Fitoterapia 82 (2011) 883–888

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Comparative pharmacokinetic and bioavailability studies of three salvianolic acids after the administration of *Salviae miltiorrhizae* alone or with synthetical borneol in rats

Xiao-Juan Lai [a], Liang Zhang [c], Jun-Song Li [a,b,*], Hang-Qing Liu [a], Xun-Hong Liu [a], Liu-Qing Di [a], Bao-Chang Cai [a,b,*], Li-Hua Chen [d]

[a] College of Pharmacy, Nanjing University of Chinese Medicine, 138 Qixia Road, Nanjing 210046, China
[b] Jiangsu Key Laboratory of Chinese Medicine Processing, Nanjing University of Chinese Medicine, 282 Hanzhong Road, Nanjing 210029, China
[c] Key Laboratory of Tea Biochemistry & Biotechnology, Ministry of Education and Ministry of Agriculture, Anhui Agriculture University, Hefei 230036, China
[d] College of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Jiangxi 330004, China

ARTICLE INFO

Article history:
Received 10 March 2011
Accepted in revised form 24 April 2011
Available online 4 May 2011

Keywords:
Salvianolic acids
Synthetical borneol
Pharmacokinetic
Bioavailability

ABSTRACT

Salviae miltiorrhizae is one of the most commonly used herbal plants in the treatment of numerous ailments including cardiovascular diseases for hundreds of years. According to the theory of traditional Chinese herbal medicine, *S. miltiorrhizae* is always used in combination with borneol to obtain better pharmacological effects. The purpose of this study was to investigate the effects of borneol on the pharmacokinetic and bioavailability of *S. miltiorrhizae*. The pharmacokinetics studying on rosmarinic acid, salvianolic acid A and salvianolic acid B which are the main active compounds of *S. miltiorrhizae* in rat plasma, was achieved using a optimal high-performance liquid chromatographic technique coupled with liquid–liquid extraction method. After administration of either single salvianolic acids or salvianolic acids in combination with borneol, plasma concentrations of rosmarinic acid, salvianolic acid A and salvianolic acid B of male Sprague–Dawley rats were determined at different time points (5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min). In comparison with salvianolic acid extract alone, there were statistically significant differences in pharmacokinetic parameters of rosmarinic acid, salvianolic acid B and salvianolic acid A, and the bioavailability of the three salvianolic acids increased by different degrees when the salvianolic acid extract and borneol were administered together. These results indicated that borneol could enhance the intestinal absorption, decrease the distribution and inhibit the metabolism of salvianolic acids.

1. Introduction

Salviae miltiorrhizae, the root of *S. miltiorrhiza Bge*, is a well-known Chinese herbal medicine. In these years, *S. miltiorrhizae* is also widely accepted as a health product for its biological activities in the western, especially in the treatment of cardiovascular disorders. As the main type of compounds of *S. miltiorrhizae*, salvianolic acids (SAs) are water-soluble components which are mainly composed of salvianolic acid B (SAB), rosmarinic acid (RA) and salvianolic acid A (SAA) (Fig. 1). These salvianolic acids have been reported to decrease the infarct area and inhibit cerebral edema of ischemic rat by improving RCBF in the ischemic hemisphere, inhibiting platelet aggregation and scavenging oxygen free radicals in rats [1,2].

Borneol is a monoterpenoid component of the medicine plant, traditionally used in the treatment of unconsciousness, usually as "Guide" drug regulating and mediating other drugs in the prescription. The natural borneol (only containing D-borneol)

* Corresponding authors at: Pharmaceutical College, Nanjing University of Chinese Medicine, PO Box. 8, No. 282, Hanzhong Road, Nanjing 210029, China. Fax: +86 25 86798281.
E-mail addresses: lijunsong1964@163.com (J.-S. Li), bccai@126.com (B.-C. Cai).

