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Bioavailability of salvianolic acid B in conscious and freely moving rats

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Abstract

Salvianolic acid B is an herbal ingredient isolated from *Salvia miltiorrhiza*. The aim of this study was to apply an automated blood sampling system coupled to a simple liquid chromatographic system to determine the bioavailability of salvianolic acid B in stress-free rats. The plasma sample (25 μl) was vortex-mixed with 50 μl of internal standard solution (chloramphenicol 10 μg/ml in acetonitrile) to achieve protein precipitation. Salvianolic acid B in the rat plasma was separated using a reversed-phase C18 column (250 mm × 4.6 mm, 5 μm) with a mobile phase of acetonitrile–methanol–20 mM NaH_2PO_4 (adjusted to pH 3.5 with H_3PO_4) (20:10:70 v/v/v) containing 0.1 mM 1-octanesulfonic acid, and the flow-rate of 1 ml/min. The UV detection wavelength was 286 nm. The concentration–response relationship from the present method indicated linearity over a concentration range of 0.5–200 μg/ml. Intra- and inter-assay precision and accuracy of salvianolic acid B fell well within the predefined limits of acceptability (<15%). The plasma sample of salvianolic acid B was further identified by LC–MS/MS in the negative ion mode using mass transition *m/z* 358.2 to the product ion *m/z* 196.9. After salvianolic acid B (100 mg/kg, i.v.; 500 mg/kg, p.o.) was given in conscious and freely moving rats, the AUC were 5030 ± 565 and 582 ± 222 min μg/ml for intravenous (100 mg/kg) and oral (500 mg/kg) doses, respectively. The oral bioavailability of salvianolic acid B in freely moving rats was calculated to be 2.3%.

Keywords: Automated blood sampling; Bioavailability; Pharmacokinetics; Salvianolic acid B; Tandem mass spectrometry

1. Introduction

The herbal extracts of *Salvia miltiorrhiza* Bunge was widely used as traditional Chinese preparation for the treatment of ischemic heart disease, heart-stroke (Sugiyama et al., 2002) and cerebrovascular diseases (Sze et al., 2005). The herbal ingredient salvianolic acid B (Fig. 1) is one of the major ingredients in the water-soluble extracts of *S. miltiorrhiza* Bunge which has been reported to reduce atherosclerosis (Wu et al., 1998), inhibit low density lipoprotein (Chen et al., 2001), improve regional cerebral blood flow, prohibit platelet aggregation (Tang et al., 2002) and protect liver against fibrosis (Zhao et al., 2004).

Determination and pharmacokinetics related to salvianolic acid B have been studied in the beagle dogs (Li et al., 2004a,b, 2005a) and human subjects (Li et al., 2005b) following intravenous administration. In addition, current pharmacokinetic reports elucidate the low oral bioavailability of magnesium lithospermate B, an active component from *S. miltiorrhiza* (Zhang et al., 2004b) and the biliary excretion of in restrained rats (Zhang et al., 2004b). A liquid chromatographic with ultraviolet detection and liquid–liquid sample extraction has recently been described to determine salvianolic acid B in plasma of anesthetic rats after oral administration (Zhang et al., 2005). Our previous report also explored the biliary excretion of unbound salvianolic acid B by microdialysis technique (Chen et al., 2005). However, the oral bioavailability of salvianolic acid B in experimental model of conscious animal has not been investigated.

The major disadvantage of previous reports on the pharmacokinetics of salvianolic acid B is that the experimental animals are either in restrained or under anesthesia. The stress associated with restraint and conventional blood sampling from conscious rodents affects their physiology, biochemistry, metabolism and protein expression (Kissinger, 2002). Automated blood sampling system has been reported (Zhu et al., 2000; Lin et al.,

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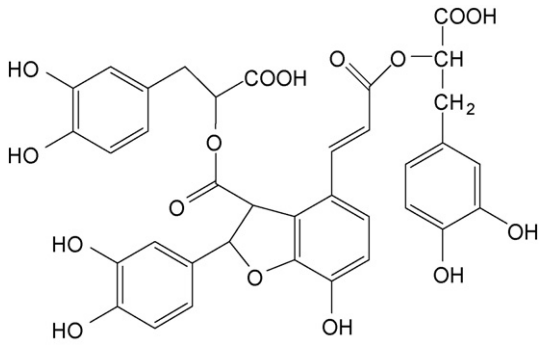

Fig. 1. Chemical structure of salvianolic acid B.

