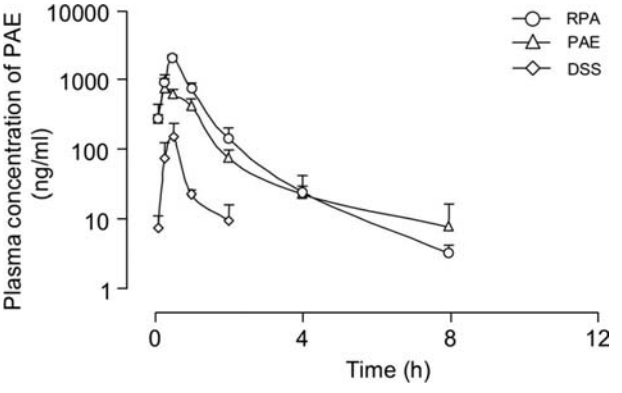

Figure 5. Logarithmic plasma concentration–time profiles of PAE in rats after oral administration of PAE, RPA extract, and DSS prescription extract with dose of 20 mg/kg, 10 g/kg (19.7 mg/kg for PAE), and 10 g/kg (13.0 mg/kg for PAE), respectively, and the administration volume was 10 ml/kg for all treated groups.

Table 3. The plasma concentration of ALB at a different time after p.o. administration of ALB, RPA extract, and DSS extract to rats (mean ± SD, $n = 5$).

	Plasma concentration (ng/ml)		
Time (h)	ALB (35 mg/kg)	RPA extract (10 g/kg)	DSS extract (10 g/kg)
0.083	271 ± 135	832 ± 103	34.3 ± 4.92
0.25	706 ± 455	2635 ± 782	183 ± 94.7
0.5	525 ± 353	3805 ± 573	457 ± 173
1	270 ± 128	1709 ± 272	76.9 ± 15.5
2	173 ± 53.0	388 ± 95.1	45.2 ± 20.2
4	72.0 ± 55.4	110 ± 13.8	26.2 ± 2.19
8	30.4 ± 20.3	25.5 ± 5.81	8.75 ± 0.51
12	18.6 ± 15.5	–	–

Table 4. The plasma concentration of PAE at a different time after p.o. administration of PAE, RPA extract, and DSS extract to rats (mean ± SD, $n = 5$).

	Plasma concentration (ng/ml)		
Time (h)	PAE (20 mg/kg)	RPA extract (10 g/kg)	DSS extract (10 g/kg)
0.083	271 ± 172	268 ± 45.5	7.37 ± 3.26
0.25	749 ± 299	894 ± 280	72.5 ± 50.5
0.5	600 ± 122	2046 ± 166	150 ± 75.7
1	416 ± 112	703 ± 180	22.1 ± 3.10
2	71.3 ± 22.0	138 ± 58.8	9.14 ± 6.20
4	37.3 ± 7.04	23.4 ± 6.19	–
8	11.4 ± 7.57	4.66 ± 0.98	–
12	–	–	–

143 ± 65 ng/ml (0.45 ± 0.11 h), respectively. Compared to the AUC_{0-t} value (1122 ± 351 and 722 ± 158 ng h/ml for ALB and PAE, respectively) after oral administration of monomers, larger AUC_{0-t} value of ALB (4755 ± 2560 ng

Table 5. The pharmacokinetic parameters of ALB in plasma after oral administration of ALB, extract of RPA, and extract of DSS prescription to rats calculated at a normalization dose of 35 mg/kg ALB (mean ± SD, $n = 5$).

PK parameters	ALB	RPA	DSS
C_{max} (ng/ml)	812 ± 370	4637 ± 2774*	226 ± 122$^{*\Delta}$
T_{max} (h)	0.25	0.40 ± 0.14	0.35 ± 0.14
AUC_{0-t} (ng h/ml)	1122 ± 351	4755 ± 2560*	411 ± 118$^{**\Delta}$
$AUC_{0-\infty}$ (ng h/ml)	1206 ± 305	4853 ± 2578*	591 ± 187$^{***\Delta}$
K_{el} (l/h)	0.24 ± 0.09	1.90 ± 0.77**	0.32 ± 0.27$^{\Delta\Delta}$
$t_{1/2}$ (h)	3.30 ± 1.50	1.46 ± 0.61*	3.87 ± 3.01
MRT (h)	4.19 ± 2.16	1.85 ± 0.81	5.44 ± 4.69
F (%)	4.96	19.5	1.69

Notes: $^{*}P < 0.05$, $^{***}P < 0.01$ vs. oral administration of ALB. $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ vs. oral administration of extract of RPA.

Table 6. The pharmacokinetic parameters of PAE in rat plasma after oral administration of PAE, extract of RPA, and extract of DSS prescription to rats calculated at a normalization dose of 20 mg/kg PAE (mean ± SD, $n = 5$).