0367-326X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.fitote.2011.04.015

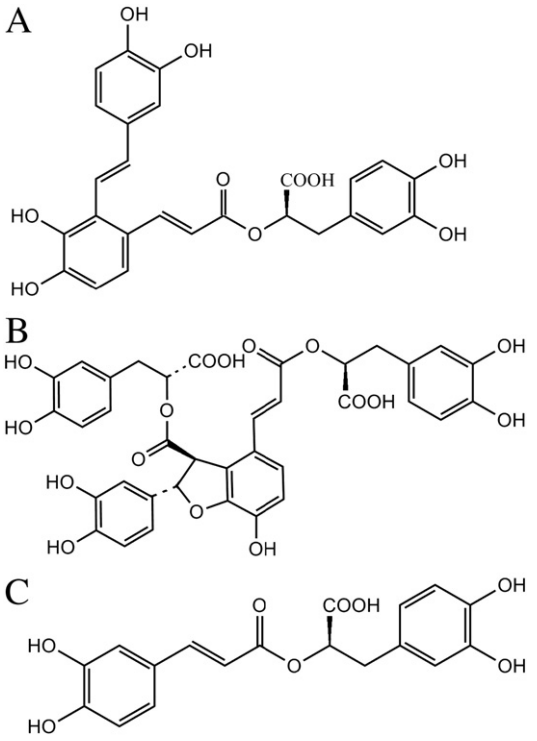

Fig. 1. Chemical structures of (A) salvianolic acid A, (B) salvianolic acid B and (C) rosmarinic acid.

has been prescribed as natural medicine for thousands of years in China. While recently synthetic borneol (including D-borneol and isoborneol) is predominately used in clinical application to replace natural borneol due to its cheaper price and abundant resource. Borneol could obviously loosen the intercellular tight junction (ICTJ), inhibit the function of P-glycoprotein (P-gp) on cell membrane, increase the fluidity of membrane and influence the permeability of bilayer lipid membrane in vitro [3]. In Dai's studies, borneol could influence oral submucous fibrosis as penetration enhancer [4].

Currently, there were a number of pharmacokinetic reports on SAB in pure drug, extract and prescription [5–15], while there was no report on simultaneous determination of RA, SAB and SAA after administration of salvianolic acid extract. In order to represent the pharmacokinetic of the whole herb extract, it is better to select several effective ingredients as the target to investigate the pharmacokinetic. Therefore, the main active compounds of *S. miltiorrhizae,* RA, SAB and SAA were selected as the object drugs to study the pharmacokinetic of the whole salvianolic acid extract. Considering the clinic application of *S. miltiorrhizae*, it was always prescribed in combination with borneol to obtain synergistic effects and diminish the possible adverse reactions. These effects have been confirmed by clinic reports, but the mechanism of synergistic effect has not been explored in depth. ADME (absorption, distribution, metabolism, excretion) is the critical pathway to study the drug–drug interaction of pharmacokinetics in vivo. In our previous studies, we found that borneol could enhance the oral absorption of salvianolic acids in situ intestinal perfusion model in rat [16]. However, there is little investigation about the influence of borneol on salvianolic acids in pharmacokinetic and bioavailability in vivo. In the present study, we investigated and compared the pharmacokinetic and bioavailability of RA, SAB and SAA alone or with borneol. It is useful to explain and predict a variety of events related to the interaction between *S. miltiorrhizae* and borneol.

This current study is to investigate how the interaction of synthetic borneol affects salvianolic acids on the rat pharmacokinetic and bioavailability in vivo. A sensitive and accurate method was established and evaluated for the determination and pharmacokinetic study of three salvianolic acids including RA, SAB and SAA in rat plasma after intravenous and oral administrations of salvianolic acid extract or compound of salvianolic acids with the addition of synthetic borneol.

2. Materials and methods

2.1. Chemicals and reagents

SA extract was purchased from Hongsheng Biology Technology Co. Ltd (Xian, China). The contents of RA, SAB and SAA were determined to be 3.92%, 50.60% and 5.71% (w/w) by HPLC, respectively. RA, SAB and SAA standards (>98%) were purchased from Ronghe Pharmaceutical Technology Development Co., Ltd. (Shanghai, China). Naringin (the internal standard IS) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Synthetical borneol was obtained from Huangpu Chemical Industry Co., Ltd. (Guangzhou, China). Methanol (HPLC grade) was obtained from TEDIA company, Inc (Fairfield, USA) and the other chemical reagents were of analytical grade or better.

