International Journal of Pharmaceutics 410 (2011) 68–74

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Danshen extract does not alter pharmacokinetics of docetaxel and clopidogrel, reflecting its negligible potential in P-glycoprotein- and cytochrome P4503A-mediated herb–drug interactions

Joo Hyun Lee [a], Yong-Jun Shin [b], Hye Jin Kim [a], Ju-Hee Oh [b], Young Pyo Jang [a,b], Young-Joo Lee [a,b,*]

[a] College of Pharmacy, Kyung Hee University, Seoul 130-701, Republic of Korea
[b] Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul 130-701, Republic of Korea

ARTICLE INFO

Article history:
Received 20 December 2010
Received in revised form 10 March 2011
Accepted 12 March 2011
Available online 21 March 2011

Keywords:
Danshen
Docetaxel
Clopidogrel
Pharmacokinetic interaction
P-glycoprotein
Cytochrome P450 3A

ABSTRACT

Danshen (*Salvia miltiorrhiza*) contains tanshinones, which inhibit P-glycoprotein (P-gp) and the cytochrome P450 (CYP) system. In the present study, we evaluated the possible pharmacokinetic interactions of Danshen extract with docetaxel and clopidogrel in rats. Docetaxel (5 mg/kg intravenously and 40 mg/kg orally) or clopidogrel (30 mg/kg orally) was administered to rats with or without oral co-administration of Danshen (400 mg/kg). Co-administration of Danshen did not affect the plasma concentration profiles and pharmacokinetic parameters of docetaxel and clopidogrel, whereas cyclosporine A, a P-gp and CYP3A inhibitor, significantly influenced the pharmacokinetics of co-administered docetaxel and clopidogrel. Orally administered Danshen had no substantial effect on the pharmacokinetics of docetaxel and clopidogrel, suggesting the negligible safety concern of Danshen in P-gp- and CYP3A-mediated interactions in vivo.

1. Introduction

Danshen, the dried root of *Salvia miltiorrhiza*, is used in Chinese medicine to treat coronary heart and cerebrovascular diseases (Zhou et al., 2005). According to Chinese medicine theory, it promotes blood flow and resolves blood stasis. The annual sales volumes of Danshen containing products have exceeded $140 million U.S. since 2002 (Lu et al., 2008). This herbal medicine is also available as a prescription or an over-the-counter drug in countries such as Singapore, Republic of Korea, India, the United Arab Emirates, Russia, Cuba, and South Africa, and as a dietary supplement in the United States (Lu et al., 2008). Thus, safety of administered Danshen is of great concern. Danshen as a treatment or dietary supplement is often administered in combination with therapeutic drugs, causing clinically important herb–drug interactions and adverse outcomes (Holbrook et al., 2005; Hu et al., 2005). For example, Danshen–anticoagulant warfarin interactions increase the international normalized ratio and the risk of bleeding in patients (Chan et al., 1995; Izzo et al., 2005; Yu et al., 1997).

The effects of Danshen on other drugs are caused by its major ingredients, namely, tanshinone I (TSI), tanshinone IIA (TSA), tanshinone IIB (TSB), dihydrotanshinone, and cryptotanshinone (CTS). Li et al. (2008) found that TSI is a substrate and inhibitor of P-glycoprotein (P-gp) using a Caco-2 monolayer model. In Caco-2 monolayer models and single pass rat intestinal perfusion models, TSA (Yu et al., 2007a), TSB (Yu et al., 2007b), and CTS (Zhang et al., 2006) are also substrates for P-gp and may also be reversing agents of P-gp. Moreover, TSI, TSA, dihydrotanshinone, and CTS are inhibitors of human CYP1A2, 2C9, 2E1, and 3A4, respectively, with different modes of inhibition (Qiu et al., 2008; Wang et al., 2010a). In addition, single-dose treatment with Danshen increased the clearance of tolbutamide, a CYP2C11 probe drug, and decreased the plasma exposure of 4-hydroxy-tolbutamide in rats (Wang et al., 2010b). Thus, there is a growing safety concern for use of Danshen when administered in combination with therapeutic drugs that are substrates for P-gp or metabolized by cytochrome P450.

