

**Plasma and Urinary Tanshinol from *Salvia
Miltiorrhiza* (Danshen), Can Be Used as
Pharmacokinetic Markers for Cardiotonic Pills, a
Cardiovascular Herbal Medicine**

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ABBREVIATIONS: PK marker, pharmacokinetic marker; TSL, tanshinol; PCA, protocatechuic aldehyde; SAA, salvianolic acid A; SAB, salvianolic acid B; SAD, salvianolic acid D; RMA, rosmarinic acid; LSA, lithospermic acid; C_{max} , maximum plasma concentration; T_{peak} , the time taken to achieve the peak concentration; $AUC_{0 \rightarrow t}$, area under concentration-time curve up to the last measured time point; $AUC_{0 \rightarrow \infty}$, $AUC_{0 \rightarrow t}$ extrapolated to infinity; k , the elimination rate constant; $T_{1/2}$, elimination half-life; MRT , mean residence time; $CL_{tot,p}$, total plasma clearance; V_{SS} , distribution volume at steady state; F , oral bioavailability; E_H , hepatic extraction; $Cum.A_e$, cumulative amount excreted during urine collection period; f_e , fraction of administered compound excreted unchanged in urine; CL_R , renal clearance; P_{app} , apparent permeability coefficient; P-gp, P-glycoprotein; MRP2, multidrug resistance-associated protein 2; f_u , unbound fraction in plasma; MW , molecular weight; $LogD$, distribution coefficient D; HBD , number of hydrogen bond donors; HBA , number of hydrogen bond acceptors; $NROTb$, number of rotatable bonds; $TPSA$, topological polar surface area; $LogS$, aqueous solubility at a given pH; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

ABSTRACT:

Cardiotonic pills are a type of cardiovascular herbal medicine. To identify suitable pharmacokinetic marker(s) for indicating systemic exposure to Cardiotonic pills, we examined the in vivo pharmacokinetic properties of putatively active phenolic acids from the component herb Danshen (*Radix Salviae Miltiorrhizae*). We also performed in vitro and in silico assessments of permeability and solubility. Several phenolic acids were investigated, including: tanshinol, protocatechuic aldehyde, salvianolic acids A, B, and D, rosmarinic acid, and lithospermic acid. Plasma tanshinol exhibited the appropriate pharmacokinetic properties in dogs, including dose-dependent systemic exposure in area under concentration-time curve (*AUC*) and a 0.5-h elimination half-life. In rats, over 60% of intravenous tanshinol was excreted intact into the urine. In humans, we found a significant correlation between the urinary recovery of tanshinol and its plasma *AUC*. The absorption rate and bioavailability of tanshinol were not significantly different whether Cardiotonic pills were given orally or sublingually. The gender-specificity in plasma *AUC* disappeared after body-weight normalization, but the renal excretion of tanshinol was significantly greater in women than in men. Protocatechuic aldehyde was predicted to be highly permeable according to in vitro and in silico studies; however, extensive presystemic hepatic elimination and degradation in the erythrocytes led to extremely low plasma levels and poor dose proportionality. Integrated in vivo, in vitro, and in silico studies on the other phenolic acids showed poor gut permeability and nearly undetectable levels in plasma and urine. In conclusion, plasma and urinary tanshinol are promising pharmacokinetic markers for Cardiotonic pills at the tested dose levels.

The use of herbal therapies is escalating worldwide. However, the basis for the therapeutic effects is often poorly interpreted, and the safety, dose, and potential herb-drug interactions require better estimation (De Smet, 2002; Fugh-Berman, 2000; Lazar, 2004). Unlike most synthetic drugs, herbal medicinal products usually contain numerous chemical constituents, especially traditional Chinese medicines that often use a combination of multiple herbs.

Determining which constituents of an herbal product have favorable drug-like properties will extend our knowledge of the basis for pharmacological efficacy and safety. An herbal constituent can be defined as drug-like when it possesses the desired potency, a wide safety margin, and appropriate pharmacokinetic (PK) properties and exists in adequate abundance in the herbal product. A deficit in these properties limits the usefulness of the herbal constituent for the herbal product. For a drug, the pharmacologic effect is attained when the drug or its active metabolite reaches and sustains an adequate concentration at an appropriate site of action; this hypothesis should also be applied to the herbal product. Both the dose levels and fates of active constituents in the body govern their target-site concentrations after administration of an herbal product. The relevant PK properties include ability of an herbal chemical to be absorbed from the site of administration and to pass through multiple biological barriers to reach the action target, sufficient metabolic stability to achieve therapeutically meaningful systemic and target-site concentrations, and appropriate metabolic lability to be eliminated effectively by the excretory processes.

Measuring systemic exposure to an herbal medicinal product is important for understanding the link between the product consumption and the medicinal effects. To implement this, the PK properties of the active constituents and major chemical constituents

should be evaluated. Active constituents that possess favorable PK properties, including a significant dose-dependent systemic exposure and an appropriate elimination half-life, qualify as pharmacokinetic markers (PK markers) for the herbal product. When the active constituent is unknown, or a suitable assay is not feasible, the major chemical constituents or the main metabolites detected in plasma or urine may be evaluated as surrogate PK markers. PK markers may be used to demonstrate systemic exposure to the herbal product in animals and/or humans. For a multi-herb product, identification of PK markers derived from each component herb is important for evaluating the combination rationality and for investigating possible synergistic interactions between the component herbs. Such studies are also relevant to designing rational dosage regimens, evaluating potential herb-drug or herb-herb interactions, and developing new formulations.

Cardiotonic pills (Fufang Danshen Diwan) are an herbal medicine recognized in the official Chinese Pharmacopoeia (The Pharmacopoeia Commission of People's Republic of China, 2005); they contain three component herbs, i.e., *Radix Salviae Miltiorrhizae* (Danshen or red root sage), *Radix Notoginseng* (Sanqi or sanchi ginseng), and *Borneolum* (Bingpian or borneol) and they are indicated for angina pectoris in coronary heart disease. The pills are prepared by combining concentrated aqueous extracts of Danshen and Sanqi and pulverized Bingpian, mixing well with heated polyethylene glycols, and dripping the mixture into cool liquid paraffin to yield pills. In Cardiotonic pills, the main constituents of Danshen are phenolic acids, including tanshinol, protocatechuic aldehyde, salvianolic acids A, B, and D, rosmarinic acid, and lithospermic acid; the constituents of Sanqi are triterpene saponins, including ginsenosides Rb₁, Rd, Rg₁, and notoginsenoside R₁; and the constituent of Bingpian is borneol (Fan et al.,

2006; Liu et al., 2006). As specified in the Chinese Pharmacopoeia, each pill (~25 mg) should contain ≥ 0.1 mg of tanshinol. Cardiogenic pills are generally well tolerated and have rare side effects, usually mild stomach discomfort and temporary dizziness. The annual sales volume of cardiogenic pills has exceeded ¥1 billion Chinese (~ \$140 million US) since 2002. This herbal medicine is also available as a prescription or an over-the-counter drug in countries including Singapore, Republic of Korea, India, the United Arab Emirates, Russia, Cuba, and South Africa, and as a dietary supplement in the United States.

To help better understanding the role of Cardiogenic pills in coronary heart disease, we have implemented relevant PK studies. The current study focused on estimating the PK properties of the main phenolic acids from Danshen. The phenols received our attention because they have properties known to benefit heart function, including antioxidation, anti-blood coagulation, coronary artery dilatation, and cell protection (Wang et al., 2007). Notably, we identified plasma and urinary tanshinol as PK markers for this herbal medicine.

Materials and Methods

Chemicals and Materials. Purified tanshinol (TSL), protocatechuic aldehyde (PCA), rosmarinic acid (RMA), and lithospermic acid (LSA) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Purified salvianolic acids A (SAA), B (SAB), and D (SAD) were obtained from the Phytochemistry Department of the Shanghai Institute of Materia Medica (Shanghai, China). The purity of these compounds was $\geq 99.0\%$ and their chemical structures are illustrated in Fig. 1.

