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Effect of sodium caprate on the oral absorptions of danshensu and salvianolic acid B

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ABSTRACT

The current study aims to investigate the effect of sodium caprate on the intestinal absorption and bioavailabilities of danshensu and salvianolic acid B, the major active components in *Salvia miltiorrhiza* Bge (Danshen). Biopharmaceutics and pharmacokinetics properties of the two compounds have been characterized by *in vitro*, *in situ* models as well as *in vivo* in rats. Based on the identified biopharmaceutics characteristics of the two compounds, effect of sodium carparate as absorption enhancer on the intestinal absorption and pharmacokinetics of danshensu and salvianolic acid B in pure compound form as well as extract form were investigated both *in vitro* and *in vivo*. Both danshensu and salvianolic acid B demonstrated very limited intestinal permeabilities, leading to oral bioavailabilities of only 11.09% and 3.90% in rats, respectively. Results from both *in vitro* and *in vivo* studies consistently indicated that sodium caprate could significantly enhance intestinal permeabilities as well as the *in vivo* bioavailabilities of both danshensu and salvianolic acid B. The current findings not only identified the usefulness of sodium caprate for the improved delivery of Danshen product but also demonstrated the importance of biopharmaceutics characterization in the dosage form development of traditional Chinese medicine.

1. Introduction

Danshen, the dried root of *Salvia miltiorrhiza* Bge has been widely used in China for many years for treating coronary heart disease such as angina pectoris and stuffiness in the chest. It also could be used in the treatment of cerebrovascular disease and hyperlipidemia, etc. The main hydrophilic components of Danshen are danshensu, protocatechuic aldehyde and salvianolic acid B. The main lipophilic components of Danshen are tanshinone IIA, cryptotanshinone and tanshinone I. In China, numerous pharmaceutical dosage forms of Danshen are commercially available. Fufang Danshen Tablet and Fufang Danshen Dripping Pill are the two most widely used products in China and have been officially listed in the Chinese pharmacopoeia (Zhou et al., 2005). Findings from us and others consistently demonstrated that the hydrophilic components e.g. danshensu and salvianolic acid B are the main components in the commercial Fufang Danshen Tablets and Fufang Danshen Dripping Pill (Zhang et al., 2002; Shi et al., 2005; Zhou et al., 2006; Ma et al., 2007; Liu et al., 2007; Wei et al., 2007; Cao et al., 2008) (Fig. 1). The main reported pharmacological activities of danshensu include: inhibition of platelet aggregation and decrease the levels of blood viscosity (Li et al., 1983); scavenging oxygen free radicals (Zhao et al., 1996); inhibition of myocardium cell apoptosis (Liu et al., 2001) and protection of the endothelial cells against homocysteinemia (Chan et al., 2004). The main pharmacological activities of salvianolic acid B include: inhibition of platelet aggregation (Li et al., 2004) as well as oxidative modification of low-density lipoprotein (LDL) (O et al., 2001; Wu et al., 1998); stimulation of nitric oxide production of the endothelial cell (O et al., 2000) and inhibition of angiotensin II-induced hyperplasia; protection of ischemia–reperfusion injury (Ouyang et al., 2001); scavenge superoxide radical and inhibit erythrocyte hemolysis (Kang et al., 2004).

Due to the promising pharmacological effects of danshensu and salvianolic acid B, more researchers started to investigate their pharmacokinetics properties. It was reported that after oral administration of Fufang Danshen Dripping Pill in rats, the pharmacokinetic model of danshensu could be described by an open two-compartment model with K_α of 4.95 h^{-1}, $T_{1/2\alpha}$ of 0.20 h, $T_{1/2\beta}$ of 3.28 h, C_{max} of 9.38 mg/l and T_{max} of 0.50 h (Hong et al., 2000). Luo et al. gave Danshen aqueous extraction to dog orally and found that danshensu could be absorbed with a T_{max} of 1.75 h and a C_{max} of 4.72 mg/l (Luo et al., 2001). The pharmacokinetic process of danshensu in human can be described by a one-compartment model with a $T_{1/2}$ of 5.54 h and K_α of 1.69 h^{-1} after sublingual administration of 250 mg Fufang Danshen Dripping Pill to 10 healthy volunteers (Pei et al., 2004). In summary, these published pharmacokinetic studies of danshensu were usually conducted by measuring the plasma concentrations of danshensu after oral

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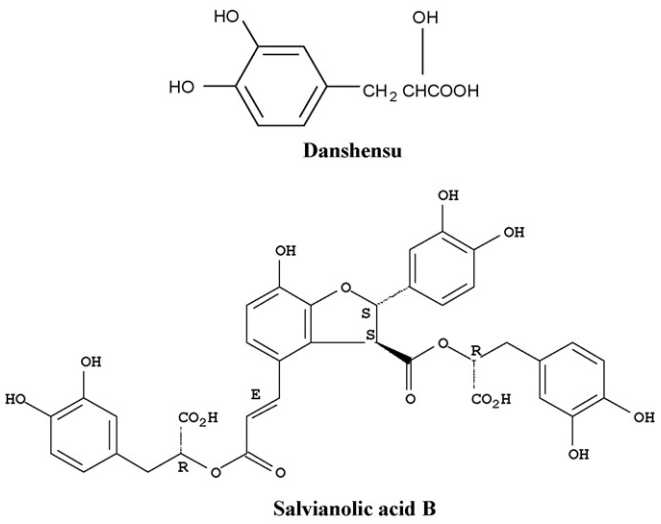

Fig. 1. Chemical structure of danshensu and salvianolic acid B.

administrations of either Danshen extract or Danshen products. Such pharmacokinetic parameters obtained may not truly reflect the pharmacokinetic behavior of danshensu since the co-occurring components in Danshen extract or Danshen products may alter the pharmacokinetics of danshensu. The pharmacokinetics of an herbal active substance may be different when administered in an extract form as compared to that when administered as individual pure compound (Chang et al., 2005). However, there is barely any report on the intestinal absorption and oral bioavailability of danshensu in pure form.

Pharmacokinetics of salvianolic acid B in pure form has been investigated in rats by Zhang et al. (2004b). In this study, the pharmacokinetic parameters were reported as $T_{1/2\alpha}$ of 22.70 min, $T_{1/2\beta}$ of 176.00 min, and AUC of 1130.00 μg min/ml after intravenous administration of 20 mg/kg salvianolic acid B. However, salvianolic acid B could only be observed in rat plasma when the oral administration dose was increased to 100 mg/kg with the mean $AUC_{0-90min}$, C_{max} and T_{max} to be 1.26 μg min/ml, 0.04 μg/ml, and 20.00 min, respectively. Based on the plasma concentration versus time profiles from different intravenous and oral doses, the absolute oral bioavailability of salvianolic acid B was calculated to be 0.02% in rat. The same study also found that a considerable amount (about 65%) of salvianolic acid B was detected in the gastrointestinal tract even 4 h after administration and more than 50% of the dose reached the lower parts of the intestine. In summary, previous studies consistently suggested a poor absorption of salvianolic acid B from the rat small intestine. However, the reasons for causing the low intestinal absorption of salvianolic acid B have not been further investigated. Such mechanistic information is essential for the further development of dosage forms with improved oral bioavailability for salvianolic acid B

Similar to western drug development, it is expected that the biopharmaceutics and pharmacokinetics characteristics are also essential for the further development of any traditional Chinese medicine preparations. The current study aims to demonstrate a systemic biopharmaceutics and pharmacokinetics characterization of the two major active ingredients from Danshen for the further development of an improved oral dosage form of Danshen. The specific objectives of the current study include: (1) To investigate the intestinal absorption and pharmacokinetics of danshensu and salvianolic acid B, using *in vitro* Caco-2 cell monolayer model, rat *in situ* intestinal perfusion model and *in vivo* pharmacokinetics in rats after both oral and intravenous administration of pure form of danshensu and salvianolic acid B; (2) To improve the intestinal absorption and bioavailabilities of danshensu and salvianolic acid B by using appropriate absorption enhancer (such as sodium caprate) identified based on the biopharmaceutics and pharmacokinetics characteristics of the two major active ingredients from Danshen.

2. Materials and methods

2.1. Chemicals and animals

Danshensu was purchased from School of Pharmacy, Fudan University (Shanghai, PR China). Salvianolic acid B was purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, PR China). Two Danshen tablets (product A manufactured by Guangzhou Yuehua pharmaceutical LTD and product B Guangzhou Meichen pharmaceutical LTD) were purchased from pharmaceutical store in China. Sodium caprate, atenolol, propranolol, homoprotocatechuic acid used as internal standard for the determination of danshensu and naringin used as internal standard for the determination of salvianolic acid B were all purchased from Sigma (St. Louis, MO, USA). Phosphoric acid was obtained from MERCK company (NJ, USA). Waters Oasis® hydrophilic–lipophilic-balanced (HLB) copolymer extraction cartridges were purchased from Waters (Milford, MA, USA). Acetonitrile (HPLC grade) were obtained from Labscan Asia, Thailand. Methanol (HPLC grade) was obtained from TEDIA company, INC., USA. Acetic acid, formic acid and ethyl acetate (analytical grade) were obtained from BDH Laboratory supplies, England. All other reagents were of at least analytical grade and used without further purification. Distilled and deionized water was used for the preparation of all solutions.