Docetaxel (Taxotere), a semi-synthetic analogue of paclitaxel (Taxol), was developed in 1981. It promotes microtubule polymer-

Abbreviations: P-gp, P-glycoprotein; CYP, cytochrome P450; TSI, tanshinone I; TSA, tanshinone IIA; TSB, tanshinone IIB; CTS, cryptotanshinone; LC–MS/MS, liquid chromatography with tandem mass spectrometry; DART, direct analysis in real time; PEG 600, polyethylene glycol of average molecular weight 600; HPLC, high-performance liquid chromatography; AUC_{last}, total area under the plasma concentration–time curve from time zero to time last; $AUC_{0-\infty}$, total area under the plasma concentration–time curve from time zero to time infinity; MRT, mean residence time; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}; CL, time-averaged total body clearance; V_{ss}, apparent volume of distribution at steady state; SD, standard deviation.

* Corresponding author at: Division of Biopharmaceutics, College of Pharmacy, Kyung Hee University, 1 Hoegi-dong Dongdaemun, Seoul 130-701, Republic of Korea. Tel.: +82 2 961 9256; fax: +82 2 966 3885.
E-mail address: yj_lee@khu.ac.kr (Y.-J. Lee).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.03.031

ization leading to cell cycle arrest at G_2/M, apoptosis, and finally, cytotoxicity (Ringel and Horwitz, 1991). However, the low extent of absolute oral bioavailability of docetaxel has limited the development for oral dosage forms due to the high affinity for the multidrug efflux pump P-gp in the mucosa of the gastrointestinal tract in human (Shirakawa et al., 1999). In mice, rats, dogs, and cancer patients, docetaxel as well as its hydroxylated metabolites is exclusively eliminated through hepatic metabolism by CYP3A and biliary excretion (Marre et al., 1996; Sparreboom et al., 1998). Inhibition of P-gp and CYP3A is regarded as a highly effective strategy to improve the oral bioavailability of docetaxel. Cyclosporine A, a well-known P-gp and CYP3A inhibitor, is used to enhance docetaxel bioavailability in dogs and rats (McEntee et al., 2003).

Clopidogrel is currently the thienopyridine antiplatelet agent predominantly used for the management of patients following percutaneous coronary intervention and stent placement (Braunwald et al., 2002). Clopidogrel has two metabolic pathways, with the primary pathway leading to the formation of the inactive clopidogrel acid metabolite through ester hydrolysis by hepatic human carboxylesterase 1 (Tang et al., 2006). Interestingly, P-gp-mediated intestinal efflux is known to cause poor oral bioavailability of clopidogrel in humans (Taubert et al., 2006). Patients with two variant alleles of *ABCB1* (gene coding P-gp) had a higher rate of cardiovascular events at 1 year than those with the *ABCB1* wild-type genotype (Simon et al., 2009).

It has become increasingly clear that the administration of cytotoxic drugs such as taxane may be associated with life-threatening vascular episodes. Antiplatelet agents are extensively used for the prevention of thrombosis in patients undergoing placement of a coronary stent as mentioned above. Therefore, in this study, we chose docetaxel and clopidogrel as drugs that have potential for co-administration with Danshen and investigated the possible pharmacokinetic interactions of Danshen–docetaxel and Danshen–clopidogrel in rats.

2. Materials and methods

2.1. Chemicals

Danshen was purchased from Sun Ten Pharmaceuticals Co., Ltd. (Taipei Hsien, Taiwan). Each gram contained the following dry herbs: 2 g of Radix Salviae miltiorrhizae (this yielded 0.66 g of dry extract) and 0.34 g of Radix Salviae miltiorrhizae. Docetaxel trihydrate and paclitaxel [internal standard for the liquid chromatography with tandem mass spectrometry (LC–MS/MS) of docetaxel] were supplied by the Central Research Institute, Shin Poong Pharmaceutical Co., Ltd. (Ansan, South Korea). Clopidogrel carboxylic acid and clopidogrel bisulfate (clopidogrel 75 mg, PLATLESS® tablet) were purchased from Toronto Research Chemicals, Inc. (Ontario, Canada) and Samjin Pharmaceutical Co., Ltd. (Seoul, South Korea), respectively. Cyclosporine A was obtained from Novartis Pharma (Tokyo, Japan). All other chemicals were of analytic grade, and solvents were of high-performance liquid chromatography (HPLC) grade.