2.2. Animals

Male Sprague–Dawley rats (290–330 g) were purchased from the Slaccas Experiment Animal Co., Ltd. (Shanghai, China). Animal welfare and experimental procedures were strictly in accordance with the Guide for the Care and Use of Laboratory Animals (US National Research Council, 1996) and the related ethics regulations of Nanjing University of Chinese Medicine. These rats were fasted for at least 12 h prior to initiating the experiments and had free access to water.

2.3. In vivo pharmacokinetic study in rats

The male Sprague–Dawley rats were randomly divided into 3 groups with six rats in each group to receive various administrations. In Group 1, rats were administered with 80 mg/kg SA extract intravenously from the tail vein. In Group 2, rats were administered with 800 mg/kg SA extract orally by gastrogavage. In Group 3, rats were administered with 800 mg/kg SA extract with the addition of 0.06% borneol orally by gastrogavage. SA extract was dissolved in normal saline solution, while borneol was first dissolved in little methanol next added into normal saline solution, too. The blood samples (0.5 mL) were obtained via the oculi chorioideae vein at 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min after administration, and then transferred into a heparinized microcentrifuge tube according to a programmed schedule. The plasma was isolated immediately by centrifugation at 4000 rpm for 10 min and subsequently stored at −20 °C until analysis.

2.4. Sample preparations and analysis

An aliquot of 200 μL of plasma sample was placed into a centrifuge tube and added with 20 μL of internal standard (50 μg/mL of naringin) and 30 μL of 5 M hydrochloride acid. The mixture was spiked with 3 mL of ethyl acetate by vortexing for 3 min and centrifugation at 3000 rpm for 5 min, and then the supernatant was transferred to a clean tube. The extraction was evaporated to dryness under a nitrogen gas stream in a 35 °C water bath. The residue was reconstituted in 100 μL of 30% methanol in 3% formic acid solution. A 20 μL of supernatant solution was injected into the HPLC system.

The analysis were performed using an Agilent 1100 HPLC system (Agilent, Germany), equipped with an on-line degasser, a quaternary solvent delivery system, an auto-sampler, a column temperature controller and a DAD detector. Chromatographic separation was carried out on a Kromasil 100-5C18 (250 mm × 4.6 mm, 5 μm, Akzo Nobel, Sweden) column. Gradient elution of the analyte consisted of methanol (A) and 0.2% (v/v) formic acid aqueous solution (B). The initial condition was A:B (35:65, v/v), linearly changed to A:B (46:54, v/v) at 21 min, then continuously changed to A:B (49:51, v/v) at 28 min, finally back to A:B (35:65, v/v) at 30 min. The column temperature was maintained at 30 °C. The flow rate was 1 mL/min and the detector operated at 286 nm. The injection volume for HPLC was 20 μL.

2.5. Method validation

2.5.1. Specificity

The specificity was evaluated by comparing chromatograms of blank plasma, blank plasma spiked with RA, SAB, SAA and IS, and plasma samples obtained from rats administrated orally or intravenously by salvianolic acid extract.

2.5.2. Linearity and sensitivity

Standard stock solutions of RA (178 μg/mL), SAB (227 μg/mL), SAA (257 μg/mL) and naringin (I S, 50 μg/mL) were prepared in methanol. For the calibration curve, six concentrations of analyte solution were prepared by dilution of the stock solution. All calibration samples were prepared by adding 50 μL working solutions and 20 μL internal standard solutions into 200 μL blank rat plasma, and then pretreated as described in Section 2.4. The LLOQ is defined as the lowest concentration on the calibration curve with a precision of 20% and accuracy of 80–120%.