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), 0.05% trypsin–EDTA, penicillin–streptomycin, and non-essential amino acids were obtained from GibcoBRI, Life and Technologies, USA. Collagen type I, sodium pyruvate and MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetraaolium bromide) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phosphate buffered saline tablets were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Six-well Transwell® insert (0.4 μm pore size, 4.71 cm^2, polycarbonate filter, Costar 3410) and plate were purchased from Corning Costar Co., NY, USA.

Male Sprague-Dawley rats (~250 g) were supplied by the Laboratory Animal Service Centre at The Chinese University of Hong Kong and fasted overnight before use. The experiments were carried out after approval by the Animal Ethics Committee of The Chinese of Hong Kong.

2.2. Cell culture, cytotoxicity test and bi-directional transport study of danshensu and salvianolic acid B in Caco-2 cell monolayer model

2.2.1. Cell culture of Caco-2 cells

Caco-2 cells from American Type Culture Collection (ATCC) were cultured in DMEM at 37 °C, supplemented with 10% fetal bovine serum and 1% non-essential amino acids, in an atmosphere of 5% CO_2 and 90% relative humidity as we described before (Zhang et al., 2004a). Cell grown in 75 cm^2 flasks was subcultured at 80–90% confluence by treating with 0.05% trypsin–EDTA. The Caco-2 suspension was seeded in collagen coated Transwell® insert at a density of 3×10^5 cells/well and cultured for 21 days prior to the transport experiments. Passage 35 was used for the transport study.

2.2.2. Preparation of transport buffer

Phosphate buffered saline plus (PBS^+) was used as the transport buffer for the transport study in Caco-2 cell monolayer model. It was prepared by dissolving a commercial available PBS tablet in

200 ml water, followed by supplementing with 90 μl of 2 M calcium chloride and 80 μl of 1 M magnesium chloride. The pH value of the buffer was adjusted to pH 7.4 by 85% of phosphoric acid.

2.2.3. Cytotoxicity tests of danshensu, salvianolic acid B and sodium caprate on Caco-2 cells

MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) test was used to estimate the potential cytotoxicities of the studied danshenu, salvianolic acid B and sodium caprate toward Caco-2 cells. The Caco-2 cells were seeded onto a 96-well plate at a seeding density of 5×10^4 cells/well in DMEM culture medium and cultured at 37 °C for 24 h. Subsequently, the culture medium was replaced with 150 μl of danshensu, salvianolic acid B or sodium caprate (combined with or without danshensu or salvianolic acid B) dissolved in PBS^+ (pH 7.4) at different studied concentrations (1, 10, 50, 100, 200, 500, 1000 and 2000 μM for danshensu or salvianolic acid B; 0.01%, 0.03%, 0.05%, 0.10%, 0.25%, 0.40%, 0.50% and 0.80% (w/v) for sodium caprate with or without addition of 100 μM of danshensu or salvianolic acid B). Blank PBS^+ (pH 7.4) was employed as a negative control. Then the 96-well plate was incubated at 37 °C for 2 h. Thereafter, 20 μl of 5 mg/ml MTT solution in PBS^+ was added to each well and the plate was incubated for another 4 h. The solutions in each well were then removed followed by dissolving the remained formazan crystals in the cells with 200 μl of DMSO. The absorbance of the mixture in the 96-well plate was then measured with a Kinetic microplate reader (Molecular Devices) at 590 nm. The cytotoxicity of each compound was calculated as the percentage of the absorbance relative to that of the negative control.

2.2.4. Stability of danshensu and salvianolic acid B in transport buffer

In order to investigate the stabilities of danshensu and salvianolic acid B in the transport buffer, 100 μM of danshensu and salvianolic acid B was prepared with PBS^+ (pH 7.4). The prepared solutions were incubated in shaking water bath at 37 °C. A 150 μl of the sample was taken at different time intervals (0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min) and the drug concentration was measured. The percentage of danshensu and salvianolic acid B remained in the transport buffer was plot against period of incubation to obtain their stability profiles in the transport buffer.

2.2.5. Effect of sodium caprate on the absorption of danshensu and salvianolic acid B in Caco-2 cell monolayer model

To investigate the enhancement effect of sodium caprate in the caco-2 monolayer model, 1.5 ml of 100 μM of danshensu/salvianolic acid B or 3 mM of Atenolol (paracellular marker) with or without the addition of sodium caprate (0.0625%, 0.125%, 0.25% w/v) in Ca^{2+}/Mg^{2+}-free PBS^+ (Ca^{2+}/Mg^{2+}-free PBS^+ was always used in the donor chamber to avoid precipitation of sodium caprate. Exclusion of Ca^{2+}/Mg^{2+} from the donor solution did not affect the integrity of the monolayers because the PBS^+ in the basolateral chamber always contained Ca^{2+}/Mg^{2+} (Anderberg et al., 1993)) was loaded at the apical side (donor chamber), respectively, and 2.6 ml of black PBS^+ was placed at basolateral side (receiver chamber). Aliquots of 0.5 ml samples were taken from receiver chambers at various time intervals (15, 30, 45, 60, 90, 120 min) after the loading. Equal volume of the blank PBS^+ buffer will be added to the receiver chambers after each sampling to maintain a constant volume in the whole course of experiment. At the end of the experiment, all samples were withdrawn from both the donor chambers and receiver chambers (Zhang et al., 2004a). The collected samples were stored at −20 °C until HPLC analysis. During the above absorption studies, the TEER values were also monitored before and at the end of experiment.

2.3. Rat in situ single pass intestinal perfusion study

2.3.1. Preparation of perfusion buffer for rat in situ single pass intestinal perfusion study

The perfusion buffer was isotonic (293 mOsm/l) and composed of 118 mM NaCl, 4.7 mM of KCl, 1.2 mM of $MgSO_4 \cdot H_2O$, 2.5 mM of $CaCl_2$, 1.2 mM of KH_2PO_4, and 25 mM of $NaHCO_3$. All the above chemicals were dissolved in water followed by adjusting the pH to 6.8 by concentrated phosphate acid. Phenol red at 10 μg/ml was added to the perfusate as a non-absorbable marker.

2.3.2. Stability of danshensu and salvianolic acid B in perfusion buffer

In order to investigate the stabilities of danshensu and salvianolic acid B in the perfusion buffer, 100 μM of danshensu and salvianolic acid B was prepared with perfusion buffer (pH 6.8). The prepared solutions were incubated in shaking water bath at 37 °C. A 150 μl of the sample was taken at different time intervals (0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min) and the drug concentration was measured. The percentage of danshensu and salvianolic acid B remained in the perfusion buffer was plot against period of incubation to obtain their stability profiles in the perfusion buffer.

2.3.3. Effect of sodium caprate on the absorption of danshensu and salvianolic acid B in rat in situ intestinal perfusion model

Rat *in situ* single pass intestinal perfusion model was set as previously described by us (Zhang et al., 2005b). Briefly, male Sprague-Dawley rats (body weight: 250–300 g) were fasted overnight with free access to water. The rats were anesthetized with an intramuscular injection of a mixture containing 60 mg/kg ketamine and 6 mg/kg xylazine. During the surgical process, the body temperature was maintained by a heating lamp. Fresh heparinized blood was collected from donor rats by cardiac puncture. Right jugular vein for infusion of donor blood was cannulated with a polyethylene tubing (0.5 mm ID, 1 mm OD. Portex Ltd., Hythe, Kent, England). The infusion rate of donor blood via right jugular vein was set at 0.25 ml/min. The small intestine was then exposed by middle incision, a 7 to 11 cm long segment of jejunum was cannulated with silicon tubing (0.5 mm ID, 1 mm OD. Portex Ltd.) connected to a peristaltic pump. The segment was then flushed with warm saline to remove intestinal contents. The mesenteric vein for collecting blood from the specified segment of intestine was cannulated with polyethylene tubing (0.5 mm ID, 1 mm OD. Portex Ltd.).

Solutions containing 100 or 600 μM danshensu, 250 or 500 μM salvianolic acid B were perfused through the intestinal lumen to investigate the permeabilities of the two studied compounds in the rat *in situ* intestinal perfusion model. In addition, 600 μM danshensu, 250 μM salvianolic acid B with the addition of 0.50% (w/v) sodium caprate were also perfused through the intestinal lumen to investigate the effect of sodium caprate on the permeabilities of the two studied compounds. Samples obtained from the mesenteric vein were collected into the pre-weighed micro tubes at every 5 min. All collected samples were weighed and centrifuged at 13,200 rpm for 5 min immediately. The plasma samples were stored at −80 °C refrigerator until analysis.