2.2. Animals

Male Sprague–Dawley rats (250–300 g, 7–8 weeks; Orient, Seoul, Korea) were used. Rats were maintained at a temperature of 25 °C and a 12 h:12 h light/dark cycle (Animal Center for Pharmaceutical Research, College of Pharmacy, Kyung Hee University, Seoul, Korea). Rats were divided randomly into groups (3–8 animals in each group) and used for pharmacokinetic studies after acclimation with free access to water and feed. All experiments were conducted according to the guidelines of the Committee on Care and Use of Laboratory Animals of the Kyung Hee University.

2.3. Direct analysis in real time–MS analysis of Danshen granules

MS analysis was done using an AccuTOF-TLC single-reflectron time-of-flight mass spectrometer (JEOL Ltd., Tokyo, Japan) equipped with a direct analysis in real time (DART) ion source (IonSense, Saugus, MA). The mass spectrometer was operated in positive-ion mode at a resolving power of 6000 (full width at half maximum). The atmospheric pressure interface potentials were set to the following values: orifice 1 = 10 V, ring lens and orifice 2 = 5 V. The rf ion guide potential and detector voltage were set to 500 V and 2200 V, respectively. The DART ion source electrode potentials were set to needle electrode = 3000 V, electrode 1 = 100 V, and electrode 2 = 100 V. Gas temperature was set to 300 °C, and helium gas flow rate was 3 L/min. Direct analysis was carried out by holding intact granules of Danshen product with a pair of forceps and placing them between the sampling orifice and the exit of the ion source. The molecular ion peaks appeared on the mass spectrum within 1 s when the sample was introduced into the DART helium gas stream. Polyethylene glycol of average molecular weight 600 (PEG 600) was used as an external reference standard for exact mass calibration.

2.4. Pharmacokinetic interactions between Danshen and docetaxel

The procedures used for the pretreatment of rats including the cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration in the intravenous study) were similar to a previously described method (Choi et al., 2010).

For the intravenous study, Danshen (suspended in normal saline) at a dose of 400 mg/kg (n = 5) or normal saline (n = 4) was administered (5 mL/kg) orally 90 min prior to the infusion of docetaxel. Docetaxel [docetaxel trihydrate was dissolved in Tween 80/ethanol/normal saline, 1:1:1.3 (v/v/v)] at a dose of 5 mg/kg was infused intravenously (2 mL/kg) for 1 min into rats. Blood samples (each approximately 420 μL) were collected via the carotid artery at 1 (end of the infusion), 5, 15, 30, 60, 90, 120, 180, 240, and 300 min after the start of intravenous infusion of docetaxel. Heparinized 0.9% NaCl injectable solution (20 U/mL; 0.3 mL) was used to flush each cannula immediately after each blood sampling to prevent blood clotting and to compensate for fluid loss. And freshly collected blood from untreated rats were injected to replace the lost volume. After centrifugation of the blood samples, plasma (each 200 μL) was stored at −80 °C until used for the analysis of docetaxel.

For the oral study, Danshen (suspended in normal saline) at a dose of 400 mg/kg (n = 8), normal saline (n = 6), or cyclosporine (dissolved in normal saline) at a dose of 10 mg/kg (5 mL/kg) (n = 4) was administered orally (5 mL/kg) 30 min prior to the administration of docetaxel. Docetaxel [docetaxel trihydrate was dissolved in Tween 80/ethanol, 1:1 (v/v)] at a dose of 40 mg/kg was administered orally (5 mL/kg) in rats using a gastric gavage tube after overnight fasting with free access to water. Blood samples (each approximately 420 μL) were collected via the carotid artery at 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 min after oral administration of docetaxel. All other procedures were similar to those used in the intravenous study.

2.5. Pharmacokinetic interactions between Danshen and clopidogrel

The procedures used for the pretreatment of rats were similar to those used in the drug interaction study between Danshen and docetaxel. As clopidogrel is a pro-drug and its active metabolite is unstable and difficult to measure, pharmacokinetic interactions were evaluated using the plasma concentration of the inactive carboxylic acid metabolite (Taubert et al., 2004). Danshen (sus-