PK parameters	PAE	RPA	DSS
C_{max} (ng/ml)	659 ± 147	2132 ± 560*	143 ± 65$^{**\Delta}$
T_{max} (h)	0.35 ± 0.14	0.40 ± 0.14	0.45 ± 0.11
AUC_{0-t} (ng h/ml)	722 ± 158	2259 ± 910*	242 ± 126$^{**\Delta}$
$AUC_{0-\infty}$ (ng·h/ml)	744 ± 154	2325 ± 911*	299 ± 118$^{**\Delta}$
K_{el} (1/h)	1.77 ± 0.35	2.51 ± 0.93	0.26 ± 0.14$^{***\Delta\Delta}$
$t_{1/2}$ (h)	0.41 ± 0.09	1.46 ± 0.61*	2.02 ± 1.51
MRT (h)	0.81 ± 0.11	1.85 ± 0.81*	2.61 ± 1.92
F (%)	1.44	4.49	0.481

Notes: $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$ vs. oral administration of ALB. $^{\Delta}P < 0.01$, $^{\Delta\Delta}P < 0.005$ vs. oral administration of extract of RPA.

h/ml, $P < 0.05$) and PAE (2259 ± 910 ng h/ml, $P < 0.05$) after oral administration of the extract of RPA and smaller AUC_{0-t} value of ALB (411 ± 118 ng h/ml, $P < 0.01$) and PAE (242 ± 126 ng h/ml, $P < 0.005$) after oral administration of the extract of DSS were obtained.

The results showed that there were also statistical differences in pharmacokinetics parameters of ALB including the C_{max}, AUC, $t_{1/2}$, and K_{el} ($P < 0.05$, $P < 0.01$) among the animals receiving ALB, the extract of RPA, and the extract of DSS. Similarly, there were statistically differences in pharmacokinetic parameters of PAE including the C_{max}, AUC, $t_{1/2}$, and K_{el} ($P < 0.05$, 0.01, 0.005, or 0.001) among the animals receiving PAE, the extract of RPA, and the animals receiving the extract of DSS.

In the animals that received the extract of RPA, the peak plasma concentrations of ALB and RPA were remarkably increased, about 5.71-fold and 20.5-fold of that in the rats administered ALB alone and extract of DSS and about 3.24-fold and 14.9-fold of that in the rats administered PAE alone and extract of DSS, respectively. Accordingly, the $AUC_{0-\infty}$ of ALB was increased to 4.02-fold and 8.21-fold and the $AUC_{0-\infty}$ of PAE were increased to 3.13-fold and 7.78-fold, respectively. The $t_{1/2}$ and mean residence time (MRT) of ALB after rats received the extract RPA were both remarkable shortened. However, the $t_{1/2}$ and MRT of PAE after rats received the extract of RPA and

DSS were both increased. Compared to the rats administered PAE, there are only remarkable difference in $t_{1/2}$ and MRT after rats received the extract of RPA. The bioavailability (F) value of ALB and PAE was 4.96 and 1.44% for rats received monomer alone, 19.5 and 4.49% for rats received extract of RPA, and 1.69 and 0.481% for rats received extract of DSS, respectively.

3. Discussion

It was reported that the bioavailability of PAE in rats after oral administration was very low [13]. Poor permeation, *p*-gp-mediated efflux, and hydrolysis via a glucosidase contributed to the poor bioavailability of PAE [14]. However, compared to the oral administration of monomers alone, the bioavailability (F) values of ALB and PAE was remarkably improved after oral administration of the extract of RPA, but decreased after oral administration of the extract of DSS. The changes in AUC might indicate that different extents of ALB and PAE were absorbed after oral administration of the extract of RPA and DSS. In addition, the relatively longer T_{max} found without remarkable statistical difference indicated possible delayed absorption processes of these two monomers after oral administration of the extract of RPA and DSS.

The influence of the drug–drug interaction on the pharmacokinetics of ALB and PAE in RPA and DSS should be considered [14]. It seemed that the manifest difference in chemical components of RPA and DSS contributed to the difference in the pharmacokinetic parameters of ALB and PAE. It was reported that 41 compounds including monoterpene glycosides, phenolic acids, phathalides, sesquiterpenoids, and triterpenes were identified or tentatively characterized from the DSS extract. Except for the common components, ALB and PAE, existing in both RPA and DSS extract, gallic acid, ferulic acid, benzoic acid, senkyunolide I, coniferyl ferulate, senkyunolide A, 3-butylphthalide, *Z*-ligustilide, *Z*-butylidenephthalide, atractylcnolide II, atractylcnolide I, and levistolide A were determined by HPLC-DAD from DSS [12,15].