2005a) in pharmacokinetic studies to minimize the stress caused by the restrain and anesthesia in rodents. Therefore, the object of this study is to assess the oral bioavailability of salvianolic acid B in a stress-free condition (in conscious and freely moving rats) with an automated blood sampling system, which may results in more meaningful pharmacokinetic profiles. In addition, a LC–MS/MS method was developed to verify the salvianolic acid B in rat plasma.

2. Experimental

2.1. Chemical and reagent

Authentic salvianolic acid B (purity 99.5%, by HPLC) was originally separated and isolated from *S. miltiorrhiza* Bunge. Solvents and reagents of liquid chromatographic grade were obtained from E. Merck (Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA, USA) was used for all preparations. Salivanolic acid B was dissolved in normal saline for drug administration in the animal experiment.

2.2. Liquid chromatography

HPLC was performed with a chromatographic pump (BAS PM-80, West Lafayette, IN, USA), a Rheodyne Model 7125 injector was equipped with a 20 μl sampling loop and an ultraviolet detector (Linear Model 340, San Jose, CA, USA). Separation was achieved by a LiChrosorb RP-18 column (Merck, 250 × 4 mm i.d.; particle size 5 μm) at room temperature (24 ± 1 °C). The mobile phases consisted of acetonitrile–methanol–20 mM NaH_2PO_4 (adjusted to pH 3.5 with H_3PO_4) (20:10:70 v/v/v) containing 0.1 mM 1-octanesulfonic acid, and the flow-rate of 1 ml/min. The detection wavelength was 286 nm. Output data from the detector were integrated using an EZChrom chromatographic data system (Scientific Software, San Roman, CA, USA).

2.3. Method validation

Calibration curves were established by using blank plasma spiked with different amounts of salvianolic acid B and chloramphenicol as the internal standard. The peak area ratios of salvianolic acid B to the internal standard versus analyte concentrations were used to calculate linear regression. The concentration–response relationship from the present method indicated linearity over a concentration range of 0.5–200 μg/ml with coefficient of estimation (r^2) at least 0.995. The intra-assay and inter-assay variabilities were determined by quantitating six replicates at concentrations of 0.5, 1, 5, 10, 50, 100 and 200 μg/ml using the HPLC method described above on the same day and six consecutive days, respectively. The lowest concentration of the linear regression defined the limit of quantitation (LOQ). The accuracy (% bias) was calculated from the mean value of observed concentration (C_{obs}) and the nominal concentration (C_{nom}) as follows: accuracy: (% bias) = $[(C_{obs} - C_{nom})/C_{nom}] \times 100$. The relative standard deviation (R.S.D.) was calculated from the observed concentrations as follows: precision (% R.S.D.) = [standard deviation (S.D.)/C_{obs}] × 100.

2.4. LC–MS/MS

LC–MS/MS analysis was performed using a Waters 2690 with a 996 photodiode array (PDA) detector together with an automatic liquid chromatographic sampler and an autoinjection system hyphenated to a Micromass Quattro Ultima tandem quadrupole mass spectrometry (Micromass, Manchester, UK) equipped with an electrospray ionization (ESI) source. For operation in the MS/MS mode, a mass spectrometer with an orthogonal Z-spray electrospray interface (ESI) was used. The infusion experiment was performed to optimize the ESI parameters using a Mode 22 multiple syringe pump (Harvard, Holliston, MA, USA). During the analyses, the ESI parameters were set as follows: capillary voltage, 2.5 kV for the negative mode; source temperature, 80 °C; desolvation temperature, 250 °C; cone gas flow, 110 l/h; and desolvation gas flow, 570 l/h. The cone voltage of *m/z* 358 was 45 V and the collision energy was 10 eV. All LC–MS/MS data were processed by the MassLynx version 3.5 NT Quattro data acquisition software. The separation was achieved using a ZORBAX Extend-C18 column (4.6 mm × 150 mm i.d.) (Agilent, USA). The solvent delivery system was kept constant at 500 μl/min and the mobile phase consisted of 20% acetonitrile and 80% 0.1 mM ammonium acetate. The volume of injection was 10 μl.