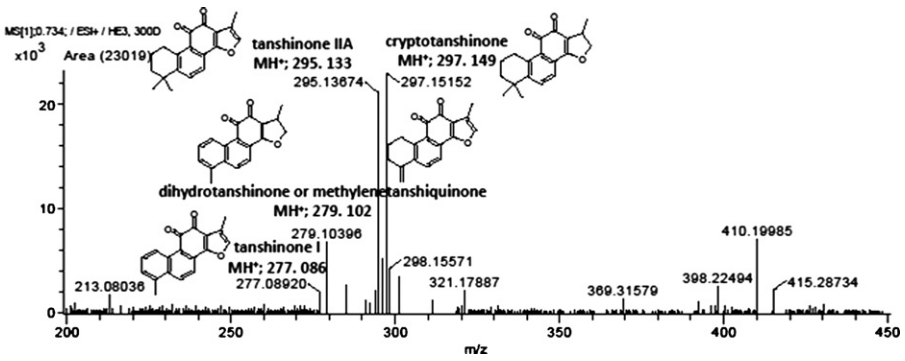

Fig. 1. Chromatogram of DART for Danshen product (batch no. 082003). TSI, TSA, dihydrotanshinone (and/or methylenetanshiquinone), and CTS were detected.

pended in normal saline) at a dose of 400 mg/kg, normal saline, or cyclosporine (dissolved in normal saline) at a dose of 10 mg/kg (6 mL/kg) was administered orally (6 mL/kg) 10 min prior to the administration of clopidogrel. Clopidogrel (PLATLESS® tablet was suspended in 0.5% carboxymethyl cellulose) at a dose of 30 mg/kg was administered orally (6 mL/kg) in rats using a gastric gavage tube after overnight fasting with free access to water. Blood samples (each approximately 420 μL) were collected via the carotid artery at 0, 5, 10, 20, 40, 60, 120, 240, 480, 960, 1440, and 2880 min after oral administration of clopidogrel.

2.6. LC–MS/MS and HPLC analyses

The concentrations of docetaxel were determined using the LC–MS/MS method with an Agilent Technologies 1200 series (Agilent, Santa Clara, CA) coupled to a Waters Quattro micro™ API mass spectrometer (Waters, Milford, MA) equipped with an electrospray ionization interface used to generate positive ions $[M+H]^+$. An aliquot of 50 μL (for intravenous samples) or 20 μL (for oral samples) of acetonitrile containing 500 ng/mL paclitaxel (internal standard) and 1 mL of tert-butyl methyl ether were added to 200 μL of plasma sample. Then, the mixture was vortex-mixed for 5 min and centrifuged at 15,000 rpm for 10 min; an aliquot (1 mL) of the supernatant was evaporated to dryness by using a Speed-Vac concentrator (Centra Vac, Vision Scientific Co., Bucheon, Korea). The residue was reconstituted with 200 μL (for intravenous samples) or 50 μL (for oral samples) of mobile phase (see below), and a 30-μL aliquot was directly injected onto a YMC-UltraHT Pro C18 column (50 mm × 2.1 mm i.d.; particle size, 2 μm; YMC Co., Ltd., Kyoto, Japan). The mobile phase, 0.1% formic acid/acetonitrile (35:65, v/v), was run at a flow rate of 0.3 mL/min. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 808.3 → 527.2 for docetaxel and *m/z* 854.3 → 104.9 for paclitaxel. The optimal mass parameters obtained were as follows: capillary voltage, 3.5 kV; cone voltage, 15 and 25 V for docetaxel and paclitaxel, respectively; source temperature, 120 °C; and desolvation temperature, 400 °C. Nitrogen was used as the desolvation and cone gas at a flow rate of 800 and 50 L/h, respectively. Collision energy was 12 and 50 eV for docetaxel and paclitaxel, respectively. The analytic data were processed by MassLynx V 4.1 software (Waters, Milford, MA).

The concentrations of clopidogrel carboxylic acid metabolite were determined by the reverse-phase HPLC system consisting of Hitachi pump L-7110 and autosampler L-7250 (Hitachi, Tokyo, Japan) coupled to a UV/Vis detector (Walnut Creek, CA). Briefly, 120 μL of acetonitrile was added to 60 μL of plasma for protein precipitation. Then, the mixture was vortex-mixed for 2 min and centrifuged at 15,000 rpm for 2 min; an aliquot (160 μL) of the supernatant was evaporated to dryness by using a Speed-Vac concentrator (Centra Vac, Vision Scientific Co., Bucheon, Korea). The residue was reconstituted with 160 μL of mobile phase (see below), and a 100-μL aliquot was directly injected into the HPLC system. Reverse-phase Supelcosil™ LC-18-DB column (250 mm × 4.6 mm; particle size, 5 μm) was used. The mobile phase, 30 mM potassium dihydrogen phosphate buffer (pH 3)/tetrahydrofuran/acetonitrile (79:2:19, v/v/v), was run at a flow rate of 1.0 mL/min. The column effluent was monitored with a UV detector at 220 nm.