2.5.3. Accuracy and precision

Accuracy was evaluated through the analytical recovery, and precision was determined through the relative standard deviation (RSD). The accuracy and the precision of the assay for intra-day and inter-day determinations were evaluated by the analysis of three concentration levels of quality control samples (n = 6) on the same day and on three consecutive validation days.

2.5.4. Extraction recovery

The extraction recoveries of analytes were determined by comparing the mean peak areas of the analytes in the pretreated quality control samples with those obtained from the pretreated blank plasma samples post-spiked with corresponding working solutions (n = 6). Three different concentration levels of analytes and IS were evaluated by analyzing six samples at each level.

2.5.5. Stability

The stability of analyte in the plasma was assessed using three concentrations (high, medium and low) of spiked samples under two conditions (room temperature, 8 h; −20 °C, 7 days).

2.6. Data and statistical analysis

Plasma concentration versus time profile was analyze by non-compartmental methods using WinNonlin software (Pharsight Corporation, Mountain View, CA, USA, Version 5.2).The pharmacokinetic parameters, including peak plasma concentration (C_{max}), time to reach C_{max} (T_{max}), the area under concentration–time curve ($AUC_{0-\infty}$), half-life of elimination ($t_{1/2,\lambda z}$), mean residence time ($MRT_{0-\infty}$) and absolute bioavailability (F) were obtained from analysis of experimental data. A p value less than 0.05 was considered to be significantly different using paired student's t-test. All data were presented as means ± SD.

3. Results

3.1. Method validation

As presented in Fig. 2, no significant endogenous peak interfering with RA, SAB, SAA and IS was obtained in blank plasma. The chromatograms were free of interfering peaks at

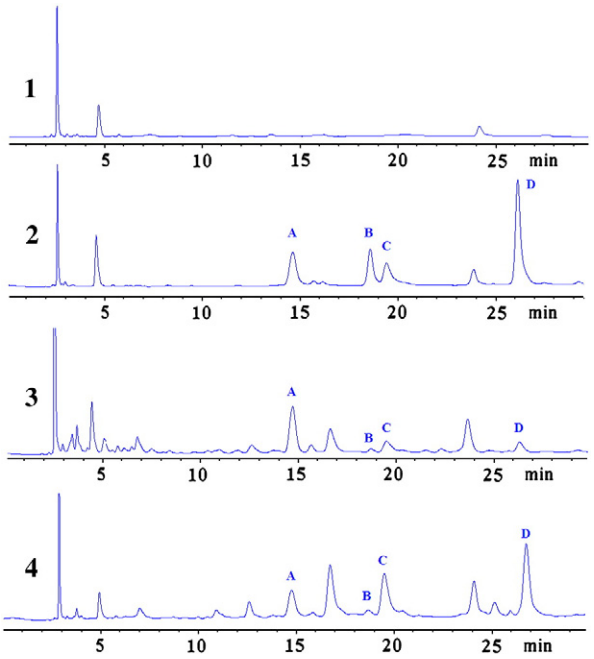

Fig. 2. Typical HPLC of salvianolic acids and naringin in rat plasma. (1) blank plasma; (2) blank plasma spiked with salvianolic acid standards (11.1 μg/mL RA, 14.2 μg/mL SAB, 16.1 μg/mL SAA) and naringin(10 μg/mL); (3) rat plasma sample collected at 1 h after oral administration of 800 mg/kg SAs; and (4) rat plasma sample collected at 20 min after intravenous administration of 80 mg/kg SAs. Chromatographic peaks: (A) naringin (internal standard), (B) rosmarinic acid, (C) salvianolic acid B and (D) salvianolic acid A.