There are several works about pharmacokinetics of PAE in different preparation [16,17]. In one of the reports, the parameters of T_{max} and $t_{1/2}$ of PAE were remarkably increased when orally administering PAE in the decoctions of RPA, but C_{max} and AUC were decreased, in comparison with that when orally administering monomer alone [16]. However, these results were not coincident with what we obtained. In that study, the concentration of PAE in final condensed decoction of RPA was 18.35 mg/ml, which was even more than 5-fold of that in our dried extract powder (3.35 mg/g) of RPA. In fact, different region source, processing method, parts, growth years, and germ plasms of RPA lead to different content of PAE [18–22]. That means the possible different components in the extracts contribute to the different results in pharmacokinetics parameters of PAE after the rats were administered extracts of RPA. All these possible factors mean that it is vital for TCM to perform good agriculture practice, good extraction practice, and good manufacture practice.

In summary, the parameters of C_{max}, AUC, and K_{el} of ALB and PAE were remarkably increased ($P < 0.05$, 0.01) during oral administration of the extract of RPA in comparison to that of the extract of DSS. The main explanation for these differences seems to be the different chemical components and the variances of compound content in both extracts. These differences, following with different interactions, affect many aspects of absorption of ALB and PAE including the metabolism of intestinal bacteria, intestinal absorption, and intestine and liver metabolism before ALB and PAE

could have onset after entering the body. It was important to investigate the reasons of these differences in pharmacokinetics of ALB and PAE between RPA and DSS. The obtained information can be used to not only evaluate impact of these differences on the efficacy and safety in clinical applications, but also to understand the compatibility mechanisms or rules of compound recipe of TCM. In the present study, we compared the pharmacokinetic properties of ALB and PAE, respectively, after oral administration of pure compounds, RPA extract, and DSS extract to rats on separate occasions. Besides ALB and PAE, more components should be included to be markers to control the quality of DSS prescription or to describe the clinical pharmacokinetic behavior of the prescription. We will investigate more compounds to achieve a global understanding of their pharmacokinetics properties in the complex prescription of DSS.

4. Experiment

4.1 Materials and reagents

The reference substance of PAE ($\geq$ 99%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China). The reference substance of ALB with purity higher than 98.5% determined by HPLC-ELSD was supplied by Beijing Institute of Radiation Medicine. The internal standard, propranolol (IS; Figure 1), was also obtained from NICPBP. Methanol (HPLC grade) was purchased from Sigma-Aldrich Co. (St Louis, MO, USA), and the other chemicals used were all analytical reagent grade.

RPA, Radix *Angelica sinensis*, Rhizoma *Chuanxiong, Poria cocos*, Rhizoma *Atractylodis macrocephalae*, and *Rhizoma Alismatis* were purchased from Pu-Sheng-Lin Pharmaceutical Limited Company (Beijing, China). All these plant materials were authenticated and the voucher specimens were deposited at 4°C before preparation. The herb materials, scrunched into powder before using, were mixed in the ratio of 16:3:8:4:4:8 and the total weight was 86 g. The mixture was decocted with 95% ethanol twice (1:5, w/v), 2 h for each time. After that, the mixture was decocted with distilled water with the same volume and time as the process treated with ethanol, all the solution obtained were put together and condensed by vacuum-drier at 50°C and then concentrated under reduced pressure at -20°C to give 24.65 g of residue. RPA was treated as above to get 20.85 g of powder with initial herb weight of 100 g. The content of ALB and PAE in DSS extract was 3.26 and 1.30 mg/g, respectively. The content of ALB and PAE in RPA extract was 3.35 and 1.97 mg/g, respectively.

4.2 Instruments and liquid chromatography-tandem mass spectrometry analytical method

The assay was performed on an Agilent LC/MSD quadrupole mass spectrometer equipped with a 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) consisting of a binary pump, an autosampler, and an automatic solvent degasser. An electrospray ionization (ESI) source was used for the analysis. Liquid chromatographic separations were performed on an Ultimate AQ-C18 column (5 μm, 5.0 mm × 2.1 mm, i.d.) from Welch Materials, Inc. (Ellicott City, MD, USA), and the $[M + Na]^+$ was detected at *m/z* 503.2 for both ALB and PAE using selected ion monitoring mode. The isocratic mobile phase, 25% of methanol aqueous solution containing 0.1% of formic acid, was run at a flow rate of 0.2 ml/min. The optimum ESI conditions included a nitrogen nebulizer pressure of 40 psi, a nitrogen drying gas temperature of 350°C at 11 L/min, spray voltage of 4500 V, a detector gain of 1600 V, and fragmentation voltage of 100 V. Mass spectra were obtained at a dwell time of 0.2 s.