2.7. Data analysis

Standard methods (Gibaldi and Perrier, 1982) were used to calculate the following pharmacokinetic parameters using the non-compartmental analysis (WinNonlin®, Pharsight, Mountain View, CA): the total area under the plasma concentration–time curve from time zero to infinity (AUC) or up to the last measured time in plasma (AUC_{last}) (Chiou, 1978), time-averaged total body clearance (CL), terminal half-life ($t_{1/2}$), and apparent volume of distribution at steady state (V_{ss}). The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly read from the experimental data. A P value of <0.05 was considered to be statistically significant using a t-test between the two means for the unpaired data, or a Dunnet's multiple range test of Statistical Package for the Social Sciences (SPSS) posteriori analysis of variance (ANOVA) among the three means for the unpaired data. All data were expressed as mean ± standard deviation (SD).

3. Results

3.1. DART–MS analysis

As DART ion source can detect various components in Danshen granules without any preparation steps such as extraction, direct contact of a granule of sample was used to obtain the whole mass spectrum. Phenanthraquinones such as TSI, TSA, dihydrotanshinone (and/or methylenetanshiquinone), and CTS were detected as protonated forms (Fig. 1). The calculated deviations of the acquired mass numbers for each phenanthraquinone were less than 4 mmu as compared with their corresponding theoretical mass numbers. The high-resolution power of AccuTOF-MS allowed the efficient confirmation of the identity of phenanthraquinones by comparison of the measured molecular mass with the corresponding theoretical molecular mass.

3.2. LC–MS/MS and HPLC analyses

There are no interfering peaks from endogenous substances at the elution times for docetaxel (1.31 min), paclitaxel (1.35 min), or clopidogrel carboxylic acid metabolite (12.0 min) (Fig. 2). Fig. 2(A) represents the typical chromatograms for blank plasma (I), docetaxel standard peak (500 ng/mL) and internal standard (paclitaxel; 500 ng/mL) (II), and a plasma sample obtained from a con-

