Pharmacokinetic Interaction between Tanshinones and Polyphenolic Extracts of *Salvia miltiorrhiza* BUNGE after Intravenous Administration in Rats

**Zeng-Jun GUO,** a,b,c **Yu ZHANG,** b **Xing TANG,** a,b **Hui LI,** b and **Qi-Shi SUN** a

a Department of Pharmacognosy, Shenyang Pharmaceutical University; b Department of Pharmaceutics, Shenyang Pharmaceutical University; 103 Wenhua Road, Shenyang 110016, China; and c Faculty of Pharmacy of School of Medicine, Xi’an Jiaotong University; Xi’an 710061, China.

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The objective of this study was to investigate the interaction between tanshinones and polyphenolic extracts of *Salvia miltiorrhiza* BUNGE in rats. The rats in the medium dose groups were given an intravenous administration of 10 mg/kg tanshinones extract-loaded emulsion (equivalent to 4.0 mg/kg tanshinone IIA (TSIIA)), 100 mg/kg polyphenolic extract solution (equivalent to 61.2 mg/kg salvianolic acid B (Sal B)) or mixed extracts-loaded emulsion (equivalent to 4.0 mg/kg TSIIA and 61.2 mg/kg Sal B). The dosage given to the low dose groups was half that of the medium dose groups, while the high dose groups received twice the dosage of the medium dose groups. The areas under the plasma concentration-time curve (AUC) of TSIIA and Sal B were considerably increased (about 2—14 fold) after intravenous administration of mixed extracts-loaded emulsion in comparison with the equivalent dose of the corresponding extract administration. An increase of about 2-fold was observed in both the low and medium dose groups for TSIIA and Sal B, while there was at least a 14- and 5-fold significant increase (*p* < 0.01) for TSIIA and Sal B in the high dose groups, respectively which was due to a significant (*p* < 0.01) reduction in total plasma clearance (*C* *F*). The peak plasma concentrations (*C* *p* *m* *) of TSIIA and Sal B were also both significantly increased (*p* < 0.01). However, no significant differences in the terminal elimination half-life (*t* *e* *) of TSIIA and Sal B in the mixed extracts-loaded emulsion groups were found compared with that of the corresponding extract groups except for the high dose groups of TSIIA (*p* < 0.05). Therefore, a pharmacokinetic interaction occurs between tanshinones and polyphenolic extracts of *Salvia miltiorrhiza* BUNGE after intravenous administration in rats, which affects the pharmacokinetic process of TSIIA and Sal B in vivo.

**Key words** *Salvia miltiorrhiza*; tanshinone IIA; salvianolic acid B; interaction

The dry roots of *Salvia miltiorrhiza*, also called “Danshen” in Chinese, are a well-known traditional Chinese medicinal herb. Because of its better performance and fewer side effects confirmed during the long-time clinical use, Danshen is widely used in traditional Chinese medicinal preparations to treat a number of coronary heart diseases, especially angina pectoris and myocardial infarction.1—4) At present, a variety of Danshen products are commercially available, such as Fufang Danshen Tablets and Fufang Danshen Dripping pills, which are the two most commonly used ones in China and these have been officially listed in the Chinese Pharmacopoeia 2005.5) According to their chemical structures, the major bioactive constituents in *S. miltiorrhiza* BUNGE can be classified into two groups: the tanshinone group, which belong to a group of lipid-soluble diterpenoids, and water-soluble polyphenolic compounds.9) The major lipophilic components are cryptotanshinone, tanshinone I and tanshinone IIA (TSIIA). Pharmacological tests have revealed that TSIIA possesses a variety of pharmacological activities including antioxidant effects,7) prevention of angina pectoris and myocardial infarction,8) as well as antineoplastic.9) anti-cancer,10,11) and anti-inflammatory effects.12) The major hydrophilic components include danshensu, protocatechuic aldehyde and salvianolic acid B (Sal B). Sal B is the most common component in the *Salvia* species and the most abundant in their aqueous extracts.13,14) Different studies have shown that Sal B has a variety of biological activities including anti-oxidation,15,16) anti-fibrotic,17) and myocardial salvage effects18); it also prevents hepatitis and uremia,19) and improves blood circulation and renal function.20—25) Considering their prominent pharmacological activities and high content in Danshen, both TSIIA and Sal B have been selected as marker components for the quality control of Danshen and many other medicinal preparations containing Danshen in the 2005 edition of Chinese Pharmacopoeia.5,12,26) The chemical structures of TanIIA and Sal B are shown in Fig. 1.