2.4. Effect of sodium caprate on the oral bioavailabilities of danshensu and salvianolic acid B in vivo

SD rats (250 g, Male) obtained from Laboratory Animal Service Centre were anaesthetised and cannulated at the jugular vein with a polyethylene tube (0.5 mm I.D., 1 mm O.D., Portex Limited, Hythe, Kent, England) that was led under the skin and exteriorized at the back of the neck. After the exposed areas were surgically sutured, the rats were placed individually and allowed to recover for 24 h and fasted for at least 12 h prior to drug administration.

The cannulated rats were randomly divided into 10 groups with five rats in each group to receive various intravenous or oral administrations. In Group 1, rats received 20 mg/kg danshensu intravenously. In Group 2, rats received 20 mg/kg danshensu orally. In Group 3, rats received 20 mg/kg danshensu with the addition of 100 mg/kg sodium caprate orally. In Group 4, rats received 50 mg/kg salvianolic acid B intravenously. In Group 5, rats received 50 mg/kg salvianolic acid B orally. In Group 6, rats received 50 mg/kg salvianolic acid B with the addition of 100 mg/kg sodium caprate orally. In Group 7, rats received 1727 mg/kg product A extract (equivalent to 20 mg/kg of danshensu) orally. In Group 8, rats received 1727 mg/kg product A extract (equivalent to 20 mg/kg of danshensu) with the addition of 100 mg/kg sodium caprate orally. In Group 9, rats received 1447 mg/kg product B extract (equivalent to 50 mg/kg of salvianolic acid B) orally. In Group 10, rats received 1447 mg/kg product B extract (equivalent to 50 mg/kg of salvianolic acid B) with the addition of 100 mg/kg sodium caprate orally.

Blood samples (0.3 ml) were withdrawn *via* the jugular vein cannula and collected in heparinized microcentrifuge tubes before dosing (to serve as a control) and at appropriate time intervals after drug administration. After each blood sampling, an equal volume of heparinized normal saline solution (20 I.U./ml) was immediately injected back into the rat via the cannula, to flush the cannula and prevent blood coagulation. The collected blood sample was centrifuged immediately at 13,200 rpm for 5 min to obtain plasma. The collected plasma samples were analyzed immediately or stored at −80 °C until analysis.

2.5. Sample preparations and analysis

2.5.1. Sample treatment and HPLC-UV analysis for samples collected from Caco-2 studies

The samples obtained from the Caco-2 cell model transport study were directly subjected to HPLC analysis without further treatment.

The liquid chromatographic system used was a Waters HPLC system (Waters, Milford, MA, USA) equipped with 2695 solvent delivery module and a 996 photodiode-array (PDA) UV detector. The chromatographic separation was achieved by a reversed-phase HPLC column (Radial-pak C_{18} cartridge, 10 cm × 8 mm I.D.; 4 μm particle size, Waters) protected by a pre-column filter (Nove-pak C_{18} Guard-pak, Waters, Milford, MA, USA). The elution gradient for HPLC analysis consisted of two solvent compositions: 0.5% acetic acid in acetonitrile (solvent A) and 0.5% acetic acid in water (solvent B). Gradient elution was carried out according to the following program: solvent A was increased from 5% to 50% in the first 20 min, and returned to 5% in 5 min. The total run time was 25 min. The sample injection volume was 100 μl. The column and auto-sampler were set at ambient temperature. The flow rate was set at 1 ml/min. The eluent was monitored by a UV detector at the wavelength of 280 nm for the two studied compounds.

2.5.2. Sample treatment and analysis for plasma samples obtained from both rat in situ intestinal perfusion study and in vivo pharmacokinetic study

2.5.2.1. For samples containing danshensu. A 150 μl of the collected rat plasma sample was added with 10 μl of 10 μg/ml of homoprotocatechuic acid (IS), 10 μl of concentrate phosphoric acid (85%) and 330 μl of water to an eppendorf tube followed by votexing and mixing thoroughly. The samples were extracted with Oasis HLB cartridge conditioned with methanol and water followed by washing with 1 ml of 5% methanol in water and eluting with 1 ml of methanol. The collected eluent was evaporated to dryness and reconstituted with 150 μl of 0.5% acetic acid in water. A 100 μl of the supernatant was injected to HPLC for analysis

A Waters HPLC system (Waters, Milford, MA, USA) equipped with 2695 solvent delivery module and a 996 photodiode-array (PDA) UV detector was used for the analysis of danshensu in various plasma samples. The chromatographic separation was achieved by using a reversed-phase HPLC column (ODS Hypersil C18 column (250 mm × 4.6 mm I.D.; 5 μm particle size, Thermo) protected by a pre-column filter (Nove-pak C_{18} Guard-pak, Waters, Milford, MA, USA). The mobile phase consisted of 0.5% acetic acid in acetonitrile (A) and 0.5% acetic acid in water. The analytes were eluted with a gradient program: 0–5 min 5% A, 5–20 min 35% A, 20–25 min 35% A, 25–28 min 5% A. The flow rate was set at 1 ml/min. All the analytes were monitored at a UV wavelength of 280 nm. The injection volume for HPLC was 100 μl.

2.5.2.2. For samples containing salvianolic acid B. A 150 μl plasma sample was aliquoted in a glass centrifuge tube and spiked with 10 μl of internal standard (200 μg/ml of naringin) and 20 μl of phosphoric acid (25%). The acidified mixture was extracted with 1 ml of ethyl acetate by vortexing for 1 min and further mixing by a rotator for 20 min. After centrifuging at 4000 rpm for 10 min, the organic layer was collected. The same extraction procedure was repeated twice and the organic layers were combined and evaporated to dryness. The residue was reconstituted in 100 μl of 30% methanol in 0.01% formic acid. A 30 μl of the supernatant was injected to LC–MS–MS system for analysis.

LC/MS/MS system consisting of an API Q-Trap mass spectrometer (Applied Biosystems, Toronto, Canada) equipped with a two Pekin-Elmer PE-200 series micro-pumps and auto sampler (Perkin-Elmer, Norwalk, CT, USA) was used to analyze the concentrations of salvianolic acid B in various plasma samples. The chromatographic separation was achieved by using a XTerra MS C18 column (3.5 μm, 2.1 mm × 100 mm) connected with a XTerra MS C18 guard column (3.5 μm, 2.1 × 10 mm) (Waters, Milford, MA, USA). The mobile phase consisted of 0.01% formic acid (30%) and methanol (70%). The injection volume was 30 μl. Negative ion mode of the mass spectrometer was used for the analysis. The working mass spectrometer parameters were: declustering potential, −36 V; entrance potential, −7.5 V; curtain gas, 40 psi; collision gas, 5 psi; ionspray voltage, −4500 V; temperature, 400 °C; ion source gas 1, 50 psi; ion source gas 2, 50 psi. Detection of ions of salvianolic acid B and naringin were performed by monitoring the transitions of m/z 717 to m/z 321 for salvianolic acid B and m/z 579 to m/z 271 for naringin.

2.6. Calculations

The apparent permeability coefficient (Papp) was calculated as $\text{Papp} = [(dC/dt) \times V]/[A \times C]$ as we described before (Zhang et al., 2005b). For Caco-2 monolayer model, "dC/dt" (μg/ml/s) is the change of the accumulated drug concentration in the receiver chamber over time, "V" (cm^3) is the volume of the solution in the receiver chamber, "A" (cm^2) represents the membrane surface area of Caco-2 monolayer (4.7 cm^2), "C" (μg/ml) is the drug loading concentration in the donor chamber. All reported values represent mean ± SD ($n = 3$); For rat *in situ* intestinal perfusion model, "dC/dt" (μg/ml/s) is the change of the drug concentration appearing in the blood collected from mesenteric vein during the perfusion experiment; "V" (cm^3) is the volume of the collected blood sample; "A" (cm^2) is the surface area of the tested small intestine and "C" (μg/ml) is the loading drug concentration in the perfusate.

2.7. Data analysis for the pharmacokinetic parameters

Plasma concentrations versus time profiles obtained from individual animals were analyzed using WinNonlin software (Pharsight Corporation, Mountain View, CA, USA, Version 2.1). The non-compartmental data analysis was employed to estimate the

following pharmacokinetic parameters: terminal elimination half-life ($t_{1/2,\lambda z}$), area under the plasma concentration versus time curve from zero to infinity ($AUC_{0-\infty}$). The peak plasma concentration (C_{max}) and the time reaching C_{max} (T_{max}) were read directly from the observed individual plasma concentration–time data. Absolute bioavailability (F) was calculated based on the $AUC_{0-\infty}$ obtained after oral and intravenous administration of each pure compound at the same dose.

2.8. Statistical analysis

Statistical significance in the Papp values and pharmacokinetic parameters obtained from various treatment groups was estimated by the analysis of variance (student t-test). A p value of less than 0.05 was considered to be significantly different. All data were expressed as mean ± SD.

3. Results and discussions

3.1. Intestinal absorptions of danshensu and salvianolic acid B

3.1.1. Caco-2 monolayer model

In comparison with the negative control for MTT test, there was no significant decrease in the percentage of absorbance observed at all the studied concentrations of danshensu and salvianolic acid B. These results suggested that danshensu and salvianolic acid B loading solutions at the concentration range of 1–2000 μM should have no cytotoxicity effect on Caco-2 cells. In addition, danshensu and salvianolic acid B were all stable in the transport buffer for Caco-2 cell study at 37 °C for at least 3 h, indicating that the loading solutions prepared in the transport buffer are suitable for the current 2 h Caco-2 cell monolayer transport study.