(Insert Figure 1 here)

Cardiotonic pills comprised *Radix Salviae Miltiorrhizae*, *Radix Notoginseng*, and *Borneolum* (lot no. 20040710, expiration: 07/2008; Tianjin Tasly Pharmaceutical Co. Ltd., Tianjin, China). Each pill (24.7 ± 1.1 mg) contained the following amounts of danshen phenolic acids (μg): 160 TSL, 61.3 PCA, 75.1 SAA, 76.4 SAB, 87.5 SAD, 35.7 RMA, and 14.8 LSA, as measured by LC-MS/MS. An injectable Cardiotonic solution was prepared by diluting an intermediate fluid extract of the Cardiotonic pill (Tasly Pharmaceutical) in a 20-fold volume of physiological saline and then passing it through a 0.22- μm nylon filter. The prepared Cardiotonic solution contained the following amounts of danshen phenolic acids (mg/ml): 1.50 TSL, 0.419 PCA, 0.393 SAA, 1.17 SAB, 1.07 SAD, 0.333 RMA, and 0.237 LSA. In addition, a separate portion of purified TSL or PCA was dissolved in physiological saline and then filtered (0.22- μm) to prepare an injectable solution of 2 mg/ml.

Experimental Animals. Animal studies were conducted according to protocols approved by the Review Committee of Animal Care and Use at the Shanghai Institute of Materia Medica.

Male beagle dogs (7.8–9.0 kg) were purchased from the Teaching and Research Farm of Shanghai Jiao Tong University (Shanghai, China) and were individually housed in stainless steel cages (1.7 m × 1.0 m × 0.9 m). Male Sprague-Dawley rats (300–350 g) were obtained from Shanghai Laboratory Animals Co. (Shanghai, China) and were housed in rat cages (48 cm × 29 cm × 18 cm; 3 rats/cage). The animals were maintained on controlled temperature (20–24°C), relative humidity (40–70%) and a 12-h cycle of light and dark. The animals were given commercial diets, except for an overnight fasting period before dosing, and filtered tap water *ad libitum*. The rats were acclimated to the facilities for 1 week before use and the dogs for 2 weeks.

Study in Beagle Dogs. The dogs were randomly assigned to three treatment groups (3 dogs/group) to receive a single oral administration of Cardiotonic pills at a dose of 2, 4, or 8 pills/kg. The doses were delivered in gelatin capsules and the animal doses were derived according to the daily dose commonly given clinically in humans. After a 7-day washout period, the dogs of the intermediate-dose group received a single intravenous bolus of the Cardiotonic solution at 0.50 ml/kg (in the left forelimb vein). Then, after another 7-day washout period, the TSL solution was given to the same group in a single intravenous bolus at 0.32 ml/kg. Serial blood samples (~250 µl collected in heparinized tubes) were taken from the right forelimb vein at 0, 5, 10, 20, 30 min, and 1, 2, 4, and 6 h. The collected blood samples were placed on ice. After centrifugation at × 6000 g for 2 min, plasma fractions were decanted and then frozen at –70°C until analyzed. For dosing and blood sampling, the dogs were temporarily restrained in slings.

Studies in Rats. Three rat studies were performed as a supplement to the dog and human studies. In the first study, 4 rats were housed in Nalgene metabolic cages (1 rat/cage) with urine collection tubes frozen to -15°C for sample stability. After an intravenous bolus dose of the TSL solution (2 mg/kg), urine samples were collected over 24 h. All urine samples were weighed before storage at -70°C .

In another study, 6 rats were randomly assigned to two groups. The animals were anesthetized with an intraperitoneal injection of pentobarbital at 50 mg/kg and maintained under anesthesia throughout the blood sampling period. In each group, either the portal vein or the right femoral vein was cannulated for a 15 min intraportal or intravenous infusion of the TSL solution (0.6 mg/rat; 20 $\mu\text{l}/\text{min}$) using a PHD 2000 microdialysis infusion pump (Harvard Apparatus, Holliston, MA). Serial blood samples (~ 120 μl collected in heparinized tubes) were taken at 0, 5, 15, 20, 30, 45 min, and 1, 2, and 4 h from a carotid artery catheter after initiation of infusion. Plasma samples were prepared as described above. Similar study was also performed for PCA.

In the third study, rats under ether anesthesia were given a single intravenous dose of the TSL solution (2 mg/kg) and then sacrificed by bleeding at the abdominal aorta at 0, 15, 30 min, and 1 and 2 h after dosing. Three animals were used for each sampling time. Selected tissues (the heart, brain, liver, and kidney) were excised, rinsed in ice-cold saline, blotted, and weighed. The tissues were homogenized in 4 volumes of ice-cold saline and homogenate samples were stored at -70°C until analysis.

Study in Human Subjects. Six male and 6 female healthy volunteers were enrolled in this study after providing written informed consent. The study was approved by the Ethics

Committee of Clinical Investigation at Tianjin University of Traditional Chinese Medicine (Tianjin, China). Subjects were between 21 and 26 years old and within 15% of ideal body weight. The volunteers were determined healthy with regard to medical history, physical examination, electrocardiogram, and routine clinical laboratory tests. The female volunteers were negative for menstruation and pregnancy. Subjects who had a significant history of drug or food allergy or intolerance to Cardiotonic pills were excluded. Any over-the-counter or prescription drugs were prohibited two weeks prior to and through the end of the study period. In addition, any alcohol-, caffeine-, tea-, or citrus-containing beverages or foods were prohibited 2 days prior to and through the end of each study period.

A single-dose, randomized, two-way crossover study was designed to compare the pharmacokinetics of TSL, particularly the absorption profiles, after oral or sublingual administration of Cardiotonic pills. Subjects were randomized to use 30 Cardiotonic pills by either swallowing whole pills with 100 ml of water (oral route) or placing the pills underneath the tongue without swallowing for 15 min (sublingual route). After a 10-day washout period, the subjects received Cardiotonic pills by the alternate route. All subjects were in a fasted state from midnight of the previous evening. Food consumption was not allowed until 4 h after dosing and strenuous physical activity was not allowed during the study. Serial blood samples (~1 ml; collected in heparinized tubes) were taken from the antecubital vein catheter at 0, 5, 10, 15, 20, 30 min, and 1, 2, 4, and 6 h after initiating drug administration. The collected blood samples were placed on ice and then centrifuged to obtain plasma samples, as describe above. Additionally, serial urine samples were collected in ascorbic acid-treated bottles pre-dose and

at 0–1, 1–3, 3–6, 6–8, 9–12, and 12–24 h post-dose. All urine samples were weighed before storing.

Pharmacokinetic Data Analysis. Plasma PK parameters were estimated by a noncompartmental method using the Kinetica™ 2000 software package (version 3.0; InnaPhase Corp., Philadelphia, PA). The C_{\max} and the T_{peak} were observed values with no interpolation. The $AUC_{0 \rightarrow t}$ was calculated by the trapezoidal rule. The $AUC_{0 \rightarrow \infty}$ was generated by extrapolating the $AUC_{0 \rightarrow t}$ to infinity using the k and the last measured concentration. The $T_{1/2}$ was calculated using the relationship $0.693/k$. The $CL_{\text{tot,p}}$ for intravenous dosing or the $CL_{\text{tot,p}}/F$ for oral dosing was estimated by dividing the administered dose by the $AUC_{0 \rightarrow \infty}$. The V_{ss} for intravenous dosing or V_{ss}/F for oral dosing was estimated by multiplying the $CL_{\text{tot,p}}$ or $CL_{\text{tot,p}}/F$, respectively, by the MRT. The F of TSL was calculated according to the following equation:

$$F = [(AUC_{\text{oral}} \times \text{Dose}_{\text{intravenous}}) / (AUC_{\text{intravenous}} \times \text{Dose}_{\text{oral}})] \times 100\% \quad (1)$$

Where the AUC_{oral} was the plasma $AUC_{0 \rightarrow t}$ value of TSL measured after an oral dose of Cardiotonic pills was given to a dog, and the $AUC_{\text{intravenous}}$ was such a value measured after a bolus intravenous dose of the TSL solution was delivered to the same animal. The E_{H} was calculated by comparing the AUC values obtained after intravenous and intraportal infusions according to the following equation:

$$E_{\text{H}} = [1 - (AUC_{\text{intraportal}}) / (AUC_{\text{intravenous}})] \times 100\% \quad (2)$$

where the $AUC_{\text{intraportal}}$ or the $AUC_{\text{intravenous}}$ was the mean of 3 rat measurements after intraportal or intravenous infusion, respectively.