2.5. Animals

All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of the National Research Institute of Chinese Medicine. Male specific pathogen-free Sprague–Dawley rats were obtained from the Laboratory Animal Center of the National Yang Ming University, Taipei. The animals had free access to food (Laboratory rodent diet 5P14, PMI Feeds, Richmond, IN, USA) and water until 18 h prior to being used in experiments, at which time only food was removed. Six Sprague–Dawley rats (280–320 g) were initially anesthetized with pentobarbital (50 mg/kg, i.p.), and remained anesthetized throughout the surgical operation. During the period of surgery, the body temperature of rats was maintained at 37 °C with a heating pad. After surgery, the rats

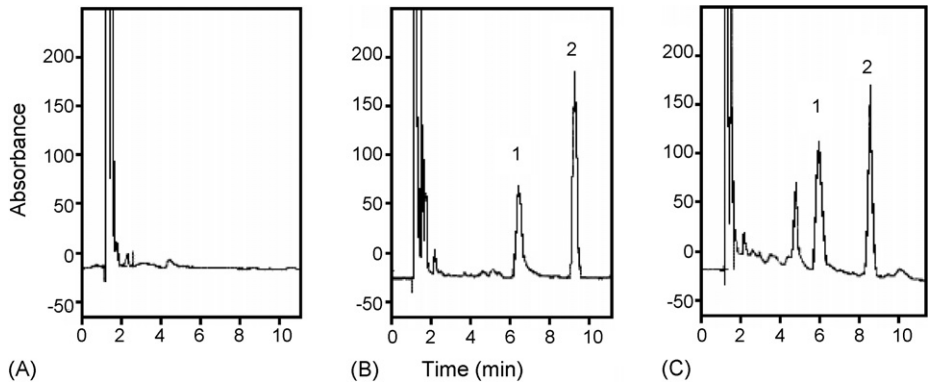

Fig. 2. Chromatograms of salvianolic acid BL: (A) shows a chromatogram of a drug-free plasma extract; (B) shows a chromatogram of a standard of salvianolic acid B (50 μg/ml); (C) shows the chromatogram of a plasma sample containing salvianolic acid B (68.8 μg/ml) collected from rat plasma 10 min after salvianolic acid B administration (100 mg/kg, i.v.). 1: salvianolic acid B; 2: internal standard (chloramphenicol).

were installed in the experimental cage and allowed to recover for 1 day.

2.6. Blood sampling and sample preparation

Salvianolic acid B (100 mg/kg, i.v.) dissolved in normal saline was given from the femoral vein for intravenous injection and 500 mg/kg of the drug was given by gastrogavage for the route of oral administration. The automated blood sampling system DR-II (Eicom Corp., Kyoto, Japan) has been employed for blood sampling in conscious and freely moving rats. A 100 μl blood sample was withdrawn from the jugular vein into a heparin rinsed vial with fraction collector according to a programmed schedule from the jugular vein at 5, 10, 30, 60, 90 min, 2–4 h after i.v. injection. Each blood sample was centrifuged at 3000 × *g* for 5 min. The resulting plasma sample (25 μl) was vortex-mixed with 50 μl of internal standard solution (chloramphenicol 10 μg/ml in acetonitrile). The protein precipitation was achieved by internal standard solution and separated by centrifugation at 8000 × *g* for 5 min. An aliquot (20 μl) of the supernatant was directly injected onto the HPLC system for analysis. Data from these samples were used to construct the pharmacokinetic curves of salvianolic acid B concentration in blood versus time. The same sample handling process was used for the determination of precision and accuracy.

Prior to using blood sampling module with experimental animals, we performed in vitro experiments to evaluate fluid delivery precision and carry-over level via the pumping tubes. Two sets of whole blood samples, which contained 10 and 25 μg/ml salvianolic acid B, respectively, were siphoned through the sampling module tubes. Each set of samples had three replicates.

2.7. Protein binding

For protein-binding of salvianolic acid B assay, the drug was given via i.v. bolus to rats with a dose of 100 mg/kg. Then, the blood sample (2 ml) was withdrawn from the heart puncture at 10 min after injection. The rat blood sample was centrifuged at 3000 × *g* under 4 °C, for 10 min. The plasma was divided into two parts; 0.1 ml of plasma was used to measure the total form concentration of salvianolic acid B (Ct). The remaining plasma was transferred to an ultrafiltration tube (Centrifree, Millipore, Bedford, MA, USA). Further centrifugation was performed at 720 × *g* for 30 min under 4° C (Eppendorf, NJ, USA) for free form measurement (C_f). Measurement of salvianolic acid B was the same as the above description. The protein-binding (*B*) of salvianolic acid B was calculated by following equation: $B = [(C_t - C_f)/C_t] \times 100\%$.