Under the current detection limit for danshensu and salvianolic acid B, there were no danshensu and salvianolic acid B being detected in the receiver chamber at a loading concentration of 100 μM after either its apical or basolateral loading onto the Caco-2 monolayer model. Both compounds could not be detected in the receiver chamber even at a loading concentration of 400 μM. This suggested that both danshensu and salvianolic acid B may be poorly absorbed orally. In a recent study with Caco-2 model by Kim et al., the Papp value of salvianolic acid B was reported to be $(0.34 \pm 0.04) \times 10^{-6}$ cm/s (apical to basolateral) and $(0.31 \pm 0.03) \times 10^{-6}$ cm/s (basolateral to apical) at a loading concentration of 50 μM of salvianolic acid B, respectively (Kim et al., 2005). Although their sensitive analytical method allows the detection of salvianolic acid B, the obtained Papp values are still only comparable to that of the paracellular markers in Caco-2 monolayer model (Grès et al., 1998).

3.1.2. Rat in situ single pass intestinal perfusion model

In all the perfusion studies, there was no significant decrease of phenol red in all the perfusates, indicating that there was no serious leakage under the current perfusion experimental conditions. Both danshensu and salvianolic acid B could be detected in the mesenteric blood after their perfusions through the rat jejunum in the rat *in situ* single pass intestinal perfusion model. The calculated Papp values for danshensu were $(7.71 \pm 1.13) \times 10^{-6}$ cm/s and $(9.81 \pm 0.93) \times 10^{-6}$ cm/s at loading concentrations of 100 and 600 μM, respectively. The calculated Papp values for salvianolic acid B were $(7.14 \pm 0.94) \times 10^{-7}$ cm/s and $(5.16 \pm 0.99) \times 10^{-7}$ cm/s at loading concentrations of 250 and 500 μM, respectively. Although danshensu and salvianolic acid B became detectable in the rat *in situ* single pass intestinal perfusion model, the obtained Papp values of the two compounds are only comparable to that of the paracellular markers (Jonker et al., 2002; Wang et al., 1997).

In summary, findings from us as well as others in both *in vitro* and *in situ* intestinal absorption models consistently suggested that the poor intestinal permeability could be the cause for poor oral bioavailabilities of both danshensu and salvianolic acid B. In general, hydrophilic compounds transport across the intestinal barrier via paracellular pathway. Since the paracellular pathway occupies less than 0.1% of the total surface area of the intestine epithelium, and the presence of tight junctions between the epithelial cells limits drug absorption, thus the intestinal absorption of these hydrophilic compounds is considered to be small. Both danshensu and salvianolic acid B are very hydrophilic with log D of −0.69 and −3.42 for danshensu, −0.34 and −2.88 for salvianolic acid B at pH 4 and 7, respectively (Calculated using Advanced Chemistry Development (ACD/Labs) Software Solaris V4.67 (1994-2004 ACD/Labs)). Therefore, their hydrophilicities may be the main reason for the poor intestinal permeabilities of danshensu and salvianolic acid B. Salvianolic acid B has larger molecular weight (MW 718) than danshensu (MW 198), thus salvianolic acid B exhibited relatively lower permeability than danshensu in the rat *in situ* intestinal perfusion study. In addition, both Caco-2 cell monolayer study and rat *in situ* intestinal perfusion study indicated that danshensu and salvianolic acid B may be transported mainly via paracellular pathway with no active transport.

3.1.3. In vivo pharmacokinetics and bioavailabilities of danshensu and salvianolic acid B in rats

The *in vivo* pharmacokinetics in rats demonstrated that both danshensu and salvianolic acid B have limited bioavailabilities (Fig. 2 and Table 1).

3.1.3.1. Danshensu. Danshensu demonstrated a rapid distribution phase and eliminated rapidly from the systemic circulation with a $t_{1/2,\lambda z}$ of 42.92 ± 11.62 min after intravenous administration. After administered orally, danshensu was absorbed and eliminated the systemic circulation rapidly, with a T_{max} of about 38.00 ± 4.47 min and a $t_{1/2,\lambda z}$ of 45.37 ± 9.23 min. However, its C_{max} (0.60 ± 0.08 μg/ml) and $AUC_{0-\infty}$ (67.86 ± 10.96 μg/ml min) were much lower than that observed after same dose of intravenous administration. The calculated bioavailability (F) of danshensu was only 11.09%.

Until now, no absolute bioavailability of danshensu has been reported after the administration of either pure compound of danshensu or Danshen extract. In our study, the peak plasma concentration of danshensu was reached at 38.00 min, the elimination half-life of danshensu was 45.37 ± 9.23 min after oral administration of pure compound of danshensu. In a recently published pharmacokinetic study of danshensu, T_{max} of danshensu was 0.80 ± 0.21 h, and the half-life ($t_{1/2}$) was 0.50 ± 0.27 h after oral administration of 200 mg/kg Danshen extract (containing 40 mg/g danshensu) (Li et al., 2008), which are similar to our observation after oral administration of pure danshensu. In Hong's study, the peak plasma concentration of danshensu was reached at 30.00 min after oral administration of Fufang Danshen Dripping Pill solution (Hong et al., 2000). However, they reported a much slower clearance of danshensu with a $t_{1/2\beta}$ of 196.80 min. Fufan Danshen Dripping Pill is a composite of *Salvia miltiorrhiza*, *Panax notoginseng* and *Cinnamomum camphora* (Guo et al., 2003). Therefore, the components in Fufang Danshen Dripping Pill are very complicated, which may affect the pharmacokinetics of danshensu. In addition, the decomposition and metabolism of other phenolic acids of Danshen may lead to the formation of danshensu, which will result in longer elimination phase after oral administration of Fufang danshen products than the pure compound of danshensu (Yuan et al., 2005; Pan et al., 2003). The reason for the different elimination half-life of danshensu after oral administration of pure form of danshensu versus Fufang danshen extract needs further investigation.

Table 1
Pharmacokinetic parameter of danshensu and salvianolic acid B in rats in various treatment groups (mean ± S.D., $n = 5$).

Parameters	Danshensu (20 mg/kg)			Salvianolic acid B (50 mg/kg)		
	Intravenous	Oral	Oral + sodium caprate (100 mg/kg)	Intravenous	Oral	Oral + sodium caprate (100 mg/kg)
T_{max} (min)	NA	38.00 ± 4.47	40.00 ± 0.00	NA	30.00 ± 0.00	30.00 ± 0.00
C_{max} (μg/ml)	NA	0.60 ± 0.08	1.12 ± 0.31*	NA	1.67 ± 0.18	1.68 ± 0.17
$AUC_{0-\infty}$ (μg/ml min)	611.92 ± 66.25	67.86 ± 10.96	113.95 ± 28.32*	3671.98 ± 681.23	143.35 ± 21.89	194.98 ± 8.70*
$t_{1/2,\lambda z}$ (min)	42.92 ± 11.62	45.37 ± 9.23	41.14 ± 8.25	65.11 ± 2.76	73.66 ± 5.68	70.66 ± 6.59

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

Caco-2 cell monolayer study and rat *in situ* intestinal perfusion study indicated that danshensu was transported via paracellular pathway which may be the main reason for the low oral bioavailability of danshensu. In addition, metabolism of danshensu could also contribute to its low oral bioavailability. Xiaohui et al. reported the metabolites of danshensu in rabbit after oral ingestion of Fufang Danshen Dripping Pill solution. It was reported that one of the main metabolites of danshensu in rabbit was isopropyl 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoate, which was proved to be a bioactive metabolite with vasodilatation effect (Xiaohui et al., 2007). It is expected that further detailed pharmacokinetic study of the active metabolites may provide more correlations to their pharmacodynamic effects *in vivo*.