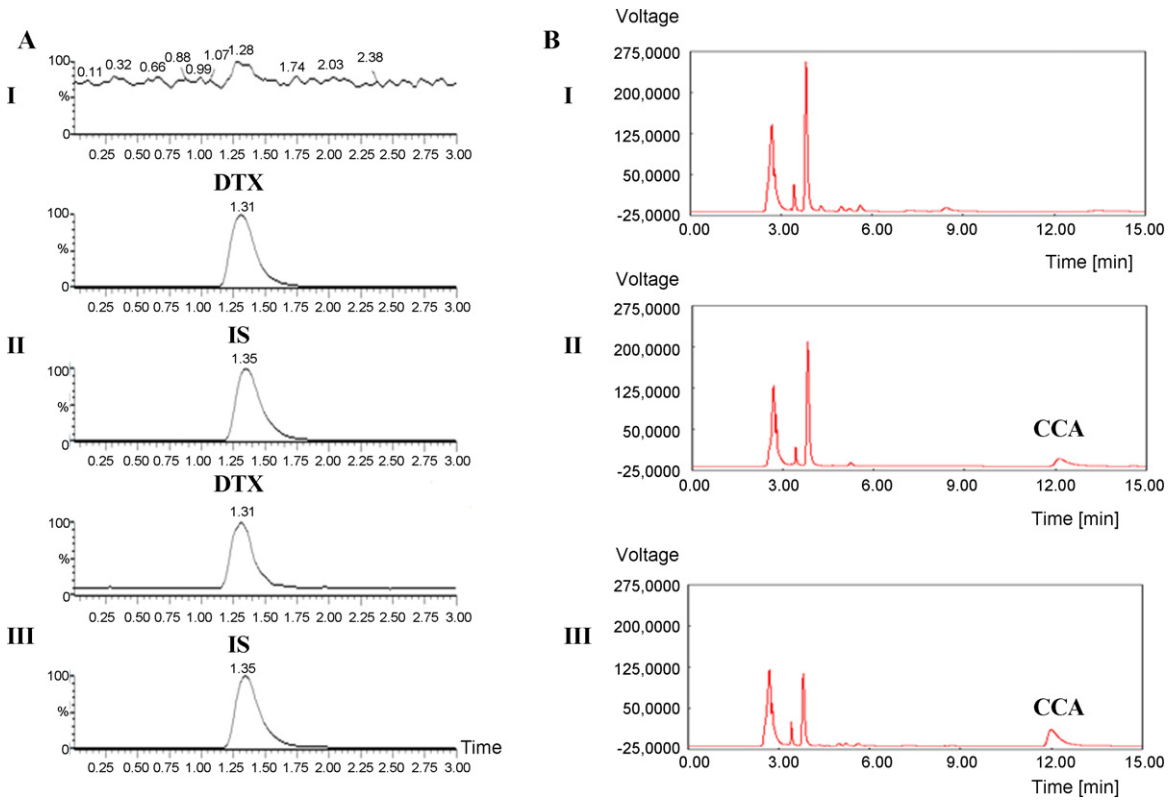

Fig. 2. Representative chromatograms of blank plasma (I), docetaxel (DTX) standard peak (500 ng/mL) and internal standard (IS) (paclitaxel; 500 ng/mL) (II), and a plasma sample obtained from a control rat 90 min after oral administration of docetaxel (40 mg/kg) (III) (A); representative chromatograms of blank plasma (I), clopidogrel carboxylic acid (CCA) metabolite standard peak (100 μg/mL) (II), and a plasma sample obtained from a control rat 60 min after oral administration of clopidogrel (30 mg/kg) (III) (B).

trol rat 90 min after oral administration of docetaxel (40 mg/kg) (III). Fig. 2(B) represents the typical chromatograms for blank plasma (I), clopidogrel carboxylic acid metabolite standard peak (100 μg/mL) (II), and a plasma sample obtained from a control rat 60 min after oral administration of clopidogrel (30 mg/kg) (III).

The calibration curves provided reliable responses from 0.005 to 100 μg/mL for docetaxel and from 0.5 to 200 μg/mL for clopidogrel carboxylic acid metabolite. The mean correlation coefficients (r^2) were greater than 0.99 (mean values, 0.997 for docetaxel and 0.999 for clopidogrel). The limit of quantification values for these analytic methods as the lowest point in the calibration curve were 0.005 and 0.5 μg/mL for docetaxel and clopidogrel carboxylic acid metabolite, respectively. The coefficients of variation of the assay were below 8.28 and 7.48% for docetaxel and clopidogrel carboxylic acid metabolite, respectively.

3.3. Pharmacokinetic interactions between Danshen and docetaxel

The mean arterial plasma concentration–time curves of docetaxel (5 mg/kg) after intravenous administration with or without oral co-administration of Danshen (400 mg/kg) are shown in Fig. 3(A). There was no significant difference between groups, and Danshen showed no significant effect on the pharmacokinetic parameters of docetaxel (Table 1).

The mean arterial plasma concentration–time curves of docetaxel (40 mg/kg) after oral administration with or without oral co-administration of cyclosporine A (10 mg/kg) or Danshen (400 mg/kg) are shown in Fig. 3(B) and the relevant pharmacokinetic parameters are listed in Table 1. Co-administration of Danshen had no substantial effect on the plasma concentration of docetaxel, whereas co-administration of cyclosporine A increased

Table 1
Pharmacokinetic parameters of docetaxel after intravenous (5 mg/kg) and oral (40 mg/kg) administrations with or without co-administration of cyclosporine A (10 mg/kg) or Danshen (400 mg/kg). Data are shown as mean ± SD.