2.8. Pharmacokinetic application

Calibration curves were constructed based on LC analyses of a standard mixture prior to each experiment. Pharmacokinetic calculations were performed on each individual set of data using the pharmacokinetic software WinNonlin Standard Edition Version 1.1 (Pharsight Corp., Mountain View, CA, USA) by non-compartmental method. The area under the concentration–time curve (AUC) was calculated according to the linear trapezoidal method.

3. Results and discussion

3.1. Chromatography

Salvianolic acid B in rat plasma was quantitated using LC coupled with ultraviolet detection. Peak scanning by the photodiode array (Waters 2695 separations module coupled to 2996 detector; Waters, Millford, MA, USA) detection revealed the maxima wavelength of salvianolic acid B was 286 nm. Fig. 2A is a chromatogram of a drug-free plasma extract showing a clean, stable baseline without interfering endogenous peaks. Run-time was set at 10 min, and no carry-over peaks were detected in subsequent chromatograms of plasma samples. Fig. 2B shows a chromatogram of a standard of salvianolic acid B (50 μg/ml). Fig. 2C is the chromatogram of a plasma sample containing salvianolic acid B (68.8 μg/ml) collected from rat plasma 10 min after salvianolic acid B administration (100 mg/kg, i.v.). The analytes were well separated using the present chromatographic conditions. The retention times were 5.9 and 8.5 min for salvianolic acid B and internal standard, respectively, and no peak

Table 1
Standard curves of HPLC assay of salvianolic acid B in rat plasma

	Slope	Intercept	r
Day 1	71.72	−0.061	0.999
Day 2	71.82	−0.036	0.999
Day 3	71.27	−0.031	0.999
Day 4	73.56	−0.078	0.999
Day 5	72.76	−0.068	0.999
Day 6	75.84	−0.052	0.999
Mean	72.83	−0.054	0.999
S.D.	1.69	0.018	0

distortions were visible. The appearance of an unidentified peak prior to the peak of salvianolic acid B which was not found in the blank plasma might be attributed to the metabolite of salvianolic acid B.

3.2. Linearity and detection limit

Linear least-square regression analysis of the calibration graph on six different days demonstrated linearity between the response and the nominal concentration of salvianolic acid B over the range of 0.5–200 μg/ml. For calculating the plasma sample of salvianolic acid B from animal study, the calibration curve based on the peak area ratios (salvianolic acid B to internal standard) versus analyte concentration of the HPLC chromatograms were constructed prior to assay. Table 1 shows the equations of the standard curves of salvianolic acid B on six different days. The results of linear regression analysis show that the correlation coefficients of all standards curves were better than 0.995. The data show the excellent repeatability of the sample analysis. The limit of detection (LOD) of salvianolic acid B in rat plasma was determined to be 0.1 μg/ml at a signal-to-noise ratio of 3. The lower limit of quantitation (LOQ) was 0.5 μg/ml.

3.3. Precision and accuracy

The intra- and inter-assay precision and accuracy values are presented in Table 2. The overall mean precision, defined by the R.S.D., ranged from 6.57 to 0.24%. Analytical accuracy, expressed as the percent difference of the mean observed values compared with known concentration varied from −5.34 to 0.02%.

3.4. Recovery and carry-over examination

The one-step extraction procedure was fairly rapid. The solvent acetonitrile gave good recovery and the average absolute recoveries of salvianolic acid B from plasma was 96.2 ± 6.9% at concentrations of 5, 10 and 50 μg/ml as shown in Table 3. The collected blood samples were determined to maintain high recovery levels, 93.04 ± 4.65 of 5 μg/ml and 99.84 ± 10.98 of 50 μg/ml. During the interval of two spiked sample collections, one blank whole blood sample was flushed through the tubes. The result of flushed blank determination indicates that no measurable carry-over was found in the automated blood sampling system.