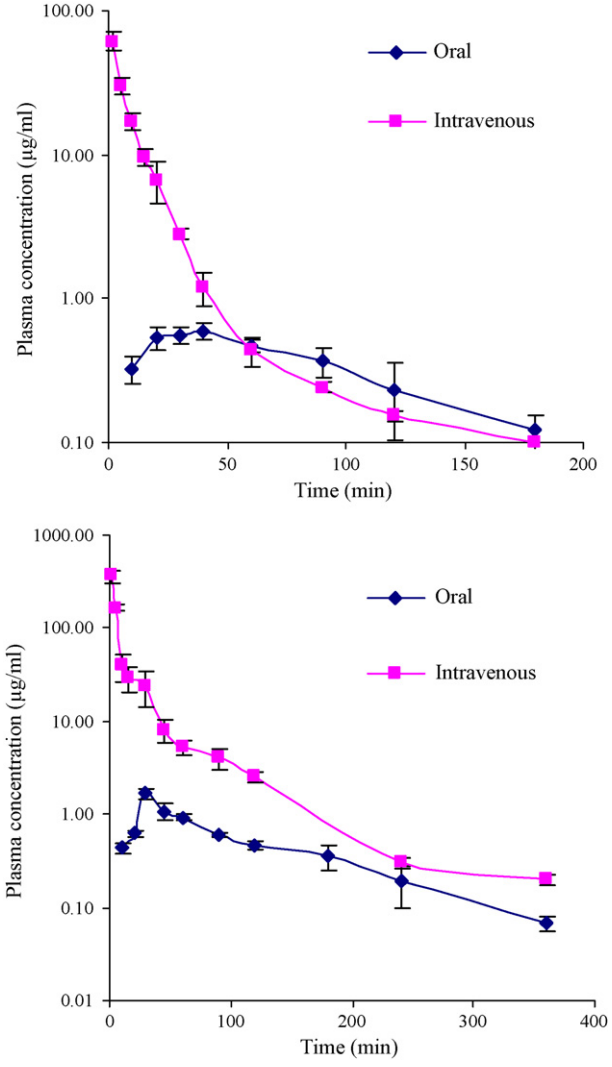

Fig. 2. Mean plasma concentration versus time profiles of danshensu (Upper) and salvianoic acid B (Lower) in rats after oral and intravenous administrations 20 mg/kg danshensu and 50 mg/kg salvianolic acid B. Each point represents mean ± SD ($n = 5$).

3.1.3.2. Salvianolic acid B. Salvianolic acid B eliminated rapidly with a half-life ($t_{1/2,\lambda z}$) of 65.11 ± 2.76 min after intravenous administration of 50 mg/kg of pure compound of salvianolic acid B. After administered orally, salvianolic acid B was absorbed quickly with a T_{max} of about 30.00 ± 0.00 min, $t_{1/2,\lambda z}$ of 73.66 ± 5.68 min after oral administration of 50 mg/kg salvianolic acid B. However, its C_{max} (1.67 ± 0.18 μg/ml) and $AUC_{0-\infty}$ (143.35 ± 21.89 μg/ml min) were extremely low when comparing to that obtained after intravenous administration at the same dose. The calculated absolute bioavailability (F) of salvianolic acid B was only 3.90%.

Our results from Caco-2 cell monolayer study and rat *in situ* intestinal perfusion study indicated that salvianolic acid B may be mainly absorbed via paracellular transport pathway. The hydrophilic property and large molecular (MW 718) size of salvianolic acid B lead to its very poor intestinal permeability as well as the low oral bioavailability *in vivo*. Zhang et al. found that about 65% of the dosed salvianolic acid B remained in the gastrointestinal tract 4 h after oral administration and more than 50% of the dosed salvianolic acid B reached the lower parts of the intestine (Zhang et al., 2004b).

Since the year of 2004, there are quite a few papers published on the pharmacokinetics of salvianolic acid B. Due to the poor oral absorption of salvianolic acid B, the absolute bioavailabilities of salvianolic acid B in majority of the published papers were obtained from the pharmacokinetics profiles of unequal intravenous and oral dose (relatively higher dose than that of intravenous). Kim et al. found a dose-dependent pharmacokinetics of salvianolic acid B after intravenous and oral administration of 10 and 50 mg/kg of pure salvianolic acid B to rats (Kim et al., 2005). Based on the pharmacokinetic profiles obtained from 50 mg/kg oral and intravenous dose, the bioavailability of salvianolic acid B was calculated to be 5.00%. Wu et al. gave pure salvianolic acid B (intravenous: 100 mg/kg; oral: 500 mg/kg) to rat and the oral bioavailability of salvianolic acid B was calculated to be 2.30% (Wu et al., 2006). These results were close to what we obtained. Although the same species of rat (Sprague-Dawley) and doses of salvianolic acid B (oral administration 50 mg/kg) were used for both studies, Kim et al. found a C_{max} of 8.93 μg/ml reached at 45.00 min, whereas a C_{max} of only 1.67 μg/ml was found at 30.00 min in our study (Kim et al., 2005). In the study by Kim et al., the elimination half-life was 103.00 min after intravenous administration of 50 mg/kg pure compound of salvianolic acid B, which was somewhat longer than that obtained in our study (Kim et al., 2005). Such different findings may result from the different animal surgical treatment (we did the surgical experiment a day ahead of the drug administration whereas Kim did not state the specific surgical time) and drug administration site (Kim et al. infuse the drug solution through the femoral vein and sample the blood via jugular vein whereas we conducted the iv bolus via jugular vein followed by saline washing before we sampled any blood from the same site). In addition, with a more sensitive LC/MS/MS method,

the concentration of salvianolic acid B at last sampling point was found to be 30.00 ng/ml in Kim et al.'s study, whereas a concentration of 70.00 ng/ml was found in ours, which was close to the LOD of our assay. Therefore, the discrepancy in half-life from the two studies could also be due to the plasma concentration variations from the terminal sampling points (Kim et al., 2005).

After oral administration of Danshen extract (Zhang et al., 2005a), it was found that salvianolic acid B reached up to its maximum concentration within 30.00 min and could be detected up to 180.00 min after oral administration. However, its elimination half-life was only 19.16 min. Due to majority of the studies using Danshen extract did not report the dose of salvianolic acid B in the extract, it is difficult to compare the result with ours.

In addition to poor intestinal permeability, another possible reason for the low bioavailability of salvianolic acid B could be its metabolism. Zhang et al. isolated four major methylated metabolites from bile collected from rats after intravenous administration of salvianolic acid B. They found that almost 100% of the dose (95% as the methylated metabolites and 5% as salvianolic acid B) was recovered in the bile within 30 h after injection (Zhang et al., 2004b; Zhang et al., 2004c). Li et al. also indicated that the majority of the metabolites of salvianolic acid B were excreted directly into the bile (Li et al., 2007). Therefore, the low bioavailability of salvianolic acid B caused by its first pass metabolism could not be excluded.

3.2. Effect of sodium caprate on the intestinal absorption and bioavailabilities of danshensu and salvianolic acid B

In general, hydrophilic compounds transport across the intestinal barrier via paracellular pathway. Since the paracellular pathway occupies less than 0.1% of the total surface area of the intestine epithelium, and the presence of tight junctions between the epithelial cells limits drug absorption, thus the intestinal absorption of these hydrophilic compounds is considered to be small. Therefore, the main cause of low bioavailabilities for danshensu and salvianolic acid B is their poor intestinal permeabilities.

In recent years, many studies have been reported on how to increase the membrane permeation of drugs with paracellular transport. Among the commonly used approaches to increase the intestinal permeability of hydrophilic drugs, the use of absorption enhancer is the most well established strategy for developing oral formulations with enhanced bioavailability. Although the mechanistic actions of paracellular permeation enhancers are still not well identified, the commonly held view is that increases in paracellular permeability result from the dilation of existing tight junction (Anderberg et al., 1993; Sawada et al., 1991). Compounds have been reported as potential paracellular enhancer include surfactants (such as polyoxyethylene esters, dodecylmaltoside), medium-chain fatty acids (such as caprylate C8, caprate C10 and laurate C12), medium-chain glycerides which generally refers to monoglycerides and diglycerides of acprylic and capric acid, steroidal detergents (such as chenodeoxycholate, ursodeoxycholate), acylcarnitines and alkanoylcholines (such as palmitoyl-DL-carnitine), chitosans (such as chitosan hydrochloride, chitosan glutamate) and and its derivatives (such as *N*-trimethyl chitosan hydrochloride, mono-*N*-carboxymethyl chitosan) (Aungst, 2000; Ward et al., 2000). Among these reported paracellular transport enhancers, sodium caprate (caprate C10: CH_3–$(CH_2)_8$–COONa), which has a natural presence in foodstuffs and dairy products, is the most extensively studied and the only absorption enhancer included in a marketed product for human use in the form of a suppository product (ampicillin) used in Japan, Sweden and Denmark. Since sodium caprate has a low molecular weight (MW 194.25), it could be absorbed from the intestine even more quickly than the drug itself. So far, there has been no report on any serious side effect of drug formulations containing sodium caprate (Aungst, 2000; Sakai et al., 1997; Lindmark et al., 1998; Takahashi et al., 1994).

3.2.1. Cytotoxicity test of sodium caprate in Caco-2 cells

Caco-2 cells were exposed to various concentrations of sodium caprate (0.01%, 0.03%, 0.05%, 0.10%, 0.25%, 0.40%, 0.50%, 0.80% w/v) with addition of 100 μM of danshensu or salvianolic acid B for 2 h. It was shown that sodium caprate at concentrations up to 0.25% (w/v) was safe to the Caco-2 cells. Chao et al. reported that cell culture models are often found to be more sensitive to the cytotoxic effects of permeation enhancers than intact intestinal membrane. Some enhancers that have demonstrated clear cytotoxic effect in Caco-2 studies only caused relatively mild damage to the intestinal tissue (Aungst, 2000; Chao et al., 1999). In the study performed by Motlekar et al., sodium caprate at a concentration of 0.125% (w/v) was found to be toxic to the cells after 12 h of incubation. However, no perceptible evidence of mucosal irritation or damage was obtained when the oral formulation containing 100 mg/kg sodium caprate was delivered to the rat *in vivo* as evidenced by histological studies (Motlekar et al., 2005).