Recently, Song et al.27) showed that the pharmacokinetics of cryptotanshinone and TSIIA in rats after oral intra-gavage of an extract of tanshinones was significantly affected by the co-present tanshinones. Also, Sal B had a lower effective concentration in vitro angiogenesis study than the crude extract.28) In clinical practice, most Danshen products used consist of tanshinone and polyphenolic extracts at the same time.5) However, there have been no reports of pharmacokinetic interactions between tanshinones and polyphenolic extracts of *S. miltiorrhiza* BUNGE in vivo.

To date, several methods have been established for the de-

**Fig. 1. The Chemical Structures of Tanshinone IIA and Salvianolic Acid B**
BUNGE in rats after intravenous administration by simultaneously monitoring the two marker components of Danshén—TSIIA and Sal B.

**MATERIALS AND METHODS**

**Materials** The tanshinones extract was obtained by supercritical CO₂ fluid extraction of *Salvia miltiorrhiza BUNGE* (Guangzhou, China), in which the content of TSIIA was determined by HPLC to be 40.1%. The residue of *Salvia miltiorrhiza BUNGE* was extracted at pH 1—2 with deionized water and further purified on an AB-8 absorbent resin (Bengbu Tianxing Resin Co., Ltd., Anhui, China). The eluate was concentrated under reduced pressure with a Model RE-3000 rotary evaporator (Shanghai Yarong Biochemistry Instrument Co., Ltd., Shanghai, China) and freeze-dried in an ZYZLA® FDU-1100/DRC-1000 freeze dryer (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) to obtain a purified polyphenolic extract, with a Sal B content of 61.2%. Acetonitrile and formic acid (HPLC grade) were supplied by Merck KGaA (Darmstadt, Germany). Diphenhydramine (internal standard) was obtained from A&A Biotechnology Co., Ltd., Shanghai, China. All other chemicals and reagents were of analytical or chromatographic grade.

**Preparation of Tanshinones Extract-Loaded Emulsion and Polyphenolic Extract Solution** Soybean oil and MCT were mixed under stirring at 70 °C to obtain the oil phase while F68, sodium oleate, and glycercol were dispersed in water for injection by stirring at 75 °C to obtain a water phase. The oil phase was added to the water phase and mixed using a high-shear mixer at 8000 rpm to prepare a coarse emulsion, and then the tanshinones extract was added, with further stirring until a primary emulsion was obtained. After adjusting the pH to approx. 7.0 with 0.1 mol/l HCl or NaOH solution, the primary emulsion was passed through a high pressure homogenizer (Niro Soavi NS10012k, Niro Soavi S.p.A., Via M. Da Erba, Italy). Finally, the preparation was gassed with N₂ and sealed in 10 ml glass bottles followed by autoclaving for 30 min at 100 °C.

The polyphenolic extract solution was prepared by dissolving the polyphenolic extract in deionized water, adding glycerol for isosmotic adjustment, and then adjusting the pH to approx. 7.0 with 0.1 mol/l HCl or NaOH solution.

**Animal Experiments and Drug Administration** Fifty-four male Wistar rats (8 weeks old, 200 ± 20 g) were obtained from the Laboratory Animal Center of Shenyang Pharmaceutical University. The experimental protocol was approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals. Rats were housed in groups of eighteen with a 12-h light/dark cycle at a temperature of 22 ± 3 °C, and relative humidity of 45—60%, for one week. Before the day of administration, the rats were fasted for 12 h but were allowed water *ad libitum*.

Animal experiments were conducted on three consecutive days. On the first day, eighteen rats were divided into three equal groups. Medium-dosed group I: 10 mg/kg tanshinones extract-loaded emulsion (equivalent to 4.0 mg/kg TSIIA), medium-dosed group II: 100 mg/kg polyphenolic extract solution (equivalent to 61.2 mg/kg Sal B) and medium-dosed group III: mixed extracts-loaded emulsion (containing 10 mg/kg tanshinones extract and 100 mg/kg polyphenolic extract, equivalent to 4.0 mg/kg TSIIA and 61.2 mg/kg Sal B), which were given to rats by intravenous injection via the femoral vein. The tanshinones extract-loaded emulsion and polyphenolic extract solution were prepared by the method described above. The mixed extracts-loaded emulsion was prepared similarly with a tanshinones extract-loaded emulsion by combining both the tanshinones and polyphenolic extracts.