Table 2
Method validation for the intra-assay and inter-assay precision (% R.S.D.) and accuracy (% bias) of the HPLC method for the determination of salvianolic acid B

Nominal concentration (μg/ml)	Observed concentration (μg/ml)	Precision (% R.S.D.)	Accuracy (% bias)
Intra-assay			
0.5	0.48 ± 0.017	3.5	−4.0
1.0	1.01 ± 0.053	5.2	1.0
5.0	4.87 ± 0.18	3.7	−2.6
10	9.92 ± 0.11	1.1	−0.8
50	49.9 ± 0.37	0.7	0.02
100	102 ± 0.41	0.4	2.0
200	200 ± 0.47	0.2	0.0
Inter-assay			
0.5	0.48 ± 0.032	6.7	−4.0
1.0	1.01 ± 0.057	5.7	1.0
5.0	4.98 ± 0.22	4.4	−0.4
10	10.1 ± 0.32	3.2	1.0
50	50.1 ± 0.28	0.6	0.2
100	101 ± 0.72	0.7	1.0
200	201 ± 0.48	0.2	0.5

Observed concentration data are expressed as mean ± S.D. ($n = 6$).

Table 3
Recovery of salvianolic acid B in rat plasma

Concentration (μg/ml)	Recovery (%)
5	93.04 ± 4.65
10	100.86 ± 5.17
50	99.84 ± 10.98

Data expressed as mean ± S.D. ($n = 4$), recovery = $(C_{\text{in plasma}}/C_{\text{in water}}) \times 100\%$.

3.5. Protein binding

Methods for studying protein binding, including equilibrium dialysis (Lin et al., 2005b) and ultrafiltration (Peng et al., 1999), make use of a semipermeable membrane that separates the protein and protein-bound drug from the free or unbound drug. The protein-bound drug is a large complex that cannot easily transverse cell or possibly even capillary membranes and therefore has a restricted distribution. The ultrafiltration method was used in this experiment and the protein-binding (B) of salvianolic acid B was calculated by $B = [(C_t - C_f)/C_t] \times 100\%$. The protein binding in rat plasma was 83.78 ± 10.5% (Table 4).

Table 4
Protein binding of salvianolic acid B in rat plasma

Experimental no.	Protein binding (%)
Rat 1	88.9
Rat 2	89.8
Rat 3	62.6
Rat 4	87.3
Rat 5	85.1
Rat 6	89.6
Average	83.78 ± 10.5

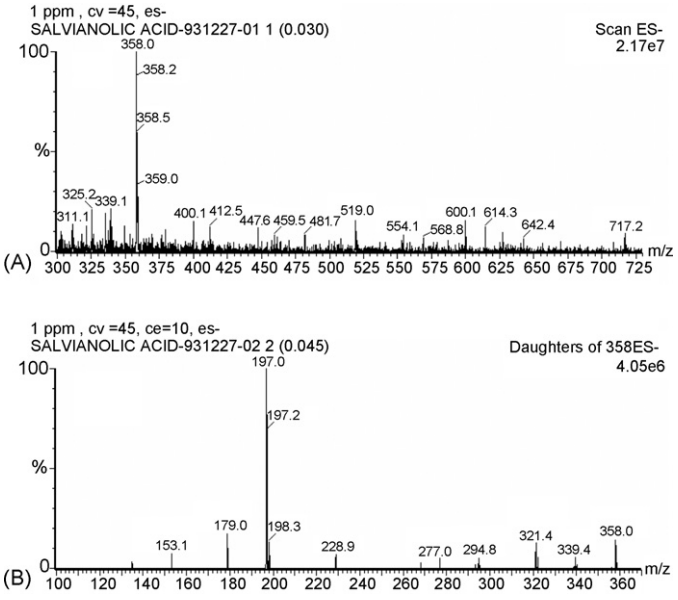

Fig. 3. Full scan mass spectrum of (A) salvianolic acid B (molecular weight 359) and (B) product ion in LC–tandem mass spectrometry of with electrospray negative-ion mode.

3.6. Verification by LC–MS/MS

We used full scan in negative ion modes (scan range from *m/z* 200 to 800) to identify the analyte and the peak of *m/z* 717 $[M-H]^-$ can been observed. The full scan mass spectrums for the determination of salvianolic acid B (precursor ion is 358 $[M-2H]^{-2}$) and the main product ion at *m/z* 197 were shown in Fig. 3. Considering the sensitivity, we therefore chose the major peak of *m/z* 358 as the precursor ion for salvianolic acid B, and this precursor ion $[M-2H]^{-2}$ may possibly result from the losing of two protons of salvianolic acid B. The mass transitions of *m/z* 358.2 → *m/z* 196.9 were employed to verify the salvianolic acid B in rat plasma after 10 min of drug administration (100 mg/kg, i.v.), and this LC–MS/MS method showed good peak symmetry and high intensity as shown in Fig. 4. The retention time of salvianolic acid B was 2.51 min.