the retention times of IS (14.68 min), RA (18.62 min), SAB (19.47 min) and SAA (26.15 min). Each calibration curvewas constructed with six different concentrations by plotting the peak areas ratios of SAs/IS against the standards concentration of SAs. The regression equations, concentration range, correlation coefficient, LOD and LOQ were listed in Table 1. The precision and accuracy for the determination of three constituents in plasma were estimated by analyzing quality control samples with low, middle and high concentrations. The intra-day precision (RSD) ranged from 2.6 to 11.9% and the inter-day precision (RSD) ranged from 3.3 to 13.8%. Analytical accuracy varied from 90.98% to 113.2%. The extraction recovery was calculated by the peak area ratios of SAs in plasma samples and the same concentration of SA standards. The mean extraction recovery of RA, SAB and SAA was 75.7%, 60.3%, 56.4%, respectively, and with RSD of less than 9.5%. The recovery of IS was 61.9% with RSD of 3.4%. The concentration of RA, SAB and SAA in plasma under two conditions deviated to less than 10% from those in freshly spiked plasma, which demonstrated a good stability of RA, SAB and SAA in the overall steps of the determination.

3.2. Pharmacokinetic study

The logarithmic plasma concentration versus time profile of RA, SAB and SAA after the intravenous and oral administrations of SA extract and compound extract with borneol in rats is shown in Fig. 3 and the pharmacokinetic data are shown in the Table 2. Compared to administration of SA extract alone, the pharmacokinetic parameters (T_{max}, C_{max}, $AUC_{0-\infty}$, $t_{1/2,\lambda Z}$ and $MRT_{0-\infty}$) were remarkably enhanced following oral administration of SA extract combined with borneol. There were significant differences ($P<0.05$) between the important pharmacokinetic parameters of the two groups.

4. Discussion

The developed and validated method was applied to the pharmacokinetic evaluation of RA, SAB and SAA in rats following intravenous and oral administrations of SAs or SAs with borneol extract. As shown in Fig. 3, compared with SAB and SAA, RA demonstrated more rapid distribution and was eliminated more rapidly from the systemic circulation with a $t_{1/2,\lambda Z}$ of (56.45 ± 0.67) min after intravenous administration. After being administered orally, RA was absorbed and eliminated more rapidly, with a T_{max1} of 10 min, a T_{max2} of 45 min and a $t_{1/2,\lambda Z}$ of (63.68 ± 13.11) min. The low content of RA in SA extract and the pharmacokinetic properties of RA leaded to only detect RA up to 180 min after oral administration by the established method. SAB and SAA were detected up to 360 min and 240 min, respectively.

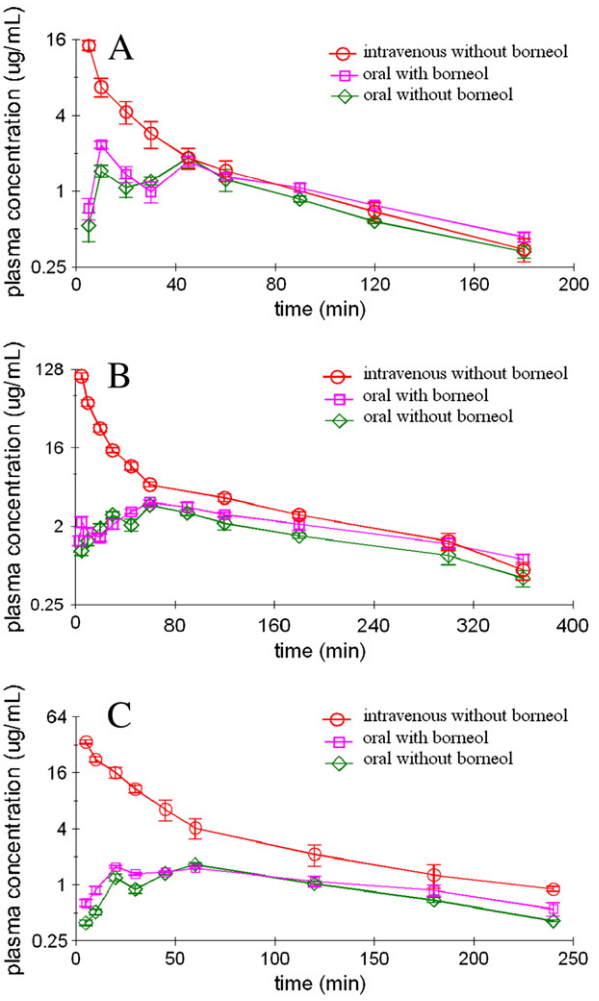

Fig. 3. Mean plasma concentration versus time profiles of RA (A), SAB (B) and SAA (C) in rats after intravenous administration of SAs at 80 mg/kg, oral administration of SAs at 800 mg/kg, and oral administration of SAs with borneol (0.03%) at 800 mg/kg. Each point represents mean ± SD (n = 6).