The low dose and high dose groups were also prepared in a similar manner to the medium dose group on the second and third days, respectively. The dosage of the low dose group was half that of the medium dose group, while that of the high dose group was twice the dosage of the medium dose group.

Blood samples (250 µl) were collected from each rat into heparinized Eppendorf tubes (2.0 ml) by puncture of the retro-orbital sinus. This was performed at 0 (predose), 0.083, 0.167, 0.250, 0.333, 0.500, 0.750, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 12.0 h after intravenous administration. Then, as soon as possible, the heparinized blood was centrifuged for 10 min at 2000×*g*, and the plasma obtained was stored frozen at −20 °C until analysis.

**UPLC/MS/MS Analysis** Frozen plasma samples were thawed at room temperature and vortexed to achieve a good mixing prior to precipitation. Then, to a 100 µl aliquot of plasma in a 2.0 ml Eppendorf tube, 50 µl of the IS and 250 µl acetonitrile was added. The mixture was vortexed for 5.0 min and then centrifuged at 10000 rpm for 5 min in a FULGOR GL-20B refrigerated centrifuge (Shanghai, China) which was kept at 4 °C. Then, a 5.0 µl aliquot of the clean supernatant was injected into the UPLC/MS/MS system for analysis.

Chromatography was performed using an ACQUITY™ UPLC system (Waters Corp., Milford, MA, U.S.A.) with a conditioned autosampler at 4 °C. The separation was carried out on an ACQUITY UPLC™ BEH C₁₈ column (50 mm×2.1 mm i.d., 1.7 µm; Waters Corp., Milford, MA, U.S.A.) maintained at a temperature of 35 °C. The analysis was carried out by gradient elution using (A) acetonitrile and (B) water (containing 0.1% formic acid) as the mobile phase.
Table 1. Transition Reactions of the Analytes and Internal Standards

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Ionization mode</th>
<th>Transition</th>
<th>Dwell (s)</th>
<th>Cone volt. (V)</th>
<th>Col. energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSIIA</td>
<td>ESI(+)</td>
<td>295.2→249.0</td>
<td>0.10</td>
<td>45.0</td>
<td>20.0</td>
</tr>
<tr>
<td>TSIIA</td>
<td>ESI(−)</td>
<td>717.3→519.3</td>
<td>0.10</td>
<td>45.0</td>
<td>20.0</td>
</tr>
<tr>
<td>I.S.</td>
<td>ESI(+)</td>
<td>256.2→166.9</td>
<td>0.10</td>
<td>30.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

The gradient started at 10% A and increased linearly to 80% A in 1.0 min, maintained at 80% for 1.0 min and then returned to the initial condition for another 1.0 min of re-equilibration. The flow rate was set at 0.35 ml/min. The injection volume was 5 μl using the partial loop mode for sample injection. A Waters ACQUITY™ TQD triple-quadrupole tandem mass spectrometer (Waters Corp., Manchester, U.K.) was interfaced to the UPLC system via electrospray ionization (ESI) interface. The capillary, extractor and RF voltages were set at 2.3 kV, 2.0 V and 0.7 V, respectively. The temperature of the source and desolvation was set at 80 and 400 °C, respectively. Nitrogen was used as the desolvation gas (500 l/h) and cone gas (50 l/h) while argon was used as the collision gas at a flow rate of 0.15 ml/min (approx. gas (500 l/h) and cone gas (50 l/h)) while argon was used as the collision gas at a flow rate of 0.15 ml/min (approx. 2.81×10⁻⁷ mbar). The multiple reaction monitoring (MRM) mode was used for quantification. The optimized conditions of MRM fragmentation transitions are listed in Table 1. All data collected in the centroid mode were acquired using Masslynx™ NT4.1 software (Waters Corp., Milford, MA, U.S.A.). Post-acquisition quantitative analyses were performed using a QuanLynx™ program (Waters Corp., Milford, MA, U.S.A.).