Dose proportionality studies on the $AUC_{0 \rightarrow \infty}$ and the C_{\max} were conducted by regression of log-transformed data (power model; Smith et al., 2000). The correlation coefficient (r^2), slope, and 90% confidence intervals for slope were calculated and inferences were made based on the theoretical slope of 1 and the confidence limits of 0.84 to 1.16.

The urinary PK parameters included $Cum.A_e$ calculated by numeric integration of the amount excreted per collection interval. The f_e was established by $Cum.A_e / \text{dose}$ and the CL_R was calculated as $Cum.A_e / AUC$.

The exposure profiles of TSL in human subjects after administration of Cardiotionic pills were compared for normal oral and sublingual routes in terms of the mean C_{\max} , T_{peak} , $AUC_{0 \rightarrow t}$, and $Cum.A_e$ values. All the results are expressed as the arithmetic mean \pm standard deviation (SD). A 2-tailed Student's t -test or a Wilcoxon rank sum test was performed, where appropriate, to compare the PK parameter estimates of the two administration routes. The Pearson correlation test was used to study the association between plasma TSL $AUC_{0 \rightarrow t}$ and relative urinary excretion (CL_R) values. A p value < 0.05 was considered statistically significant.

Stability in Canine Erythrocytes and Plasma. To assess the stability of PCA in erythrocytes and plasma, heparinized whole blood (5 ml; freshly collected from dogs and maintained at 37°C) was spiked with PCA to yield an initial blood concentration of 150 ng/ml. The spiked blood sample was incubated at 37°C and was sampled in 500 μ l aliquots at 5, 10, 30, and 60 min. After sampling, the blood samples were immediately centrifuged at 6°C to separate the plasma and the blood cells for analysis. After removing leukocytes and residual plasma, the erythrocytes were lysed by sonication on ice. The concentrations of PCA were

measured both in the lysed erythrocytes and in the plasma to determine the in vitro degradation $T_{1/2}$ of the compound. As a control, the metabolic stability of PCA was also measured after PCA was spiked at 150 ng/ml into plasma alone. In addition, the blood/plasma ratio was determined for all the tested danshen phenolic acids from the ratio of the spiked whole blood concentration to the measured plasma concentration.

Permeability Study in Caco-2 Monolayers. Caco-2 cells (passage number 34–36; American Type Culture Collection, Manassas, VA) were cultured at 37°C in an atmosphere of 5% CO₂ and 90% relative humidity in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% MEM nonessential amino acids. After harvesting at 90% confluence, the cells were seeded onto 0.4- μ m Millicell[®]-PCF filter inserts (12-mm diameter; Cork, Ireland) at a density of 1×10^5 cells/cm². The culture media were changed the day after seeding and every other day thereafter. The integrity of the Caco-2 monolayers on 21 days postseeding was monitored by measuring the transepithelial electrical resistance and only monolayers with values $\geq 400 \Omega \cdot \text{cm}^2$ were used.

The monolayers were washed three times with pre-warmed HBSS (37°C;) before use. Bidirectional transport experiments were conducted in triplicate for each danshen phenolic acid. The compounds were individually added to the donor compartment at a concentration of 50 μ M in HBSS. After 1 h of incubation, samples were collected from both the receiver compartment and the donor compartments and the recovery of the tested compound was determined. The monolayer permeability and the expression of ATP-dependent drug transporters, P-gp and MRP2, were checked as described previously with commonly used

probe compounds and specific inhibitors (Hubatsch et al. 2007). The P_{app} expressed in cm/s was calculated according to the following equation:

$$P_{app} = (\Delta Q / \Delta t) / (A \times C_0) \quad (3)$$

where the $\Delta Q/\Delta t$ is the linear appearance rate of the tested compounds on the receiver side, the A is the surface area of the cell monolayer, and the C_0 is the initial concentration of the tested compound on the donor side.

Plasma Protein Binding. A rapid ultrafiltration method (13362 × g, 3 min, 37°C; Guo et al., 2006) was used to assess the f_u of TSL in rat or human plasma. TSL was tested at concentrations of 12.3, 36.9, 111, and 333 ng/ml. Nonspecific binding of TSL to the ultrafiltration membrane was negligible.

In Silico Assessment of permeability and solubility. Chemoinformatic approaches were also used to assess the absorption properties of the tested danshen phenolic acids. The $LogS$ and the $LogD$ at a given pH were calculated with ACD/aqueous solubility v8.02 and ACD/LogD v8.02 software, respectively, via the ACD/I-Lab service (Advanced Chemistry Development Inc., Toronto, Canada). The $TPSA$, the HBD , the HBA , and the $NROTB$ were determined using the Molinspiration Property Calculator (Molinspiration Cheminformatics, Bratislava, Slovak Republic). Lipinski's Rule of five (Lipinski et al., 1997) was used as a guide to predict the compound permeability. Compound permeability was also assessed with regard to molecular-surface properties ($TPSA$ and $NROTB$). The aqueous solubility S was expressed in M.

LC-MS/MS analysis. A validated bioanalytical method was used on the basis of LC-MS/MS, which was capable to measure simultaneously TSL, PCA, SAA, SAB, SAD,

RMA, and LSA. The sample clean-up involved ethyl acetate-based extraction of hydrochloride acid-treated biological samples (100 μ l; including those of plasma, plasma water, erythrocyte homogenate, urine, or cell culture media).

The LC–MS/MS system consisted of an ACQUITY UPLC separation module (Waters, Milford, MA) and a TSQ Quantum triple stage quadrupole mass spectrometer (Thermo Fisher, San Jose, CA) with an electrospray ionization interface. The chromatographic separation was achieved on an Agilent Eclipse Plus 5 μ m C₁₈ column (50 mm \times 2.1 mm i.d.; Chadds, PA). The mobile phases (delivered at 0.4 ml/min) consisted of water (containing 25 mM formic acid) for *A* and acetonitrile/isopropanol (80:20, v/v, containing 25 mM formic acid) for *B*. A binary escalation gradient elution was performed, which consisted of five isocratic segments, i.e., 0–2.5 min at 1% *B*, 2.5–10min at 10% *B*, 10–10.5 min at 50% *B*, 10.5–12 min at 80% *B*, and 12–14 min at 1% *B*. Following each isocratic segment, *B* immediately changed to the next condition. The precursor-to-product ion pairs used for selected reaction monitoring in the negative-ion electrospray ionization mode of TSL, PCA, SAA, SAB, SAD, RMA, and LSA, were m/z 197 \rightarrow 135, 137 \rightarrow 108, 493 \rightarrow 185, 717 \rightarrow 519, 417 \rightarrow 197, 359 \rightarrow 161, and 537 \rightarrow 295, respectively. The linear dynamic ranges for determination of the tested compounds were 8–2000 ng/ml, except for SAA and SAB (16–2000 ng/ml). Assay validation was conducted to demonstrate that the performance characteristics of the newly developed method were suitable and reliable for the intended applications.

Results

Absorption and Pharmacokinetics of Danshen Phenolic Acids in Dogs. PK data were calculated for the danshen phenolic acids after an intravenous bolus of the Cardiotonic solution was given to dogs (Table 1). Plasma levels of the danshen phenolic acids declined rapidly after dosing, indicated by the short $T_{1/2}$ observed between 0.31 and 1.08 h. The $CL_{\text{tot,p}}$ values of TSL, SAA, SAB, SAD, RMA, and LSA ranged from 0.48 to 1.22 l/h·kg, which fluctuated around the average canine hepatic and renal plasma flow rates (0.93 and 0.65 l/h·kg, respectively; Davies and Morris, 1993). The distribution of these danshen phenolic acids within the blood were basically limited to the plasma, indicated by the blood/plasma concentration ratios between 0.50 to 0.68. The mean V_{SS} values for TSL, SAB, SAD, RMA, and LSA ranged from 0.22 to 0.33 l/kg; values less than canine total body water (0.60 l/kg) suggested these compounds were predominantly restricted to the extracellular fluid. However, SAA had a V_{SS} of 0.93 l/kg, indicative of a relatively greater tissue distribution. PCA was exceptional because it was detected at much lower plasma levels (70.7–88.4 ng/ml at 5 min), had a much faster CL (3.83 l/h·kg), and had a larger V_{SS} (4.64 l/kg) than the other danshen phenolic acids.