Bimodal phenomenon appeared in rats after oral administration of SA extract. As shown in RA, the time to reach C_{max1} (1.45 ± 0.16) μg/mL and C_{max2} (1.86 ± 0.33) μg/mL was 10 min (T_{max1}) and 45 min (T_{max2}). As shown in SAB, the time to reach C_{max1} (2.69 ± 0.23) μg/mL and C_{max2} (3.38 ± 0.07) μg/mL was 30 min (T_{max1}) and 60 min (T_{max2}). As shown in SAA, the time to reach C_{max1} (1.19 ± 0.10) μg/mL and C_{max2} (1.66 ± 0.07) μg/mL was 20 min (T_{max1}) and 60 min (T_{max2}), respectively. It was reported that double peak phenomenon in plasma concentration–time curve of SAB was found at 1.0 h (430 ± 300) ng/mL and 4.0 h (330 ± 190) ng/mL after oral administration of SA extract in beagle dogs [9]. This phenomenon was identical to what we obtained. The reasons for this phenomenon were mainly summed up to two points. On one hand, SAs could undergo enterohepatic circulation. A few papers reported that SAB and its methylated metabolites were mostly and rapidly excreted into rat bile after both oral and intravenous administrations, and

Table 1
Regression data and limits of detection and quantification for RA, SAB and SAA.

Analytes	Calibration graph	Linear range (μg/mL)	R^2	LLOQ (μg/mL)	LLOD (μg/mL)
RA	Y = 0.0810X − 0.0148	0.18–44.50	0.9994	0.18	0.05
SAB	Y = 0.0714X − 0.0132	0.23–56.75	0.9988	0.23	0.06
SAA	Y = 0.1709X − 0.0509	0.25–64.25	0.9996	0.25	0.03

Table 2
Pharmacokinetic parameters for RA, SAB and SAA in rats after intravenous administration of SAs at 80 mg/kg, oral administration of SAs at 800 mg/kg, and oral administration of SAs with borneol (0.03%) at 800 mg/kg (mean ± SD, n = 6).

Parameters	RA			SAB			SAA		
	Intravenous	Oral	Oral + borneol	Intravenous	Oral	Oral + borneol	Intravenous	Oral	Oral + borneol
$t_{1/2,\lambda Z}$ (min)	56.45 ± 0.67	63.68 ± 1.11	70.02 ± 9.33	92.82 ± 13.60	113.59 ± 12.37	145.95 ± 13.07*	85.17 ± 8.17	92.15 ± 9.79	114.62 ± 12.89
$AUC_{0-\infty}$ (μg·ml^{-1}.min)	425.57 ± 61.36	185.15 ± 20.61	225.25 ± 10.75*	2956.26 ± 123.04	674.74 ± 90.97	901.93 ± 92.19*	1307.47 ± 137.60	277.15 ± 7.19	348.87 ± 42.15*
$MRT_{0-\infty}$ (min)	51.43 ± 2.36	104.19 ± 2.52	123.22 ± 9.76*	66.61 ± 10.55	187.50 ± 14.48	224.70 ± 10.32*	75.21 ± 3.54	157.47 ± 7.67	179.79 ± 18.69
T_{max1} (min)	NA	10.00 ± 0.00	10.00 ± 0.00*	NA	30.00 ± 0.00	5.00 ± 0.00	NA	20.00 ± 0.00	20.00 ± 0.00*
C_{max1} (μg·ml^{-1})	NA	1.45 ± 0.16	2.38 ± 0.15*	NA	2.69 ± 0.23	2.23 ± 0.32	NA	1.19 ± 0.10	1.55 ± 0.05*
T_{max2} (min)	NA	45.00 ± 0.00	45.00 ± 0.00	NA	60.00 ± 0.00	60.00 ± 0.00	NA	60.00 ± 0.00	60.00 ± 0.00
C_{max2} (μg·ml^{-1})	NA	1.86 ± 0.33	1.74 ± 0.16	NA	3.38 ± 0.07	3.77 ± 0.19*	NA	1.66 ± 0.07	1.51 ± 0.04