3.2.2. Effect of sodium caprate on the integrity of Caco-2 cell monolayer and the absorption of paracellular marker in the Caco-2 monolayer model

The effect of sodium caprate on the TEER of Caco-2 cell monolayer was examined at 120 min. It was found that sodium caprate in the concentration range of 0.0625–0.25% (w/v) reduced the TEER of Caco-2 monolayer in a dose-dependent manner. At the concentration of 0.25% (w/v) the TEER of the Caco-2 monolayer was decreased by 56.80%, indicating the role of sodium caprate on the tight junctions of the cells. Chao et al. also reported that the addition of sodium caprate at the concentration range of 0.25–0.48% (w/v) quickly reduced the TEER of Caco-2 monolayer by 40.00–65.00%. However, recovery of TEER was recorded upon wash-out of sodium caprate from donor chamber, suggesting that the permeability-enhancing effect of sodium caprate on the Caco-2 cells is readily reversible (Chao et al., 1999).

In addition, the permeation-enhancing activity of sodium caprate was investigated by determining its effect on the transport of paracellular marker compound (atenolol). The Papp of atenolol across Caco-2 was doubled with addition of 0.0625% (w/v) of sodium caprate (from $(1.02 \pm 0.14) \times 10^{-6}$ cm/s to $(2.11 \pm 0.21) \times 10^{-6}$ cm/s), and increased at 20-fold at concentrations of 0.25% (w/v) of sodium caprate (from $(1.02 \pm 0.14) \times 10^{-6}$ cm/s to $(2.18 \pm 0.19) \times 10^{-5}$ cm/s), indicating that sodium caprate did demonstrate significant enhancement effect for this typical paracellular marker. The above results confirmed that sodium caprate could enhance the absorption of compound which transport through paracellular pathway.

When 100 μM danshensu or salvianolic acid B were loaded with the addition of different concentration of sodium caprate at the apical side of Caco-2 cell monolayer model, the absorption transport of both danshensu and salvianolic acid B increased significantly. Without of the addition of sodium caprate, both danshensu and salvianolic acid B could not be detected at the basolateral side, but with the addition of 0.25% (w/v) of sodium caprate, the Papp values of danshensu and salvianolic acid B were increased to $(13.20 \pm 1.30) \times 10^{-6}$ cm/s for danshensu, and $(41.40 \pm 4.40) \times 10^{-6}$ cm/s for salvianolic acid B.

3.2.3. Effect of sodium caprate on the intestinal absorption of danshensu and salvianolic acid B in rat in situ intestinal perfusion model

Concentrations of 600 μM danshensu and 250 μM salvianolic acid B were chosen for the current study based on the considerations of the studied drug solubility in the perfusion buffer,

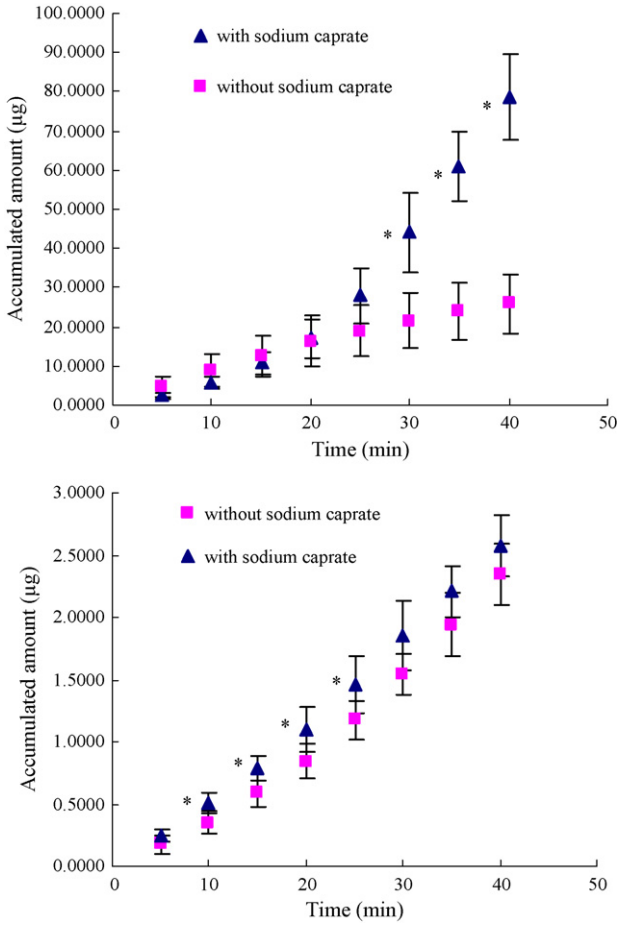

Fig. 3. Effect of sodium caprate (0.5% w/v) on the accumulated amount of danshensu or salvianolic acid B in mesenteric blood after perfuse 600 μM danshensu (Upper) or 250 μM salvianolic acid B (Lower) in rat *in situ* single pass intestinal perfusion model. Each point represents mean ± SD ($n = 5$). *$p < 0.05$.

detection limit of the studied compounds in mesenteric blood and drug concentration that will allow us to see the significant effect. The effect of sodium caprate on the absorption of danshensu and salvianolic acid B was also investigated using rat *in situ* intestinal perfusion model. Fig. 3 shows the effect of sodium caprate (0.50% w/v) on the accumulated amount of danshensu in the mesenteric blood. It is obvious that the accumulated amount of danshensu in the mesenteric blood was significantly increased with the addition of sodium caprate. The calculated Papp value of danshensu was significantly increased from $(9.81 \pm 0.93) \times 10^{-6}$ cm/s to $(3.23 \pm 0.23) \times 10^{-5}$ cm/s with the addition of sodium caprate. Although no significant difference was found with the Papp value of salvianolic acid B with the addition of sodium caprate (from $(7.13 \pm 0.94) \times 10^{-7}$ cm/s to $(8.16 \pm 0.71) \times 10^{-7}$ cm/s), the mean accumulated amount of salvianolic acid B in the mesenteric blood did demonstrate a trend of increase with the addition of sodium caprate (0.50% w/v) (Fig. 3).

Although enhancement effect of sodium caprate observed in Caco-2 cell monolayer model did not differ too much between danshensu and salvianolic acid B, such enhancement obtained from *in situ* perfusion model for danshensu was more significant than that of salvianolic acid B. This may be due to the fact that the cell culture models are usually more sensitive to the permeability enhancement effect of permeation enhancer than intact intestine membrane (Aungst, 2000; Chao et al., 1999). Therefore, the sensitive Caco-2 cell model could not differentiate the enhancement effect between danshensu and salvianolic acid B in presence of sodium caprate. However, the intestinal tissue may not be as sensitive to sodium caprate as *in vitro* cells. In rat *in vivo* studies, Lindmark et al. pointed out that permeability enhancement would not result in a significant increase in the case of larger molecules (MW > 1200) (Lindmark et al., 1998). The molecule weight of salvianolic acid B (MW 718) is much larger than that of danshensu (MW 198), which could also provide an explanation on the less significant absorption enhancement effect on salvianolic acid B through rat intestine with the same addition of sodium caparate.

3.2.4. *Effect of sodium caprate on the in vivo pharmacokinetics of danshensu and salvianolic acid B*

As shown in Fig. 4 and Table 1, increased intestinal absorption was observed after oral administration of danshensu with the addition of 100 mg/kg of sodium caprate. The C_{max} was almost doubled ($p < 0.05$) and the $AUC_{0-\infty}$ was significantly increased from 67.86 ± 10.96 to 113.95 ± 28.32 μg/ml min ($p < 0.05$). The calculated bioavailability of danshensu was increased from 11.09% to 18.62%. Similarly, when salvianolic acid B was administered with the addition of 100 mg/kg of sodium caprate, its $AUC_{0-\infty}$ was significantly increased from 143.35 ± 21.89 to 194.98 ± 8.70 μg/ml min ($p < 0.05$). The bioavailability of salvianolic acid B was increased from 3.90% to 5.31% (Fig. 4 and Table 1).

In the present study, 100 mg/kg of sodium caprate was used to evaluate the intestinal absorption enhancement of danshensu and salvianolic acid B *in vivo*. The major concern would be its potential toxicity to the intestinal tissue. According to the previous report on oral formulation containing 100 mg/kg of sodium caprate delivered to the rat, there is no perceptible evidence of mucosal irritation or damage was obtained as shown in the histological studies (Motlekar et al., 2005). It has been reported that intestinal permeability enhancement brought about *in vitro* by enhancers may not be replicable *in vivo* (Scott-Moncrieff et al., 1994). This may be partly explained by assuming that the enhancer delivered into the GI tract tends to be diluted quickly by the luminal content, resulting in reduced efficacy. However, in our study, the enhancing effect of sodium caprate on *in vitro* Caco-2 cell monolayer model and on rat *in situ* intestinal perfusion model was confirmed *in vivo*. It is suggested that increased absorption of danshensu and salvianolic acid B was not caused by damage of the GI epithelium after the oral administration of sodium caprate at the administered dose.