(Insert Table 1 Here)

In contrast to the results from the intravenous administration, after oral administration of Cardiotonic pills to dogs, only TSL was detected in plasma at dose levels of 2, 4, and 8 pills/kg. Although the PCA content (0.49 mg) in 8 pills was comparable to that of TSL (1.28 mg), the plasma concentrations of PCA were very low, with a mean C_{max} of 18.7 ng/ml for PCA compared to 1034 ng/ml for TSL. The other compounds, including SAA, SAB, SAD, RMA,

and LSA, in canine plasma were below the quantification limits (16 ng/ml for SAA or SAB or 8 ng/ml for the other compounds) of our bioanalytical methods.

Plasma TSL concentration versus time curves after oral administration Cardiogenic pills at different dose levels (2–8 pill/kg, each pill contained 0.16 mg of TSL) are shown in Fig. 2 and the PK parameters are summarized in Table 2. TSL reached its C_{\max} within 0.5 h after oral administration, indicating that this phenolic acid was rapidly absorbed. The TSL $T_{1/2}$ was 0.40–0.55 h, which was comparable to those observed after intravenous administrations of the injectable TSL solution (0.44 h) and the injectable Cardiogenic solution (0.51 h). Log-transformed plots of $AUC_{0 \rightarrow \infty}$ and C_{\max} versus the tested dose of Cardiogenic pills were analyzed with power regression (Fig. 2). The results indicated that $AUC_{0 \rightarrow \infty}$ increased linearly over the Cardiogenic pill dose range with a correlation coefficient (R^2) of 0.975, a slope of 1.008, and a 90% confidence interval of 0.892–1.123 (falling completely in the critical range of 0.839–1.169). Whereas, the C_{\max} of TSL was directly related to dose, but nonlinearly. The mean systemic bioavailability of TSL after oral administration of Cardiogenic pills was around 40%.

(Insert Figure 2 and Table 2 Here)

When TSL was given intravenously to dogs in the Cardiogenic solution (Table 1), it displayed PK behavior similar to that observed when it was given intravenously as pure TSL (Table 2); this suggested that the other herbal constituents and metabolites did not significantly affect TSL concentrations. Collectively, plasma TSL of Danshen origin was a valid PK marker for Cardiogenic pills after oral administration to dogs at doses of 2–8 pills/kg.

Eliminations and Tissue Distribution of TSL in Rats. More than 60% of intravenously administered TSL was excreted in an intact form into rat urine, suggesting that renal excretion provided a major elimination route for the compound. The CL_R value exceeded the product of the rat glomerular filtration rate (0.31 l/h/kg) and f_u (75%) by ~6.0-fold, indicating that active tubular secretion of TSL occurred in rats. In addition, after intraportal or intravenous infusion of the TSL solution to rats, a comparison of $AUCs$ was used to determine the extent of presystemic hepatic elimination. The results showed that the mean E_h in rats was only about 5%, indicating that TSL underwent minimal presystemic hepatic elimination.

A rapid tissue distribution was observed, indicated by the highest concentrations of TSL measured at 5 min after intravenous administration. Tissue and plasma concentrations at 5 min postdose were ranked as follows: kidney (29755 ng/g) > plasma (2630 ng/ml) > heart (325 ng/g) > liver (165 ng/g) > brain (38 ng/g). The concentrations of TSL in the studied tissues declined rapidly at rates similar to those observed in plasma. At every sampling time, the maximum concentrations were measured in the kidney, a major eliminating tissue for TSL. Moreover, the $T_{1/2}$ in the kidney was almost the same as that in plasma (both around 0.27 h), while the data for the other tissues were about 0.20 h. Thus, plasma TSL decline reflected the compound decline in the body; this is an advantageous property for human PK studies.

Comparative Pharmacokinetics of TSL after Oral or Sublingual Administration of Cardiotonic Pills to Human Subjects. All 12 human subjects completed the entire protocol. After administration of Cardiotonic pills via either oral (swallowing) or sublingual routes, TSL was the only danshen phenolic acid detected in plasma and urine samples. Plasma and urinary PK parameters of TSL are summarized in Table 3 and the plasma concentration-time curves

are illustrated in Fig. 3. Comparisons were made between the two administration routes, and between women and men.

(Insert Table 3 and Figure 3 Here)

TSL was not measured in the first 0.2 h following the oral administration of Cardiotonic pills; then TSL was absorbed rapidly, reaching peak levels at approximately 1.3 h after dosing. In women, the plasma C_{\max} values of TSL ranged from 19.3 to 49.8 ng/ml, and was significantly higher than that observed in men (range 14.6 to 27.1 ng/ml; $p < 0.05$). In addition, the mean $AUC_{0 \rightarrow 6h}$ in women was 72.8 h·ng/ml, also significantly greater than that observed in men (43.3 h·ng/ml; $p < 0.05$). However, when the dose was corrected for body weight, the gender difference was insignificant in these data. In addition, no significant gender differences were observed in the dose-independent plasma PK parameters, $T_{1/2}$, MRT , $CL_{\text{tot,p}}/F$, and V_{SS}/F . As to urine PK data, the renal excretion of intact TSL during the 12-h period following dosing was significantly greater in women than in men with respect to $Cum.A_e$ and f_e ($p < 0.05$). However, mean CL_R did not exhibit gender difference, indicative of 0.50 ± 0.13 l/h·kg in women and 0.45 ± 0.03 l/h·kg in men, which accounted for 23 to 46% of the total systemic $CL_{\text{tot,p}}/F$. As was found in rats, TSL might also be subject to renal secretion in humans; CL_R values for TSL were greater than the product of the glomerular filtration rate (0.11 l/h/kg) and f_u (85%) by about 5-fold.

As shown in Table 3, the two different dosing regimens of Cardiotonic pills given at the same dosage resulted in comparably high and robust plasma concentrations of TSL. In both women and men no significant differences were observed between sublingual and oral administration routes in the PK data, including: $AUC_{0 \rightarrow 6h}$, T_{peak} , C_{\max} , $T_{1/2}$, MRT , $CL_{\text{tot,p}}/F$,

V_{SS}/F , $Cum.A_e$, f_e , and CL_R . Due to the small number of human subjects, there was insufficient power for determining bioequivalence of the two dosing regimens.

Irrespective of the administration routes, the urinary $Cum.A_{e0 \rightarrow 12h}$ plotted as function of the plasma $AUC_{0 \rightarrow 6h}$ (Fig. 3) revealed a significant correlation (Pearson correlation coefficient = 0.86, $p < 0.001$, $n = 24$). A similar correlation was found between an abbreviated $Cum.A_{e0 \rightarrow 3h}$ and $AUC_{0 \rightarrow 6h}$, with a Pearson correlation coefficient = 0.74 and $p < 0.01$ ($n = 24$).

Intake and Elimination of PCA by Canine Erythrocytes and Its Presystemic Hepatic Elimination in Rats. Due to our findings that the $CL_{tot,p}$ of PCA exceeded the canine hepatic plasma flow rate (1.09 l/h/kg) by 4.3-fold and that only about 1% of intravenously administered PCA was excreted intactly into rat urine, we examined the distribution and stability of the compound in canine erythrocytes and plasma. As shown in Fig. 4, 5 min after PCA was added to heparinized whole blood (150 ng/ml; 37°C), the compound was extensively distributed into and decayed in erythrocytes, resulting in reduced plasma concentrations (101 ng/ml in plasma and 68 ng/ml in erythrocytes) compared with the theoretical initial plasma concentration of 259 ng/ml and 0 ng/ml erythrocyte. Thereafter, the concentrations of PCA in plasma and in erythrocytes decreased, indicated by the short $T_{1/2}$ values of 0.29 h in plasma and 0.17 h in erythrocytes. However, when PCA was spiked into plasma, it was stable without any obvious decline in the measured concentration. These results, together with the high membrane permeability of PCA (shown below in the Caco-2 data), suggested that the low plasma levels and the rapid $CL_{tot,p}$ were due, at least in part, to extensive uptake and degradation by erythrocytes. In addition, we also found that PCA underwent substantial presystemic hepatic elimination in rats, demonstrating the mean E_h about 60%.