NA: not applicable.
* $p<0.05$.

implied that SAB underwent hepatobiliary excretion [4,10]. On the other hand, SAs could be absorbed in multi-sites at different rates. According to the previous report on the in situ intestinal perfusion model in rat, the differences among the absorptions of SAs in different intestinal sites were considered to be significant [16].

As shown in Table 2, in comparison with SA extract alone, increased intestinal absorption of RA and SAA was observed after oral administration of SA extract with the addition of borneol. The C_{max1} was significantly increased from (1.45 ± 0.16) μg/mL to (2.38 ± 0.15) μg/mL, from (1.19 ± 0.10) μg/mL to (1.55 ± 0.05) μg/mL, respectively ($P<0.05$). A shorter time to reach the C_{max1} of SAB was from 30 min to 5 min. The results above indicated that borneol could promote the speed and intensity of intestinal absorption after the administration of SA extract with the addition of borneol. In addition, a longer $t_{1/2,\lambda Z}$ and $MRT_{0-\infty}$ of RA, SAB and SAA ($P<0.05$) imply a slower distribution and elimination when SA extract is co-administered with borneol, which resulted from the inhibition of borneol against metabolism of CYP3A and exocytosis of P-gp [17].

As shown in Fig. 3, the $AUC_{0-\infty}$ of the parent components (RA, SAB and SAA) obviously increased when SA extract was combined with borneol. In the earlier study, it was known that RA, SAB and SAA agreed with linear kinetics. So the absolute bioavailability (F) values of RA, SAB and SAA, derived from the AUC of rats that received SA extract orally alone in comparison to the $AUC_{0-\infty}$ of SA extract intravenously, were calculated to be 5.29%, 3.05% and 2.50%, respectively. Until now, no absolute bioavailability of RA and SAA has been reported after the administration of RA and SAA either in pure or in SA extract. However, the absolute bioavailability of SAB had been calculated to be 3.90% or 5.0% in rat with oral administration of SAB in pure form [5,6], 1.07% in beagle dog with oral administration of SA extract [9], and 5.6% in rabbit with oral administration of SAB tablets [18]. Data consistently suggested an extremely low bioavailability of SAs, which resulted from poor absorption of SAs from the intestine and the first pass elimination of SAs in the liver and intestine [6,10,12]. However, compared with the oral administration of SA extract alone, the absolute bioavailability (F) values of RA, SAB and SAA were remarkably improved by 21.61%, 33.77% and 17.90%, respectively, after oral administration of SA extract with the addition of borneol, mainly for the reasons that borneol could enhance the intestinal absorption, decrease the distribution and inhibit the metabolism of SAs.

In summary, there were statistically significant differences ($P<0.05$) in pharmacokinetic parameters of RA, SAB and SAA including the C_{max}, $AUC_{0-\infty}$, $t_{1/2,\lambda Z}$ and $MRT_{0-\infty}$ among the rats orally administered with SA extract alone and with SA extract with the addition of borneol. The pharmacokinetic results obtained indicate that combining the medicine can lead to differences in the pharmacokinetics. This might help as a guide when using compound prescriptions of traditional Chinese medicines.

Acknowledgments

This research was supported by the Technological Project of Chinese Medicine Administration of Jiangsu Province

(NO.HL07077) and the Scientific and Technological Innovation Team Program of "Qing-Lan Project" of Jiangsu Province.