3.7. Pharmacokinetics of salvianolic acid B

Fig. 5 illustrates the concentration versus time profiles of salvianolic acid B with a single intravenous and oral dose

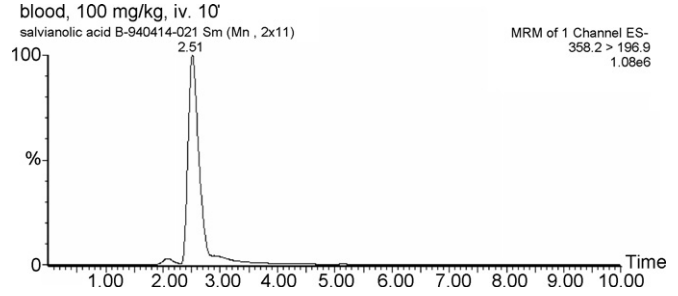

Fig. 4. Chromatogram of salvianolic acid B monitoring the transition of the molecular ion *m/z* 358.2 to the product ion *m/z* 196.9 in rat plasma.

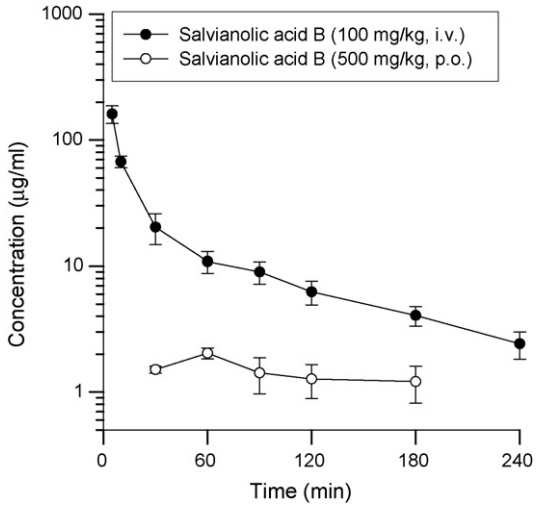

Fig. 5. Concentration vs. time curves of salvianolic acid B after drug administration (100 mg/kg, i.v. and 500 mg/kg, p.o.) in rats.

administered to six individual rats for each group. The AUC were 5030 ± 565 and 582 ± 222 min μg/ml for intravenous (100 mg/kg) and oral (500 mg/kg) doses, respectively (Table 5). The linear logarithm has been found in the elimination phase of the pharmacokinetic curve. Therefore, the rest of the time points should have the same slope. The oral bioavailability $(AUC_{p.o.}/dose)/(AUC_{i.v.}/dose)$ of salvianolic acid B in freely moving rats was calaculted about 2.3%. Obvious linear pharmacokinetic curve was not found in oral administration which may be caused by the disposition fact of polyphenolic compound. Because the chemical structure of salvianolic acid B is constructed by two ester functional groups for the connection of polyphenolic complex, enzymatic biodegradation and phase II biotransformation can be performed regularly in the polyphenolic complex (Scalbert et al., 2002).

The herbal medicine possesses many pharmacological actions in vitro and in vivo, and therefore it is worth to investigate the detail oral bioavailability and pharmacokinetics of salvianolic acid B. Serum levels of salvianolic acid B after intravenous injection had been monitored in beagle dogs (Li et al., 2004a,b, 2005a) and human subjects (Li et al., 2005b). Linear relationship ($r = 0.947$) was observed between AUC and doses after intravenous administration at the doses of 3, 6 and 12 mg/kg of salvianolic acid B, but the clearance and volume of distribution values obtained at the high dose were lower than those at the low dose. These data suggested a saturated distribution and metabolism might occur at the high dose (Li et al., 2004a). Pharmacokinetics of salvianolic acid B (200 mg/kg) after oral admin-

Table 5
Pharmacokinetic data after salvianolic acid B administration (100 mg/kg, i.v. and 500 mg/kg, p.o.) in rats

Parameters	100 mg/kg, i.v.	500 mg/kg, p.o.
AUC (min μg/ml)	5030 ± 565	582 ± 222
C_{max} (μg/ml)	910 ± 380	1.5 ± 0.5
$t_{1/2}$ (min)	105 ± 19	248 ± 11