(Insert Figure 4 Here)

Permeability and Solubility of the Danshen Phenolic Acids Assessed in Vitro and In Silico. Table 4 summarizes the P_{app} values measured in Caco-2 cell monolayers for the danshen phenolic acids. These data were compared with those of reference compounds, including antipyrine, atenolol, caffeine, doxorubicin, norfloxacin, ranitidine, reserpine, and testosterone. A sigmoid correlation was obtained between the percent dose absorbed after oral administration in humans and the corresponding log of the P_{app} value measured in Caco-2 cell monolayers (data not shown). Based on this finding, a compound might be absorbed poorly, moderately, or well with a P_{app} value of $< 1 \times 10^{-6}$, $1-10 \times 10^{-6}$, or $> 10 \times 10^{-6}$ cm/s, respectively. Accordingly, the measured P_{app} values indicated that the membrane permeability of TSL and that of PCA were moderate and good, respectively, but the other danshen phenolic acids were poorly transported. The Caco-2 cells had high expression levels of the multidrug transporters, P-gp and MRP2. Comparable bidirectional P_{app} values, which were not affected by the initial concentration on donor side (C_0 ; data not shown), suggested a passive diffusion mechanism for TSL and PCA across the Caco-2 monolayer. The ratios of the P_{app} values for basolateral→apical and apical→basolateral were ~2.5 for SAD; this was probably caused by the extremely low transport and the low concentrations measured may have affected the accuracy. Metabolism of the compounds was not detected in the study.

(Insert Table 4 Here)

The preceding Caco-2 data suggested that the gastrointestinal absorption of the danshen phenolic acids was characterized by passive diffusion. Accordingly, we performed a chemoinformatic assessment of physicochemical properties to further understand the

mechanisms governing intestinal absorption. Table 5 summarizes the calculated molecular and structural descriptors of the danshen phenolic acids. The tested compounds could be defined as highly soluble, indicated by the calculated aqueous solubility values at pH 5.0 (6–3789 mM) or pH 7.0 (102–5051 mM). The solubility values were greater than the initial concentrations (C_0) in the Caco-2 study (0.05 mM) and the compound-specific concentrations calculated for human oral dosing (0.008–0.242 mM; Varma et al., 2004) or for sublingual dosing (0.412–12.1 mM). TSL had favorable properties for supporting acceptable permeability, including: molecular weight (MW < 500 Da), hydrogen-bonding capacity (HBA + HBD < 12, TPSA < 140 Å²), and molecular flexibility (NROTBS < 10) (Veber et al., 2002). The lipophilicity of TSL at pH 7.0 was poor compared with the value at pH 5.0, suggesting that the duodenum might be a favorable absorption site for the compound. PCA showed the best permeability potential among the tested danshen phenolic acids with regard to the molecular weight, hydrogen-bonding capacity, lipophilicity, and molecular flexibility. SAA, SAB, SAD, RMA, and LSA possessed unfavorable traits including total hydrogen bond counts ranging from 13 to 25, TPSA values ranging from 145 to 278 Å², and LogD ranging from -3.8 to -0.5 (except for SAA); these traits underlie their poor permeability. In addition, SAB had the unfavorable traits of high molecular weight (MW = 718 Da) and molecular flexibility (NROTBS = 14).

(Insert Table 5 Here)

Discussion

In the current study we evaluated the PK properties of the phenolic acids from Danshen after oral administration of Cardiotonic pills. For comparison, the injectable Cardiotonic solution, as well as pure TSL, was administered intravenously. We found that TSL was detected in canine plasma, the total exposure increased linearly over the dose range 2 to 8 pills/kg, and the peak exposure was directly related to the dose. TSL was absorbed rapidly from the gastrointestinal tract ($T_{\max} = 0.5$ h) and had an acceptable bioavailability (40%). A short plasma half-life ($T_{1/2} = 0.5$ h) indicated that TSL was rapidly eliminated from the body. The elimination rate of plasma TSL was comparable to that in different rat tissues, and its concentration in the heart was about one tenth of the plasma concentration. Based on these PK properties, we conclude that plasma TSL from Danshen is a suitable PK marker for Cardiotonic pills administered orally at clinical doses in both animals and humans.

There was good correlation in humans between the urinary recovery ($Cum.A_{e0 \rightarrow 12h}$) of TSL and its plasma $AUC_{0 \rightarrow 6h}$, independent of the administration route chosen for Cardiotonic pills. Our rat studies indicated that a substantial fraction (>60%) of intravenously administered TSL was eliminated by renal excretion. We also found that TSL concentrations were quite high in the rat kidney compared to plasma or other tissues. Accordingly, we conclude that urinary TSL can also be a reliable surrogate PK marker in humans for Cardiotonic pills. Of clinical importance, we found that the correlation between the plasma $AUC_{0 \rightarrow 6h}$ and $Cum.A_{e0 \rightarrow 3h}$ was comparable to that using $Cum.A_{e0 \rightarrow 12h}$; the mean $Cum.A_{e0 \rightarrow 3h}$ accounted for approximately 73% of mean $Cum.A_{e0 \rightarrow 12h}$. Compared to plasma TSL, urinary TSL possesses some merits as a PK marker in humans because it is noninvasive, less

expensive, and straightforward to measure.

Several presystemic processes can affect the bioavailability of herbal chemicals. These include the solubility in the gastrointestinal fluid, membrane permeability, degradation in the gastrointestinal tract, transporter-mediated intestinal efflux, presystemic gut wall metabolism, and presystemic hepatic metabolism. In addition to TSL, we tested several other phenolic acids (PCA, SAA, SAB, SAD, RMA, and LSA) in the Cardiotonic pills. However, these danshen phenolic acids were only slightly or not detectable in canine or human plasma after oral administration of Cardiotonic pills at the tested dose levels.

The Caco-2 and chemoinformatic data suggested that PCA possesses better membrane permeability than TSL and good aqueous solubility. However, plasma and urine levels of PCA were much lower than those of TSL after oral administration of Cardiotonic pills, despite the comparable amounts of PCA and TSL present in the administered herbal medicine. The systemic exposure to PCA showed poor dose proportionality. We found that PCA was subject to extensive presystemic hepatic elimination and degradation in the erythrocytes. Purified PCA can be biotransformed *in vivo* into protocatechuic acid (Xu et al., 2007a); aldehyde oxidase, xanthine oxidase, and aldehyde dehydrogenase may mediate the metabolism (Panoutsopoulos and Beedham 2005). In the current study, we found the concentration of protocatechuic acid in plasma and urine was in the low nanogram per milliliter range after administration of Cardiotonic pills. Notably, we found that PCA was subject to extensive presystemic hepatic elimination and degradation in the erythrocytes. Collectively, plasma PCA was not considered an adequate PK marker for Cardiotonic pills at the tested dose level due to its poor PK properties.

Unfavorable hydrogen bond capacities of SAA, SAB, SAD, and LSA were associated with poor membrane permeability, which could result in the low bioavailability from the gastrointestinal tract. This might explain our limited detection of these Danshen phenolic acids in plasma and urine of animals or humans after oral administration. Our results are consistent with the PK studies by Zhang et al. (2004), who revealed that the oral bioavailability of SAB was extremely low (0.02%), and Wang et al. (2008), who reported that the oral bioavailability of LSA was only 1.15%. Although we found that RMA showed potentially better membrane permeability than SAA, SAB, SAD, and LSA, this compound was also poorly detected in plasma and urine at the tested dose levels of Cardiotonic pills. This result is also consistent with several studies that suggested intact RMA was poorly absorbed in the small intestine (Baba et al., 2004), and the majority of the compound was degraded in the colon by the gut microflora (Nakazawa and Ohsawa, 1998; Baba et al., 2005). In addition, we did not detect TSL in plasma or urine in rats orally receiving SAA, SAB, SAD, RMA, and LSA on separate occasions, which is in agreement with the observation with oral SAB in rats by Xu et al. (2007b).