In order to evaluate the enhancement effect of sodium caprate on the Danshen product, commercial Danshen tablet with or without the addition of sodium caprate was given to rats. With the addition of 100 mg/kg of sodium caprate, increased intestinal absorption of both danshensu and salvianolic acid B in Danshen tablet were also observed. As shown in Fig. 4 and Table 2, the C_{max} of danshensu was significantly increased from 0.70 ± 0.04 to 1.21 ± 0.19 μg/ml ($p < 0.05$), and the $AUC_{0-\infty}$ of danshensu was significantly increased from 59.88 ± 5.68 to 108.66 ± 6.51 μg/ml min ($p < 0.05$). The $AUC_{0-\infty}$ of salvianolic acid B was significantly increased from 176.75 ± 15.07 to 247.78 ± 17.55 μg/ml min ($p < 0.05$) as shown in Fig. 4 and Table 2. The addition of sodium caprate in the Danshen product did increase the intestinal absorption of danshensu and salvianolic acid B in Danshen product, suggesting that sodium caprate could serve as a potential intestinal absorption enhancer for Danshen oral product.

It was also noticed that the mean values of $AUC_{0-\infty}$ (176.75 ± 15.07 μg/ml min) of salvianolic acid B after oral administration of Danshen tablet (Table 2) in the current study was a bit higher than that obtained after oral administration of pure salvianolic acid B ($AUC_{0-\infty}$: 143.35 ± 21.89 μg/ml min) (Table 1), however, with no significant difference. Similarly, when comparing danshensu pharmacokinetic parameters between its pure compound form and tablet form (Tables 1 and 2), there is no significant difference in T_{max}, C_{max}, $AUC_{0-\infty}$ and $t_{1/2,\lambda z}$. It appears that the co-

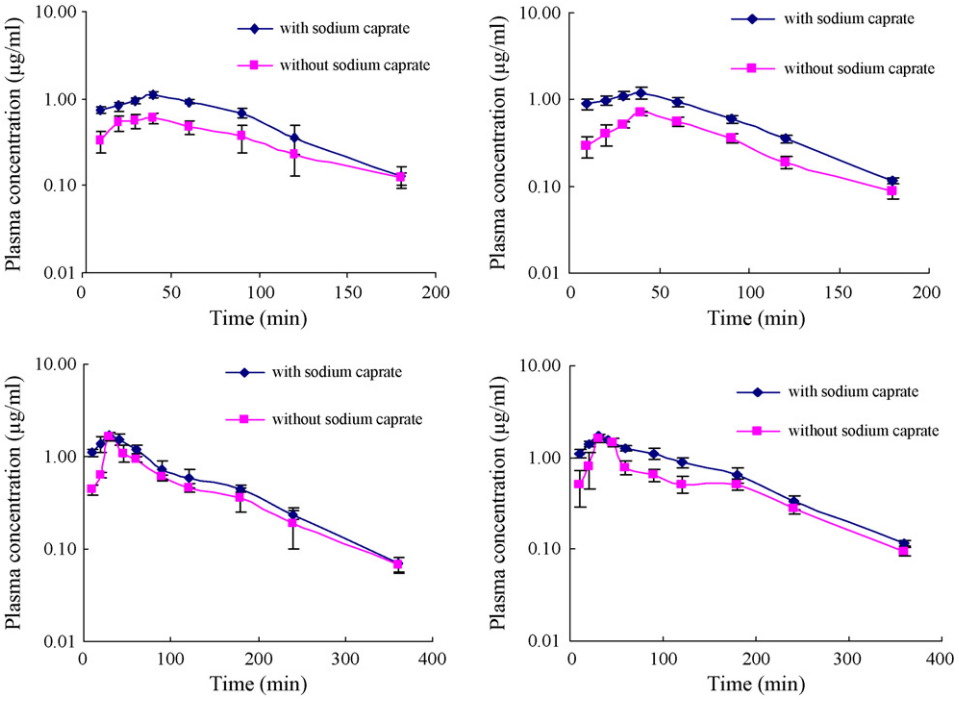

Fig. 4. Mean plasma concentration versus time profiles after oral administration of 20 mg/kg danshensu (Upper left: pure danshensu; Upper right: Danshen tablet extraction) and 50 mg/kg salvianolic acid B (Lower left: pure salvianolic acid B; Lower right: Danshen tablet extraction) with or without sodium caprate (100 mg/kg) ($n = 5$).

Table 2
Pharmacokinetic parameter of danshensu and salvianolic acid B in rats after oral administration of Danshen commercial product with or without the addition of sodium caprate (mean ± S.D., $n = 5$).

Parameters	Danshen tablet (danshensu 20 mg/kg)		Danshen tablet (salvianolic acid B 50 mg/kg)	
	Without sodium caprate	With sodium caprate (100 mg/kg)	Without sodium caprate	With sodium caprate (100 mg/kg)
T_{max} (min)	40.00 ± 0.00	40.00 ± 0.00	33.00 ± 6.71	30.00 ± 0.00
C_{max} (μg/ml)	0.70 ± 0.04	1.21 ± 0.19*	1.70 ± 0.09	1.73 ± 0.06
$AUC_{0-\infty}$ (μg/ml min)	59.88 ± 5.68	108.66 ± 6.51*	176.75 ± 15.07	247.78 ± 17.55*
$t_{1/2,\lambda z}$ (min)	46.36 ± 5.33	40.25 ± 2.89	73.49 ± 2.73	74.41 ± 5.02

* $p < 0.05$.

occurring components in the extract may not significantly affect the bioavailabilities of danshensu and salvianolic acid B.

4. Conclusions

Both danshensu and salvianolic acid B possess poor intestinal permeabilities, which are mainly due to their hydrophilic properties and paracellular absorption transport pathway. Current findings from *in vitro*, *in situ* and *in vivo* consistently demonstrated that sodium caprate could increase the intestinal absorption and bioavailabilities of danshensu and salvianolic acid B, which suggested the usefulness of sodium caprate as the potential absorption enhancer for danshensu and salvianolic acid B. Addition of sodium caprate in the Danshen product further proved the usefulness of sodium caprate for enhancing the oral absorption of danshensu and salvianolica acid B in Danshen product. Our study not only identifies the role of sodium caprate as the absorption enhancer for the improved oral delivery of Danshen but also demonstrate the importance of biopharmaceutics and pharmacokinetics characterization of the active ingredients in the further dosage form development of traditional Chinese medicine products.

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References

Anderberg, E.K., Lindmark, T., Artursson, P., 1993. Sodium caprate elicits dilatations in human intestinal tight junctions and enhances drug absorption by the paracellular route. Pharm. Res. 10, 857–864.
Aungst, B.J., 2000. Intestinal permeation enhancers. J. Pharm. Sci. 89, 429–442.
Cao, J., Wei, Y.J., Qi, L.W., Li, P., Qian, Z.M., Luo, H.W., Chen, J., Zhao, J., 2008. Determination of fifteen bioactive components in Radix Rhizoma Salviae Miltiorrhizae by high-performance liquid chromatography with ultraviolet and mass spectrometric detection. Biomed. Chromatogr. 22, 164–172.
Chan, K., Chui, S.H., Wong, D.Y., Ha, W.Y., Chan, C.L., Wong, R.N., 2004. Protective effects of danshensu from the aqueous extract of *Salvia miltiorrhiza* (Danshen) against homocysteine-induced endothelial dysfunction. Life Sci. 75, 3157–3171.
Chang, Q., Zuo, Z., Ho, W.K., Chow, M.S., 2005. Comparison of the pharmacokinetics of hawthorm phenolics in extract versus individual pure compound. J. Clin. Pharmacol. 45, 106–112.
Chao, A.C., Nguyen, J.V., Broughall, M., Griffin, A., Fix, J.A., Daddona, P.E., 1999. *In vitro* and *in vivo* evaluation of effects of sodium caprate on enteral peptide absorption and on mucosal morphology. Int. J. Pharm. 191, 15–24.
Grès, M.C., Julian, B., Bourrié, M., Meunier, V., Roques, C., Berger, M., Boulenc, X., Berger, Y., Fabre, G., 1998. Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. Pharm. Res. 15, 726–733.
Guo, Z.X., Jia, W., Gao, W.Y., Xu, Z.H., Zhao, L.B., Xiao, P.G., 2003. Clinical investigation of composite Danshen Dripping Pill for the treatment of angina pectoris. Zhongguo Tianran Yaowu 1, 124–128.
Hong, X., Mi, S.Q., Wang, N.S., 2000. Pharmacokinetics study of danshensu in compound Danshen Dripping Pill. Zhongyao Xinyao yu Linchuang Yaoli 11, 286–288.

Jonker, C., Hamman, J.H., Kotzé, A.F., 2002. Intestinal paracellular permeation enhancement with quaternised chitosan: *in situ* and *in vitro* evaluation. Int. J. Pharm. 238, 205–213.