Data are expressed as mean ± S.E.M. from six individual experiments for each group.

istration of Radix *Salviae miltiorrhizae* extract was investigated in anesthetic rats (Zhang et al., 2005). Results showed that salvianolic acid B in conformation with a two-compartment model with first-order absorption. The salvianolic acid B reached the maximum concentration within 0.5 h and could be detected up to 180 min after oral administration. Zhang et al. (2004a) first evaluated the bioavailability of salvianolic acid B in restrained rats. Intravenous injection via tail vein at 20 mg/kg and oral administration by gastric intubation at 100 mg/kg were performed to estimate the bioavailability. A low C_{max} of 0.041 ± 0.007 μg/ml and very small AUC value were found during a limited period of 60 min after oral administration. The absolute bioavailability was calculated to be 0.02%. In addition, they also found more than 60% of dosed salvianolic acid B remained in the gastrointestinal tract even 4 h after oral administration, which indicated the poor absorption of salvianolic acid B in rat small intestine. Moreover, they discovered that most of the administered salvianolic acid B was excreted into bile and metabolized into the four methylated forms (Zhang et al., 2004b).

Our previous report (Chen et al., 2005) using microdialysis technique indicated that the concentrations of unbound salvianolic acid B in bile were significantly higher than those in blood, suggesting an active transport of salvianolic acid B, which might go through the liver into the bile duct. The hepatobiliary excretion of salvianolic acid B was defined as the bile-to-blood distribution (k value), which was calculated by dividing the area under the concentration–time curve (AUC) of salvianolic acid B in bile by that in blood ($k = AUC_{bile}/AUC_{blood}$). The bile-to-blood distribution ratio was 1.55 ± 0.21 at the dose of 100 mg/kg. In present work, we assess the bioavailability of salvianolic acid B in a stress-free rat model. The salvianolic acid B reached the maximum concentration within 1 h and could be monitored up to 180 min after oral administration, which was similar to the results from anesthetic rats (Zhang et al., 2005). The oral bioavailability of salvianolic acid B in this study was calculated approximately 2.3%, which is greater than previous report (Zhang et al., 2004b). This discrepancy probably results from the different blood collection methods. Zhu et al. (2005) had observed the differences for pharmacokinetic parameters of carbamazepine and its main metabolites obtained by manual and automated blood sampling methods. They concluded that the dissimilarities can be contributed to physical stress, which influenced blood flow and distribution impacting drug absorption, distribution and metabolism. Lee and Sarna (1997) also reported the stress may delay gastric emptying and slow the absorption of drugs in the gastrointestinal tract.

In summary, an automated blood sampling system connected to a simple LC assay using UV detection was developed to monitor the plasma levels of salvianolic acid B in conscious and freely moving rats. In addition, we have verified salvianolic acid B in rat plasma by a LC–MS/MS method.

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References

Chen, Y.F., Jaw, I., Shiao, M.S., Tsai, T.H., 2005. Determination and pharmacokinetic analysis of salvianolic acid B in rat blood and bile by microdialysis and liquid chromatography. J. Chromatogr. A 1088, 140–145.

Chen, Y.H., Lin, S.J., Ku, H.H., Shiao, M.S., Lin, F.Y., Chen, J.W., Chen, Y.L., 2001. Salvianolic acid B attenuates VCAM-1 and ICAM-1 expression in TNF-treated human aortic endothelial cells. J. Cell. Biochem. 82, 512–521.

Kissinger, P.T., 2002. The history of automatic blood sampling at BAS. Curr. Sep. 19, 113–115.

Lee, C., Sarna, S.K., 1997. Central regulation of gastric emptying of solid nutrient meals by corticotropin releasing factor. Neurogastroent. Motil. 9, 221–230.

Li, X., Yu, C., Sun, W., Liu, G., Jia, J., Wang, Y., 2004a. Pharmacokinetics of magnesium lithospermate B after intravenous administration in beagle dogs. Acta Pharmacol. Sin. 25, 1402–1407.

Li, X., Yu, C., Sun, W., Liu, G., Jia, J., Wang, Y., 2004b. Simultaneous determination of magnesium lithospermate B, rosmarinic acid, and lithospermic acid in beagle dog serum by liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. 18, 2878–2882.

Li, X., Yu, C., Sun, W., Lu, J., Xuan, L., Wang, Y., 2005a. Liquid chromatography/tandem mass spectrometry for the determination of magnesium lithospermate B in beagle dog serum. J. Pharm. Biomed. Anal. 38, 107–111.