As illustrated in the product label, Cardiotonic pills can be taken either orally or sublingually. Sublingual administration of a drug may bypass some exposure to the presystemic drug-metabolizing enzymes and transporters that is encountered when the drug is orally administered by swallowing. In addition, the bioavailability of a drug taken orally may be more variable due to functional polymorphisms involved in presystemic metabolism and transportation. Accordingly, we performed a crossover, random-order, open-label human study to examine whether sublingual administration of Cardiotonic pills might change the

bioavailability or absorption rate of the Danshen phenolic acids. Our results indicated that the sublingual administration did not improve the poor bioavailability of SAA, SAB, SAD, RMA, and LSA. This finding was not surprising, considering the poor PK properties exhibited by these compounds in the Caco-2 cells. Although PCA was subject to extensive presystemic hepatic elimination, its bioavailability was not improved by the sublingual administration, this may attributed, at least in part, to the significant intake and elimination of the compound by the erythrocytes. Focusing on TSL, we found that the sublingual administration showed a bioavailability comparable to oral administration. The plasma C_{max} , T_{peak} , and AUC of TSL, as well as the urinary $Cum.A_e$, were not significantly different between the sublingual and oral routes. This result was supported by our findings that in rats a high percentage of TSL escaped first-pass hepatic elimination and in Caco-2 cells there was no indication of TSL transport by P-gp or MRP2. The finding that TSL was absorbed similarly after sublingual and oral administrations of Cardiotonic pills may be clinically relevant for patients with swallowing difficulties. However, further studies with the other component herbs in Cardiotonic pills, Sanqi and Bingpian, are required to confirm this potential benefit; in addition, the practical benefit of the sublingual route should be studied in patients.

The therapeutic strategy for relief of angina is based on improvement of the balance between myocardial oxygen supply and demand. TSL has been shown to dilate coronary arteries (Dong and Jiang, 1982), inhibit platelet aggregation (Li et al., 1983), improve microcirculation (Cheng et al., 1987), and protect the myocardium from reperfusion injury of ischemic heart (Han et al., 2008). These cardiovascular actions may be produced primarily by TSL inhibition of extracellular Ca^{2+} entry into both cardiac cells (Cao et al., 2003) and vascular

smooth muscle cells (Lam et al., 2007). Other effects of TSL may also include scavenging oxygen-free radicals (Zhao et al., 2008), and protecting the endothelial cells against homocysteinemia (Chan et al., 2004). In the current study, we identified plasma TSL and urinary TSL as PK markers of orally administered Cardiotonic pills based on PK properties evaluated in vivo (in experimental animals and in humans), in vitro, and in silico. However, our study did not address whether TSL is a principle factor in the putative antianginal actions of Cardiotonic pills.

In summary, although an herbal product usually contains numerous chemical constituents, its principle medicinal factors most likely possess favorable drug-like properties. In the current study, we examined the PK properties of putatively active phenolic acids from Danshen to identify suitable compound(s) that could indicate systemic exposure to oral Cardiotonic pills. We found that plasma TSL was a suitable PK marker and urinary TSL was a surrogate PK marker for oral Cardiotonic pills. The other danshen phenolic acids were unsuitable due to unfavorable PK properties. In addition, sublingual administration of the pills neither changed the absorption rate and bioavailability of TSL nor improved the poor bioavailability of the other tested danshen compounds, compared with the oral administration. Three points are worth mentioning: (1) In addition to PK properties, the dose level, dosage form, and route of administration affect the suitability of an herbal constituent measured in plasma or urine as a PK marker for the herbal product. (2) PK markers from the other component herbs also need to be identified and a combination of PK markers will provide a more complete characterization of the systemic exposure to Cardiotonic pills. (3) Although a substantial fraction (> 60%) of

intravenous TSL was eliminated in intact form by renal excretion, the biotransformation of this danshen phenolic compound remains to be revealed.

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Footnotes

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Legends for figures

FIG. 1. Chemical structures of the main danshen phenolic acids present in Cardiotonic pills.

FIG. 2. Plasma concentration-time profiles of TSL in dogs following a single oral dose of Cardiotonic pills (1, 2, and 3) or following a single intravenous bolus of the injectable TSL solution (4). Dose proportionality was tested by power regression analysis of the $AUC_{0 \rightarrow \infty}$ (5) and C_{max} (6) of TSL. The test doses of Cardiotonic pills were in pills/kg.

FIG. 3. Plasma TSL concentration-time curves in male (1 and 2) and female (3 and 4) human subjects after receiving a single dose of Cardiotonic pills (30 pills/subject) via normal oral (1 and 3) or sublingual (2 and 4) routes, as well as correlation between urinary excretion of TSL, $Cum.A_{e0 \rightarrow 12h}$, and plasma TSL $AUC_{0 \rightarrow 6h}$ (5) and that between abbreviated urinary excretion of TSL, $Cum.A_{e0 \rightarrow 3h}$, and $AUC_{0 \rightarrow 6h}$ (6). In (5) and (6), the data from the two administration routes were combined.

FIG. 4. Stability of PCA in canine erythrocytes and plasma. The concentration changes of PCA as a function of time in erythrocytes (dashed line) and plasma (dotted line) were measured by first adding the compound to canine whole blood at an initial concentration of 150 ng/ml. After incubation at 37°C for the indicated time period, the erythrocytes and plasma were separated by centrifugation for PCA measurements. The solid line represents the stability of PCA in canine plasma at 37°C; here the compound was added into isolated plasma at an initial concentration of 150 ng/ml and the sample was used for PCA measurements.

TABLE 1

Plasma pharmacokinetics of TSL, PCA, SAA, SAB, SAD, RMA, and LSA in dogs following an intravenous bolus dose of the injectable Cardiotonic solution at 0.50 ml/kg

PK Parameter	TSL	PCA	SAA	SAB	SAD	RMA	LSA
C_{\max} (ng/ml)	3607±249	78.3±9.1	456±22	1842±168	2423±192	545±45	438±15
$AUC_{0 \rightarrow t}$ (h·ng/ml)	1545±179	51.7±3.7	166±13	475±74	628±61	129±21	115±16
$AUC_{0 \rightarrow \infty}$ (h·ng/ml)	1549±180	54.9±4.1	200±30	489±74	635±60	133±22	123±17
MRT (h)	0.50±0.02	1.21±0.04	0.94±0.44	0.26±0.08	0.23±0.01	0.18±0.01	0.29±0.09
$T_{1/2}$ (h)	0.51±0.05	1.08±0.05	0.97±0.32	0.45±0.13	0.41±0.04	0.31±0.01	0.33±0.09
$CL_{\text{tot,p}}$ (l/h·kg)	0.48±0.06	3.83±0.28	1.00±0.15	1.22±0.20	0.93±0.09	1.28±0.22	0.98±0.15
V_{SS} (l/kg)	0.24±0.03	4.64±0.39	0.93±0.29	0.33±0.15	0.22±0.03	0.23±0.06	0.28±0.07

For $AUC_{0 \rightarrow t}$, the value of t is defined as the last quantifiable point; these were 4, 4, 2, 2, 2, 1, and 1 h for TSL, PCA, SAA, SAB, SAD, RMA, and LSA respectively.

TABLE 2

Plasma pharmacokinetics of TSL in dogs after an oral dose of Cardiotonic pills or an intravenous bolus dose of the injectable TSL solution

PK Parameter	Dose of TSL from Oral Cardiotonic Pills (mg/kg) [pills/kg]			Intravenous TSL Dose (mg/kg)
	0.32 [2]	0.64 [4]	1.28 [8]	0.64
C_{\max} (ng/ml)	329 ± 122	774 ± 50	1034 ± 219	2791 ± 500
T_{peak} (h)	0.44 ± 0.10	0.33 ± 0.00	0.50 ± 0.00	—
$AUC_{0 \rightarrow t}$ (h·ng/ml)	211 ± 18.7	447 ± 22.7	909 ± 158	1143 ± 82.6
$AUC_{0 \rightarrow \infty}$ (h·ng/ml)	223 ± 17.1	454 ± 19.8	911 ± 157	1145 ± 82.8
MRT (h)	0.90 ± 0.16	0.73 ± 0.04	0.99 ± 0.12	0.46 ± 0.03
$T_{1/2}$ (h)	0.40 ± 0.05	0.44 ± 0.04	0.55 ± 0.07	0.44 ± 0.03
$CL_{\text{tot,p}}$ (l/h·kg)	—	—	—	0.51 ± 0.04
V_{SS} (l/kg)	—	—	—	0.24 ± 0.03
F (%)	—	39.7 ± 1.2	—	—

For $AUC_{0 \rightarrow t}$, the value of t is defined as the last quantifiable point; these were 2, 2, 4, or 4 h after the low, intermediate, high oral dose of Cardiotonic pills, or the intravenous dose of the injectable TSL solution, respectively.