Typical MRM chromatograms of TSIIA and Sal B are shown in Fig. 2. Three channels were used for recording the response, channel I for TSIIA with a typical retention time of 1.97 min, channel II for IS with a typical retention time of 1.11 min, and channel III for Sal B with a typical retention time of 0.94 min. TSIIA, Sal B and I.S. were well separated with excellent peak shapes, and no interfering peaks were observed in the blank plasma and in all samples tested. The lower limit of quantitation (LLOQ) for determination of TSIIA and Sal B in plasma, defined as the lowest concentration analyzed with an accuracy within ±20% and a precision ≤20%, were 0.90 ng/ml and 0.15 μg/ml, respectively. The coefficient of variation was less than 9.87% for TSIIA and less than 9.75% for Sal B.

Pharmacokinetic Study The pharmacokinetic parameters were calculated using DAS (drug and statistics) version 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China) using non-compartmental pharmacokinetic methods. The maximum plasma concentration (C₀(0.083)) was directly obtained from the observed concentration–time data (0.083 h). The elimination rate constant (kₑ) was calculated by linear regression of the terminal points of the semi-log plot of plasma concentration against time. The elimination half-life (t½ₑ) was calculated using the formula \( t_{½ₑ} = \frac{0.693}{kₚ} \). The area under the plasma concentration–time curve (AUC₀→∞) to the last measurable plasma concentration (Cₑ) was calculated by the linear trapezoidal rule. AUC₀→∞ was obtained from \( AUC_{0→t} + t × Cₚ / kₚ \), where Cₑ was the last measurable drug concentration. Total plasma clearance (CLₑ) was calculated as Dose/AUC₀→∞.

Statistical Analysis The data are presented as mean with standard error (S.E.) values for the individual groups. An unpaired Student’s t-test was used to determine any significant differences. Differences were considered to be significant at level of p<0.05.

RESULTS The mean plasma concentration–time profiles of TSIIA in the groups after intravenous administration of tanshinones extract-loaded emulsion and mixed extracts-loaded emulsion are shown in Fig. 3. The mean pharmacokinetic parameters of TSIIA are listed in Table 2. Significant differences in the total plasma clearance (CLₑ) and C₀(0.083) were found between the tanshinones extract-loaded emulsion groups and the mixed extracts-loaded emulsion groups for each of the dose levels (p<0.01). The total plasma clearance (CLₑ) was signifi-
significantly lower in the mixed extracts-loaded emulsion groups \((p<0.01)\), while \(C_{0.083h}\) was significantly higher in the mixed extracts-loaded emulsion groups \((p<0.01)\). As far as the \(\text{AUC}\) was concerned, the \(\text{AUC}\) of TSIIA in the medium and low dose group III was approximately 2 times \((p<0.05)\) higher than in the medium and low dose groups I, however, the \(\text{AUC}\) of the high dose group III was found to be \(3595.9\pm579.9 \text{ mg}^{-1} \text{l}^{-1} \text{h}^{-1}\), which was significantly higher (about 14 times) than that of the matched group \((p<0.01)\).

With regard to elimination, the terminal elimination half-life \((t_{1/2})\) of the mixed extracts-loaded emulsion group was increased compared with that of the equivalent tanshinones extract-loaded emulsion group. Although the terminal elimination half-life \((t_{1/2})\) increased by 38% in the low dose group and by 46% in the medium dose group, the difference was not statistically significant \((p>0.05)\). However, there was a significant difference between the two high dose groups (increase of 120%, \(p<0.05)\).

Sal B pharmacokinetics was estimated following intravenous administration of three dose levels of polyphenolic extract solution and mixed extracts-loaded emulsion, separately. The mean plasma Sal B concentration–time profiles are shown in Fig. 3, and the mean pharmacokinetic parameters are given in Table 3.

It was found that the pharmacokinetic parameters \((C_{0.083h}, CL \text{ and } AUC)\) in the mixed extracts-loaded emulsion groups were significantly different from the polyphenolic extract solution groups for each dose level \((p<0.01)\). A marked increase \((p<0.01)\) was observed in both the \(\text{AUC}\) and \(C_{0.083h}\) in rats given the mixed extracts-loaded emulsion compared with the rats receiving the polyphenolic extract solution alone. However, the total plasma clearance \((CL_t)\) of the mixed extracts-loaded emulsion group was significantly reduced compared with that of the polyphenolic extract solution group \((p<0.01)\). As for \(t_{1/2}\), there was no significant difference between corresponding dose levels.