4.3 Animals

Male Sprague–Dawley rats (200–220 g) were obtained from the Animal Center of Academy of Military Medical Sciences (Beijing, China). The rats were maintained in an air-conditioned animal quarter at room temperature of 22 ± 1°C and relative humidity 50–70% under 12–12 h light–dark cycles. The animals were acclimatized to the facilities for 5 days, and then fasted with free access to water for 12 h prior to the experiment.

4.4 Samples preparation

Thawed plasma samples (50 μl) were spiked with 10 μl of internal standard solution (300 ng/ml) in 1.5 ml eppendorf centrifuge tubes. The mixture was vortexed for 5 min and 1 ml of ethyl acetate was added. After another 5 min of vortex and centrifugation at 13,000 *g* for 10 min at 4°C, an 850 μl supernatant was transferred and evaporated to dryness under N_2 flow at 40°C. The residue was reconstituted in 50 μl of methanol–water (25:75, v/v) mixture with vortex mixing for 5 min, and the centrifugation procedure was then repeated. Finally, a 30-μl aliquot of supernatant was injected to HPLC for analysis.

4.5 Validation of liquid chromatography-tandem mass spectrometry analytical method

4.5.1 Linearity

Primary stock solution of PAE, ALB, and IS was prepared by dissolving accurately weighed PAE, ALB, and IS in methanol to yield a final concentration of 1 mg/ml, 1 mg/ml, and 100 μg/ml, respectively. Working solutions were prepared daily by appropriate dilution in distilled water. Plasma calibration standards (3, 10, 30, 100, 300, 1000, and 3000 ng/ml) were prepared by spiking 5 μl of corresponding working solution mixture to 45 μl of blank plasma. The plasma was treated as described in Section 4.4.

4.5.2 Accuracy and precision

The accuracy and precision were evaluated by QC samples at low (5 ng/ml), medium (200 ng/ml), and high (2500 ng/ml) concentrations which were prepared as same as calibration standards. The *intra*-day accuracy and precision of the assay were assessed by analyzing QC samples at three concentrations in three replicates and the *inter*-day was determined by analyzing QC samples in 3 days.

4.5.3 Recovery and matrix effect

The recovery of PAE and ALB was determined by comparison of the peak areas between samples spiked with analytes in blank plasma samples (5, 200, and 2500 ng/ml) and in post-extraction at the corresponding concentrations. The recovery of IS was determined in the same way. Five samples at each concentration level were evaluated.

Matrix effect was calculated by comparing analytes peak areas obtained from supplemented plasma samples after liquid–liquid extraction with those obtained from pure mobile phase preparations ($n = 5$) at three QC levels.

4.6 In vivo *pharmacokinetic study*

Sprague–Dawley rats were divided randomly into six groups (five rats in each group). Two groups of rats were intravenously administered ALB and PAE, respectively, with dose of 3 mg/kg/ml. Other four groups of rats were orally administered of DSS extract, RPA extract, ALB, and PAE with doses of 10 g/kg, 10 g/kg, 35 mg/kg, and 20 mg/kg, respectively, and the administered volume was 10 ml/kg of body weight. Regarding the sensitivity of the analytical method based on preliminary study and avoiding possible dose-dependent nonlinear pharmacokinetics, 10 g/kg of RPA extract and DSS extract were taken as dosage in which the content was 33.5 and 32.6 mg/kg for ALB

and 19.7 and 13.0 mg/kg for PAE in RPA extract and DSS extract, respectively. According to the extract dosage, the oral dosage of monomers was 35 and 20 mg/kg for ALB and PAE, respectively. The volume of oral administration was 10 ml/kg to reduce the effect of concentration on absorption.

The 120-μl blood samples were collected in heparinized eppendorf tube via the orbital sinus under light ether anesthesia before dosing and subsequently at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 12 h following oral administration. After centrifuging at 5000*g* for 10 min, the plasma samples were obtained and frozen at −30°C until analysis.

Plasma concentration–time data were analyzed for the calculation of pharmacokinetic parameters by noncompartmental analysis with the Kinetica™ 2000 software package (version 3.0, InnaPhase Corp. Philadelphia, PA, USA). The peak concentration (C_{max}) and the time of peak concentration (T_{max}) after p.o. dosing were obtained directly from the data without interpolation. The area under plasma concentration–time curve (AUC_{0-t}) up to the last measured time point was calculated by the trapezoidal rule. The value of $AUC_{0-\infty}$ was generated by extrapolating AUC_{0-t} to infinite using K_{el} and the last measurable concentration C_t, where K_{el} is the elimination rate constant. The terminal elimination half-life ($t_{1/2}$) was calculated using the relationship $0.693/K_{el}$. The MRT was determined by $AUMC_{0-\infty}/AUC_{0-\infty}$. All results were expressed as arithmetic mean ± standard deviation (SD). An unpaired Student's *t*-test was used for comparison. Significance was accepted as $p < 0.05$.

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