Kang, D.G., Oh, H., Sohn, E.J., Hur, T.Y., Lee, K.C., Kim, K.J., Kim, T.Y., Lee, H.S., 2004. Lithospermic acid B isolated from *Salvia miltiorrhiza* ameliorates ischemia/reperfusion-induced renal injury in rats. Life Sci. 75, 1801–1816.

Kim, H.H., Kim, J., Ji, H.Y., Kim, Y.C., Sohn, D.H., Lee, B.M., Lee, H.S., 2005. Pharmacokinetics of lithospermic acid B isolated from *Salvia miltiorrhiza* in rats. J. Toxicol. Environ. Health A. 68, 2239–2247.

Li, C.Z., Yang, S.C., Zhao, F.D., Yang, Y.Q., Yang, C.X., Zhang, D.C., 1983. Anticoagulant studies of *Danshen*. Zhongxiyi Jiehe Zazhi 3, 297–299.

Li, M., Zhao, C., Wong, R.N., Goto, S., Wang, Z.M., Liao, F.L., 2004. Inhibition of shear-induced platelet aggregation in rat by tetramethylpyrazine and salvianolic acid B. Clin. Hemorheol. Microcirc. 31, 97–103.

Li, W., Li, Z.W., Han, J.P., Li, X.X., Gao, J., Liu, C.X., 2008. Determination and pharmacokinetics of danshensu in rat plasma after oral administration of danshen extract using liquid chromatography/tandem mass spectrometry. Eur. J. Drug Metab. Pharmacokinet. 33, 9–16.

Li, X., Yu, C., Lu, Y., Gu, Y., Lu, J., Xu, W., Xuan, L., Wang, Y., 2007. Pharmacokinetics, tissue distribution, metabolism, and excretion of depside salts from *Salvia miltiorrhiza* in rats. Drug Metab. Dispos. 35, 234–239.

Lindmark, T., Schipper, N., Lazorová, L., de Boer, A.G., Artursson, P., 1998. Absorption enhancement in intestinal epithelial Caco-2 monolayers by sodium caprate: assessment of molecular weight dependence and demonstration of transport routes. J. Drug Target. 5, 215–223.

Liu, M., Li, Y.G., Zhang, F., Yang, L., Chou, G.X., Wang, Z.T., Hu, Z.B., 2007. Chromatographic fingerprinting analysis of Danshen root (Salvia miltiorrhiza Radix et Rhizoma) and its preparations using high performance liquid chromatography with diode array detection and electrospray mass spectrometry (HPLC-DAD-ESI/MS). J. Sep. Sci. 30, 2256–2267.

Liu, X.Q., Luo, X.T., Ouyang, X.A., 2001. Research advance of Fufang Danshen dosage forms. Shizhen Guoyi Guoyao 12, 474–475.

Luo, X.J., Bi, K.S., Zhou, S.Y., Wei, Q.H., Zhang, R.H., 2001. Determination of danshensu, a major active compound of Radix *Salvia miltiorrhiza* in dog plasma by HPLC with fluorescence detection. Biomed. Chromatogr. 15, 493–496.

Ma, H.L., Qin, M.J., Qi, L.W., Wu, G., Shu, P., 2007. Improved quality evaluation of Radix *Salvia miltiorrhiza* through simultaneous quantification of seven major active components by high-performance liquid chromatography and principal component analysis. Biomed. Chromatogr. 21, 931–939.

Motlekar, N.A., Srivenugopal, K.S., Wachtel, M.S., Youan, B.B., 2005. Oral delivery of low-molecular-weight heparin using sodium caprate as absorption enhancer reaches therapeutic levels. J. Drug Target. 13, 573–583.

O, K., Cheung, F., Sung, F.L., Zhu, D.Y., Siow, Y.L., 2000. Effect of magnesium tanshinoate B on the production of nitric oxide in endothelial cells. Mol. Cell Biochem. 207, 35–39.

O, K., Lynn, E.G., Vazhappilly, R., Au-Yeung, K.K., Zhu, D.Y., Siow, Y.L., 2001. Magnesium tanshinoate B (MTB) inhibits low-density lipoprotein oxidation. Life Sci. 68, 903–912.

Ouyang, X.S., Takahashi, K., Komatsu, K., Nakamura, N., Hattori, M., Baba, A., Azuma, J., 2001. Protective effects of *Salvia miltiorrhiza* on angiotensin II-induced hypertrophic responses in neonatal rat cardiac cells. Jpn. J. Pharmacol. 87, 289–296.

Pan, G.X., Zhang, B.L., Gao, X.M., 2003. Determination of danshensu in rat serum after oral administer compound Salvia Recipe. Zhongguo Zhongyao Zazhi 28, 562–565.

Pei, W.J., Zhao, X.F., Zhu, Z.M., Lin, C.Z., Zhao, W.M., 2004. Study of the determination and pharmacokinetics of compound Danshen Dripping Pill in human serum by column switching liquid chromatography electrospray ion trap mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 809, 237–242.

Sakai, M., Imai, T., Ohtake, H., Azuma, H., Otagiri, M., 1997. Effects of absorption enhancers on the transport of model compounds in Caco-2 cell monolayers: assessment by confocal laser scanning microscopy. J. Pharm. Sci. 86, 779–785.

Sawada, T., Ogawa, T., Tomita, M., Hayashi, M., Awazu, S., 1991. Role of paracellular pathway in nonelectrolyte permeation across rat colon epithelium enhanced by sodium caprate and sodium caprylate. Pharm. Res. 8, 1365–1371.

Scott-Moncrieff, J.C., Shao, Z., Mitra, A.K., 1994. Enhancement of intestinal insulin absorption by bile salt-fatty acid mixed micelles in dogs. J. Pharm. Sci. 83, 1465–1469.

Shi, Z.H., He, J.T., Yao, T.T., Chang, W.B., Zhao, M.P., 2005. Simultaneous determination of cryptotanshinone, tanshinone I and tanshinone IIA in traditional Chinese medicinal preparations containing Radix salvia miltiorrhiza by HPLC. J. Pharm. Biomed. Anal. 37, 481–486.

Takahashi, K., Murakami, T., Yumoto, R., Hattori, T., Higashi, Y., Yata, N., 1994. Decanoic acid induced enhancement of rectal absorption of hydrophilic compounds in rats. Pharm. Res. 11, 1401–1404.

Wang, Y., Aun, R., Tse, F.L., 1997. Absorption of D-glucose in the rat studied using *in situ* intestinal perfusion: a permeability-index approach. Pharm. Res. 14, 1563–1567.

Ward, P.D., Tippin, T.K., Thakker, D.R., 2000. Enhancing paracellular permeability by modulating epithelial tight junctions. Pharm. Sci. Technol. Today 3, 346–358.

Wei, Y.J., Li, S.L., Li, P., 2007. Simultaneous determination of seven active components of Fufang Danshen Tablet by high performance liquid chromatography. Biomed. Chromatogr. 21, 1–9.

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.

Wu, Y.T., Chen, Y.F., Hsieh, Y.J., Jaw, I., Shiao, M.S., Tsai, T.H., 2006. Bioavailability of salvianolic acid B in conscious and freely moving rats. Int. J. Pharm. 326, 25–31.

Xiaohui, Z., Xinfeng, Z., Xin, Z., Shixiang, W., Yinmao, W., Jianbin, Z., 2007. Determination of the main bioactive metabolites of Radix salvia miltiorrhizae in compound Danshen dripping pills and the tissue distribution of Danshensu in rabbit by SPE-HPLC-MSn. J. Sep. Sci. 30, 851–857.

Yuan, D., Pan, Y.N., Fu, W.W., Makino, T., Kano, Y., 2005. Quantitative analysis of the marker compounds in *Salvia miltiorrhiza* root and its phytomedicinal preparations. Chem. Pharm. Bull. 53, 508–514.

Zhang, H., Yu, C., Jia, J.Y., Leung, S., Siow, Y.L., Man, Y.K., Zhu, D.Y., 2002. Contents of four active components in different commercial crude drugs and preparations of Danshen (*Salvia miltiorrhiza*). Zhongguo Xinyao Zazhi 23, 1163–1168.

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

Zhang, L., Lin, G., Chang, Q., Zuo, Z., 2005b. Role of intestinal first-pass metabolism of baicalein in its absorption process. Pharm. Res. 22, 1050–1058.

Zhang, L., Zheng, Y., Chow, M.S., Zuo, Z., 2004a. Investigation of intestinal absorption and disposition of green tea catechins by Caco-2 monolayer model. Int. J. Pharm. 287, 1–12.

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

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

Zhao, B.L., Jiang, W., Zhao, Y., Hou, J.W., Xin, W.J., 1996. Scavenging effects of *Salvia miltiorrhiza* on free radicals and its protection for myocardial mitochondrial membranes from ischemia–reperfusion injury. Biochem. Mol. Biol. Int. 38, 1171–1182.

Zhou, L., Chow, M., Zuo, Z., 2006. Improved quality control method for Danshen products—consideration of both hydrophilic and lipophilic active components. J. Pharm. Biomed. Anal. 41, 744–750.

Zhou, L., Zuo, Z., Chow, M.S., 2005. Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J. Clin. Pharmacol. 45, 1345–1359.