TABLE 3

*Plasma and urine PK data for TSL in human subjects that received a single dose (30 pills/subject) of
Cardiotonic pills via the normal oral route or the sublingual route*

According to LC-MS/MS measurements, the 30 Cardiotonic pills contained 4.8 mg of TSL .

PK Parameter	Normal Oral Administration		Sublingual Administration	
	Man	Woman	Man	Woman
	<i>Plasma data</i>			
C_{max} (ng/ml)	19.3 ± 5.1*	31.3 ± 10.6	18.6 ± 5.8*	30.3 ± 6.4
C_{max} (ng/ml) /D-W	263 ± 78	322 ± 95	255 ± 89	315 ± 67
T_{peak} (h)	1.33 ± 0.52	1.17 ± 0.68	1.40 ± 0.55	0.83 ± 0.26
$AUC_{0→6h}$ (h·ng/ml)	43.3 ± 3.9*	72.8 ± 30.5	42.7 ± 4.7 ^Δ	63.4 ± 20.8
$AUC_{0→∞}$ (h·ng/ml)	48.5 ± 3.4*	77.6 ± 31.6	47.3 ± 4.9 ^Δ	68.2 ± 22.1
$AUC_{0→∞}$ (h·ng/ml)/D-W	655 ± 63	810 ± 318	644 ± 79	712 ± 223
$T_{1/2}$ (h)	1.16 ± 0.42	1.27 ± 0.21	1.13 ± 0.38	1.22 ± 0.45
MRT (h)	2.50 ± 0.45	2.43 ± 0.35	2.60 ± 0.69	2.33 ± 0.57
$Cl_{tot,p}/F$ (l/h·kg)	1.54 ± 0.14	1.47 ± 0.79	1.57 ± 0.18	1.63 ± 0.88
V_{SS}/F (l/kg)	3.87 ± 0.91	3.41 ± 1.33	4.16 ± 1.43	2.48 ± 0.93
	<i>Urine data</i>			
$Cum.A_{e0→3h}$ (μg)	1017 ± 217*	1386 ± 228	892 ± 262 ^Δ	1319 ± 374
[% excreted]	[72 ± 11]	[78 ± 5.8]	[64 ± 17]	[79 ± 9.9]
$Cum.A_{e0→12h}$ (μg)	1400 ± 140*	1795 ± 387	1330 ± 130	1668 ± 416
[% excreted]	[100 ± 0.3]	[100 ± 0.0]	[100 ± 0.0]	[100 ± 0.1]
f_e (%)	29.3 ± 2.9*	37.4 ± 8.1	25.8 ± 5.5 ^Δ	34.8 ± 8.7
CL_R (l/h·kg)	0.45 ± 0.03	0.50 ± 0.13	0.44 ± 0.03	0.51 ± 0.12

D-W; the Dose-weights were: 0.074 ± 0.003 and 0.097 ± 0.012 mg/kg for males and females, respectively. The symbols Δ and

* represent $p < 0.1$ and 0.05, respectively, when comparing with women. TSL was not detected in the urine samples collected during 12–24 h after administration of Cardiotonic pills

TABLE 4

Apparent permeability for tested danshen phenolic acids in Caco-2 cell monolayers

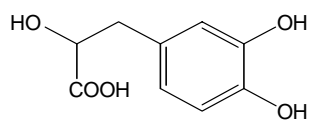
Chemical	P_{app} ($\times 10^{-6}$ cm/s)	
	Apical→basolateral	Basolateral→apical
TSL	1.22 \pm 0.14	0.903 \pm 0.044
PCA	12.1 \pm 0.8	21.8 \pm 0.6
SAA	0.0595 \pm 0.0114	0.0554 \pm 0.0052
SAB	0.0200 \pm 0.0050	0.0377 \pm 0.0197
SAD	0.0662 \pm 0.0082	0.164 \pm 0.069
RMA	0.523 \pm 0.301	0.550 \pm 0.003
LSA	0.0816 \pm 0.0275	0.0413 \pm 0.0274

TABLE 5

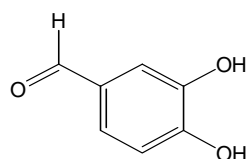
Physicochemical properties relevant to the absorption properties of danshen phenolic acids

Chemical	<i>LogS</i> (pH 5.0)	<i>LogS</i> (pH 7.0)	<i>HBD</i>	<i>HBA</i>	<i>TPSA</i>	<i>LogD</i> (pH5.0)	<i>LogD</i> (pH7.0)	<i>NROTb</i>
TSL	0.58	0.70	4	5	98	-1.5	-3.4	3
PCA	-1.08	-0.99	2	3	57	1.1	1.0	1
SAA	-2.22	-0.81	7	10	185	2.0	0.6	9
SAB	0.14	0.14	9	16	278	-2.1	-2.6	14
SAD	0.38	0.38	6	10	182	-2.3	-3.8	9
RMA	-0.52	0.44	5	8	145	-0.5	-1.9	7
LSA	0.27	0.27	7	12	211	-2.5	-3.3	9

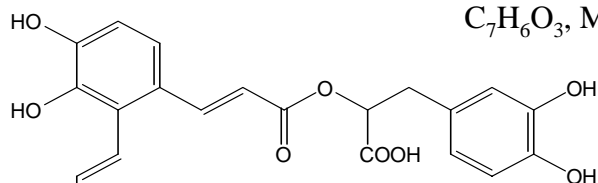
Figure 1



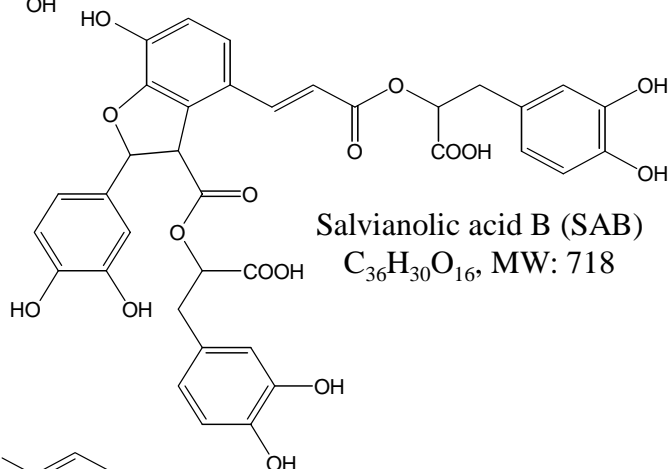
Tanshinol (TSL)
 $C_9H_{10}O_5$, MW: 198



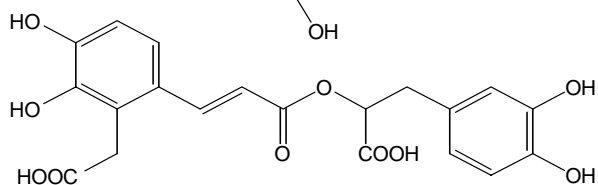
Protocatechuic aldehyde (PCA)
 $C_7H_6O_3$, MW: 138



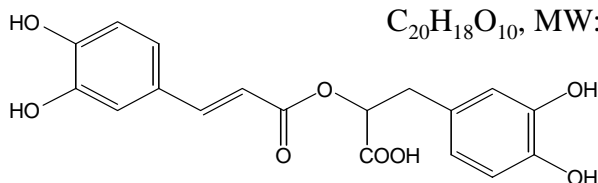
Salvianolic acid A (SAA)
 $C_{26}H_{22}O_{10}$, MW: 494



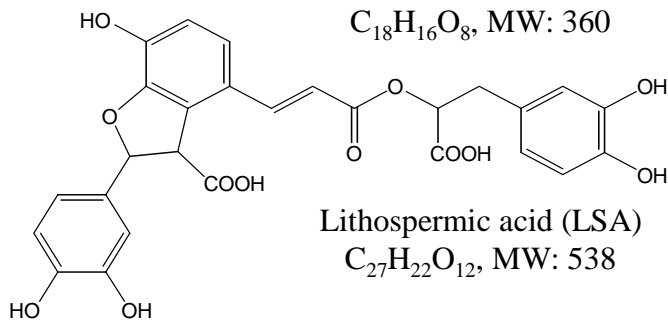
Salvianolic acid B (SAB)
 $C_{36}H_{30}O_{16}$, MW: 718