References

[1] Tang MK, Ren DC, Zhang JT, Du GH. Effect of salvianolic acids from *Radix Salviae miltiorrhizae* on regional cerebral blood flow and platelet aggregation in rats. Phytomedicine 2002;9:405.
[2] Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutat Res 2005;579:200.
[3] Li L, Yuan Y, Jiang XH. Absorption mechanism of liposoluble components of *Salvia miltiorrhiza* with Caco-2 cell model. Chin Pharm J 2006;41:108.
[4] Dai JP, Chen J, Bei YF. Influence of borneol on primary mice oral fibroblasts: a penetration enhancer may be used in oral submucous fibrosis. J Oral Pathol Med 2009;38:276.
[5] Wu YT, Chen YF, Hsieh YJ, Jaw I, Shiao MS, Tsai TH. Bioavailability of salvianolic acid B in conscious and freely moving rats. Int J Pharm 2006;326:25.
[6] Chen YF, Jaw I, Shiao MS, Tsai TH. Determination and pharmacokinetic analysis of salvianolic acid B in rat blood and bile by microdialysis and liquid chromatography. J Chromatogr A 2005;1088:140.
[7] Zhou L, Chow MS, Zuo Z. Effect of sodium caprate on the oral absorptions of danshensu and salvianolic acid B. Int J Pharm 2009;379:109.
[8] Kim HH, Kim J, Ji HY, Kim YC, Sohn DH, Lee BM, et al. Pharmacokinetics of lithospermic acid B isolated from *Salvia miltiorrhiza* in rats. J Toxicol Environ Health A 2005;68:2239.
[9] Han DE, Gao ZD, Zhao D, Wang L, Li N, Li TT, et al. Liquid chromatography mass spectrometry for the determination of salvianolic acid B, a natural compound from the herb Danshen in rat plasma and application to pharmacokinetic study. Biomed Chromatogr 2009;23:1073.
[10] Ma L, Ren WC, Dong J, He H, Chen XJ, Wang GJ. Pharmacokinetic of salvianolic acid B after intravenous administration in rats. Chin J Clin Pharmacol Ther 2007;12:1231.
[11] Gao DY, Han LM, Zhang LH, Fang XL, Wang JX. Bioavailability of salvianolic acid B and effect on blood viscosities after oral administration of salvianolic acids in beagle dogs. Arch Pharmacol Res 2009;32:773–9.
[12] Zhang Y, Akao T, Nakamura N, Hattori M, Yang XW, Duan CL, et al. Magnesium lithospermate B is excreted rapidly into rat bile mostly as methylated metabolites, which are potent antioxidants. Drug Metab Dispos 2004;32:752–7.
[13] Zhang J, Yu HL, Sheng YX, Li L, Ye M, Guo D. HPLC determination and pharmacokinetic studies of salvianolic acid B in rat plasma after oral administration of *Radix Salviae miltiorrhizae* extract. Biomed Chromatogr 2005;19:15.
[14] Zhang Y, Akao T, Nakamura N, Duan CL, Hattori M, Yang XW, et al. Extremely low bioavailability of magnesium lithospermate B, an active components from *Salvia miltiorrhiza* in rat. Planta Med 2004;70:138.
[15] Yang XN, Wang YJ, Liu YS, Tang X. Pharmacokinetics of salvianolic acids after intravenous injection, with and without *Panax quinquefolium* protopanaxadiol saponins, inrats. J Ethnopharmacol 2008;117:408.
[16] Lai XJ, Liu HQ, Li JS, Di LQ, Cai BC. Intestinal absorption properties of three components in salvianolic acids extract and the effect of borneol on absorption in rats. Acta Pharm Sin 2010;45:1576.
[17] Xiao YY, Chen ZP, Ping QN, Chen HX. The enhancing effect of borneol on the absorption of tetramethylpyrazine. Acta Pharm Sin 2009;44:915.
[18] Ma Y, Wang T. A solid-phase extraction method for high-performance liquid chromatographic determination of salvianolic acid B in rabbit plasma: application to pharmacokinetic study. Biomed Chromatogr 2007;21:217.