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**Table 2. Mean Pharmacokinetic Parameters of TSIIA after Intravenous Administration of Tanshinones Extract-Loaded Emulsion and Mixed Extracts-Loaded Emulsion (Mean±S.E., n=6)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(AUC \text{ (} \mu \text{g} \text{l}^{-1} \text{h}^{-1})</th>
<th>(C_{0.083h} \text{ (} \mu \text{g} \text{l}^{-1})</th>
<th>(t_{1/2} \text{ (h)})</th>
<th>(CL_t \text{ (l h}^{-1} \text{kg}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>High dose group I</td>
<td>247.2±62.7</td>
<td>276.2±50.4</td>
<td>2.17±0.34</td>
<td>32.4±3.7</td>
</tr>
<tr>
<td>Medium dose group I</td>
<td>109.5±36.5</td>
<td>58.9±10.3</td>
<td>2.35±0.58</td>
<td>36.5±3.2</td>
</tr>
<tr>
<td>Low dose group I</td>
<td>75.2±28.7</td>
<td>42.3±12.4</td>
<td>2.27±0.47</td>
<td>26.6±2.5</td>
</tr>
<tr>
<td>High dose group III</td>
<td>3595.9±579.9**</td>
<td>6697.0±788.3**</td>
<td>4.79±1.29*</td>
<td>2.2±0.8**</td>
</tr>
<tr>
<td>Medium dose group III</td>
<td>250.6±73.7*</td>
<td>344.8±83.2**</td>
<td>3.43±0.94*</td>
<td>16.0±2.7**</td>
</tr>
<tr>
<td>Low dose group III</td>
<td>166.3±40.8*</td>
<td>237.6±72.0**</td>
<td>3.13±0.88</td>
<td>12.0±1.5**</td>
</tr>
</tbody>
</table>

\* \(p<0.05\); \*\* \(p<0.01\) compared with the corresponding dose level, \(AUC\): area under the plasma concentration–time curve from 0 h to infinity.

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![Fig. 3. The Mean Plasma Concentration–Time Profiles of Tanshinone IIA (a, c, e) and Sal B (b, d, f)](image-url)
Recent research indicated that TSIIA may possess and these were the three hydroxylated metabolites of II A and przewaquinone A were identified in the bile samples. Zhang et al. observed that the methylated metabolites were excreted into bile, probably through a transporter-dependent mechanism. In addition, all the methylated metabolites were characterized as meta-O-methylated products. COMT (catechol O-methyltransferase) seemed to be responsible for the metabolic transformation of Sal B.

As shown in Tables 2 and 3, i.v. co-administration of tanshinones and polyphenolic extracts increased the AUC and reduced the CL of TSIIA and Sal B, compared administration as single agents. Since both TSIIA and Sal B are mainly metabolized in liver and excreted into bile, we suggest that competitive distribution and metabolism might occur after i.v. co-administration of tanshinones extract and polyphenolic extract, resulting in a reduction in the clearance of TSIIA and Sal B. With the increase of dose, the competition appears to be dose-dependent. The AUC of TSIIA and Sal B in the low dose group III was 2.21- and 2.23-fold that found in the low dose group I and low dosed group II, separately. While, a 14.55- and 5.38-fold increase was observed in the high dose group III, respectively.

Cryptotanshinone is another active component of diterpenoid tanshinones. A previous study reported that cryptotanshinone could be metabolized to TSIIA in pigs and in vitro. TSIIA is a dehydrogenated metabolite of cryptotanshinone. Thus, The TSIIA profile may be affected by cryptotanshinone being metabolized to TSIIA. When mixed extracts-loaded emulsion was administrated to rats, not only was there competitive metabolism of TSIIA, but also much of the cryptotanshinone was metabolized to TSIIA. There is another possibility that Sal B altered the TSIIA profile by accelerating the TSIIA generating process from cryptotanshinone. All resulted in a rapid increase in the original plasma concentration of TSIIA over a short time, especially for the high dose group.

The pharmacokinetic interaction between tanshinones and polyphenolic extracts of S. miltiorrhiza Bunge in rats was investigated. It was found that an interaction between them occurred after intravenous administration in rats, which resulted in the reduced elimination of both TSIIA and Sal B. The mechanism of this interaction needs further study. A better understanding of the herb–drug interaction requires a great deal of work further in order to minimize or avoid therapeutic failures. And it may provide useful information to avoid unexpected increase of the plasma drug concentration in the clinical practice.

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REFERENCES