Salvianolic acid D (SAD)
 $C_{20}H_{18}O_{10}$, MW: 418



Rosmarinic acid (RMA)
 $C_{18}H_{16}O_8$, MW: 360



Lithospermic acid (LSA)
 $C_{27}H_{22}O_{12}$, MW: 538

Figure 2

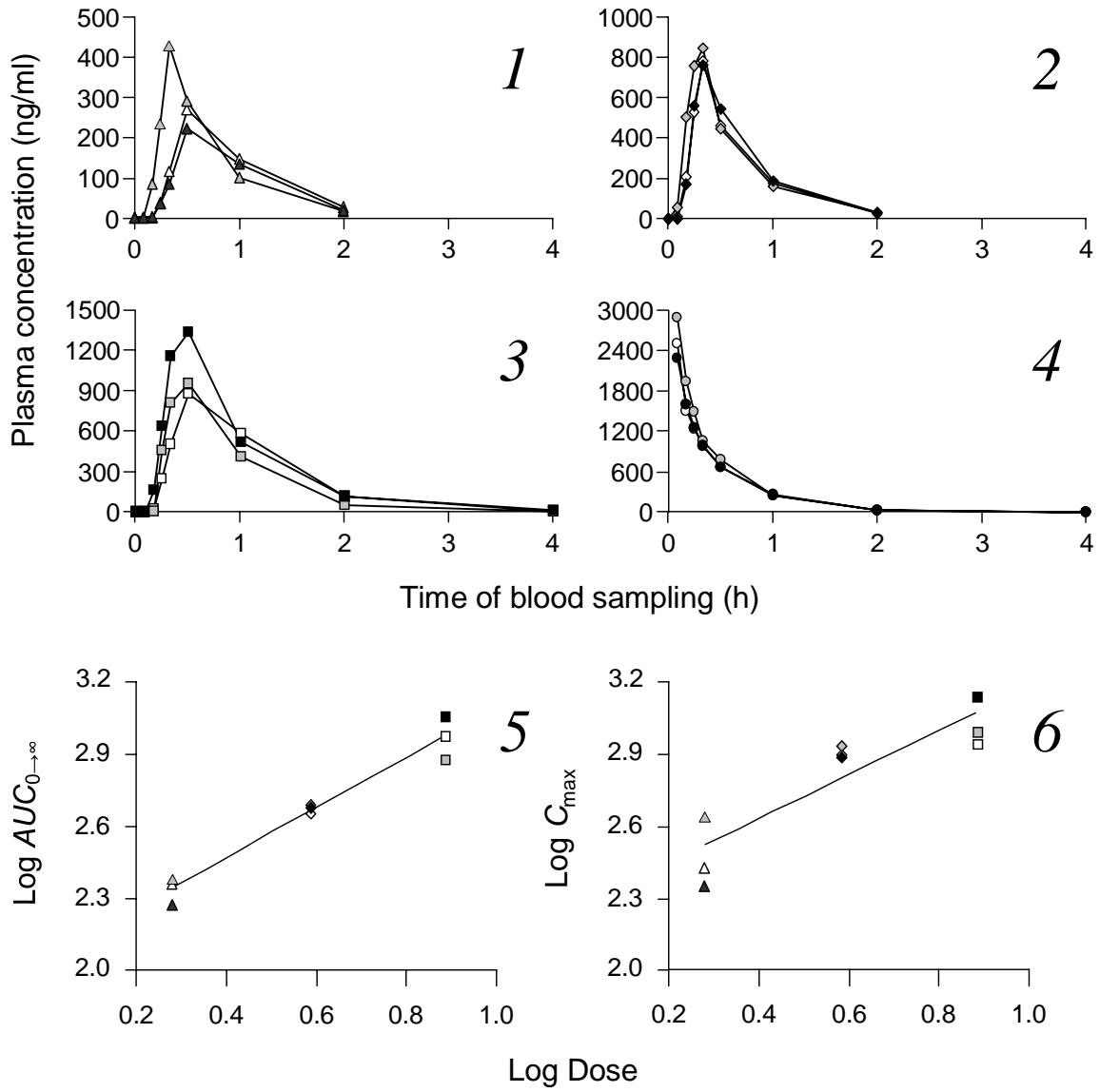


Figure 3

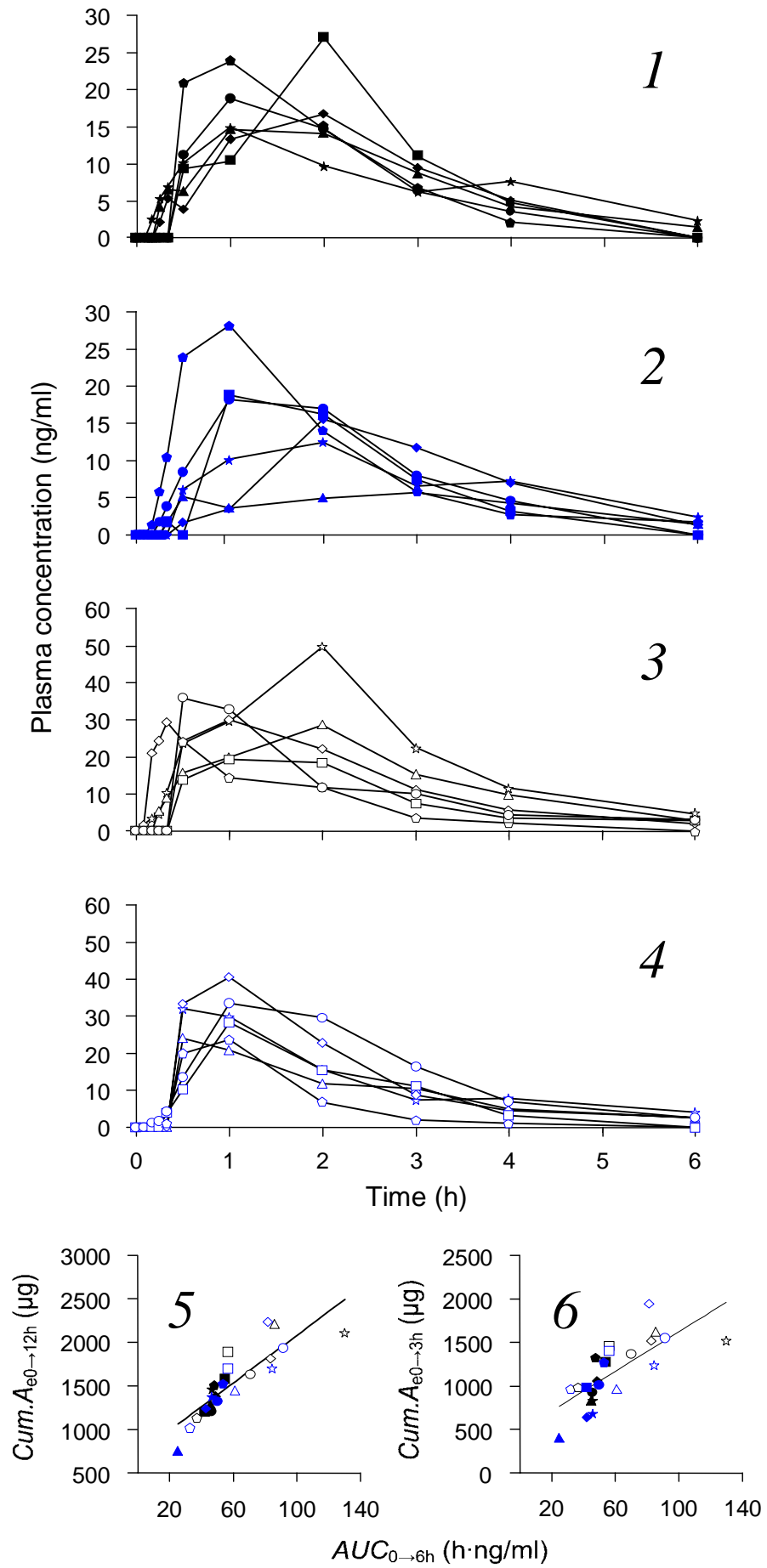


Figure 4