Li, X., Yu, C., Cai, Y., Liu, G., Jia, J., Wang, Y., 2005b. Simultaneous determination of six phenolic constituents of danshen in human serum using liquid chromatography/tandem mass spectrometry. J. Chromatogr. B 820, 41–47.

Lin, L.C., Yang, K.Y., Chen, Y.F., Wang, S.C., Tsai, T.H., 2005a. Measurement of daphnoretin in plasma of freely moving rat by liquid chromatography. J. Chromatogr. A 1073, 285–289.

Lin, Z.J., Musiano, D., Abbot, A., Shum, L., 2005b. In vitro plasma protein binding determination of flunarizine using equilibrium dialysis and liquid chromatography–tandem mass spectrometry. J. Pharm. Biomed. Anal. 37, 757–762.

Peng, S.X., Henson, C., Wilson, L.J., 1999. Simultaneous determination of enantioselective plasma protein binding of aminohydantoins by ultrafiltration and chiral high-performance liquid chromatography. J. Chromatogr. B 732, 31–37.

Scalbert, A., Morand, C., Manach, C., Rémésy, C., 2002. Absorption and metabolism of polyphenols in the gut and impact on health. Biomed. Pharmacother. 56, 276–282.

Sugiyama, A., Zhu, B.M., Takahara, A., Satoh, Y., Hashimoto, K., 2002. Cardiac effects of Salvia Miltiorrhiza/Dalbergia Odorifera mixture, an intravenously applicable Chinese medicine widely used for patients with ischemic heart disease in China. Circ. J. 66, 182–184.

Sze, F.K., Yeung, F.F., Wong, E., Lau, J., 2005. Does Danshen improve disability after acute ischaemic stroke? Acta Neurol. Scand. 111, 118–125.

Tang, M.K., Ren, D.C., Zhang, J.T., Du, G.H., 2002. Effect of salvianolic acids from Radix Salviae miltiorrhizae on regional cerebral blood flow and platelet aggregation in rats. Phytomedicine 9, 405–409.

Wu, Y.J., Hong, C.Y., Lin, S.J., Wu, P., Shiao, M.S., 1998. Increase of vitamin E content in LDL and reduction of atherosclerosis in cholesterol-fed rabbits by a water-soluble antioxidant-rich fraction of *Salvia miltiorrhiza*. Arterioscler. Thromb. Vasc. Biol. 18, 481–486.

Zhang, J., Yu, H., Sheng, Y., Li, L., Ye, M., Guo, D., 2005. HPLC determination and pharmacokinetic studies of salvianolic acid B in rat plasma after oral administration of Radix Salviae Miltiorrhizae extract. Biomed. Chromatogr. 19, 15–18.

Zhang, Y., Akao, T., Nakamura, N., Duan, C.L., Hattori, M., Yang, X.Y., Liu, J.X., 2004a. Extremely low bioavailability of magnesium lithospermate B, an active component from *Salvia miltiorrhiza*, in rat. Plant. Med. 70, 138–142.

Zhang, Y., Akao, T., Nakamura, N., Hattori, M., Yang, X.W., Duan, C.L, Liu, J.X, 2004b. Magnesium lithospermate B is excreted rapidly into rat bile mostly as methylated metabolites, which are potent antioxidants. Drug Metab. Dispos. 32, 752–757.

Zhao, J.F., Liu, C.H., Hu, Y.Y., Xu, L.M., Liu, P., Liu, C., 2004. Effect of salvianolic acid B on Smad3 expression in hepatic stellate cells. Hepatobil. Pancr. Dis. Int. 3, 102–105.

Zhu, Y., Chiang, H., Wulster-Radclife, M., Hilt, R., Wong, P., Kissinger, C.B., Kissinger, P.T., 2005. Liquid chromatography/tandem mass spectrometry for the determination of carbamazepine and its main metabolite in rat plasma utilizing an automated blood sampling system. J. Pharm. Biomed. Anal. 38, 119–125.

Zhu, Y., Huang, T., Cregor, M., Long, H., Kissinger, C.B., Kissinger, P.T., 2000. Liquid chromatography with multichannel electrochemical detection for the determination of trans-resveratrol in rat blood utilizing an automated blood sampling device. J. Chromatogr. B 740, 129–133.