Parameter	Intravenous administration		Oral administration		
	Control ($n=4$)	With Danshen ($n=5$)	Control ($n=6$)	With cyclosporine A ($n=4$)	With Danshen ($n=8$)
AUC_{last} (μg/mL min)	385 ± 97	393 ± 79	11.4 ± 6.0	197 ± 11[a]	14.1 ± 6.5
$AUC_{0-\infty}$ (μg/mL min)	397 ± 93	401 ± 77	12.7 ± 5.9	315 ± 147[a]	15.4 ± 6.8
Terminal half-life (min)	199 ± 104	196 ± 81			
MRT (min)	30.5 ± 28.9	22.6 ± 10.1			
C_{max} (μg/mL)			0.0814 ± 0.0565	0.657 ± 0.045[a]	0.0693 ± 0.0332
T_{max} (min)			47.5 ± 22.1	330 ± 60[a]	54.4 ± 21.1
CL (mL/min/kg)	13.1 ± 2.7	12.8 ± 2.3			
V_{ss} (mL/kg)	411 ± 412	301 ± 161			

AUC_{last}: total area under the plasma concentration–time curve from time zero to time last; $AUC_{0-\infty}$: total area under the plasma concentration–time curve from time zero to time infinity; MRT: mean residence time; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; CL: time-averaged total body clearance; V_{ss}: apparent volume of distribution at steady state.

[a] $P<0.01$ *vs.* control (Student's *t*-test for intravenous administration and one-way ANOVA for oral administration).

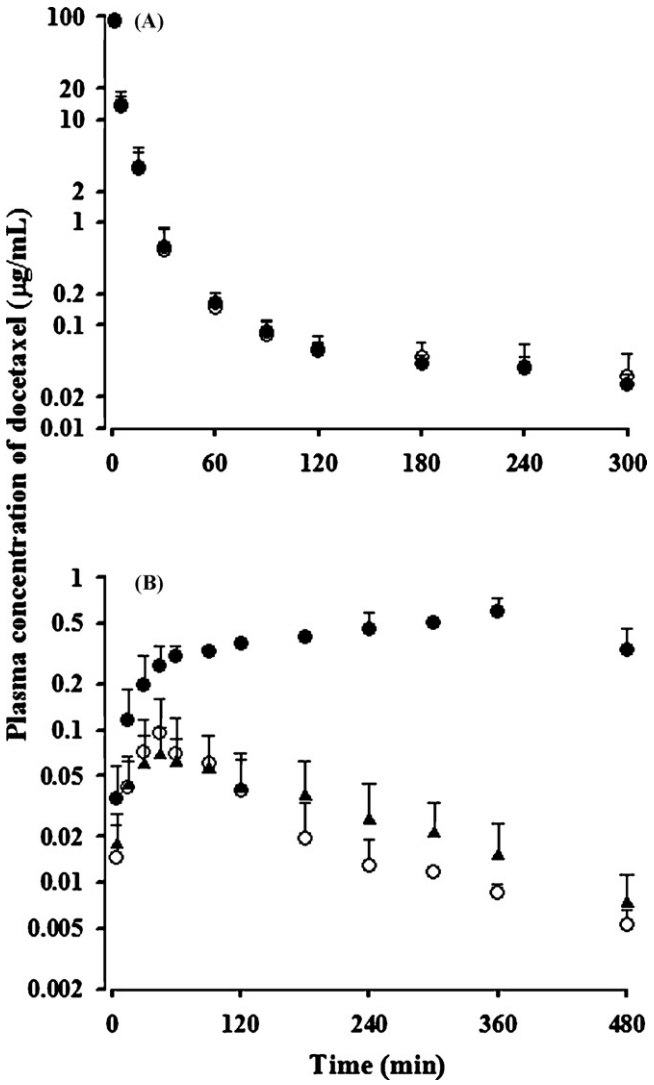

Fig. 3. Mean plasma concentration–time curves of docetaxel after intravenous administration (5 mg/kg) with (●) or without (○) co-administration of Danshen (400 mg/kg) (A) and after oral administration (40 mg/kg) with co-administration of cyclosporine A (10 mg/kg) (●) or Danshen (400 mg/kg) (▲) and without any co-administration (○) (B). Bars represent SD.

the plasma concentration of docetaxel dramatically. Administration of Danshen did not significantly change the pharmacokinetic parameters of docetaxel, as similarly observed in the intravenous study. However, the AUC_{last}, $AUC_{0-\infty}$, C_{max}, and T_{max} of docetaxel in the cyclosporine A treatment group increased by 1628%, 2380%, 707%, and 595%, respectively.

3.4. Pharmacokinetic interactions between Danshen and clopidogrel

The mean arterial plasma concentration–time curves of clopidogrel carboxylic acid metabolite after oral administration of clopidogrel (30 mg/kg) with or without oral co-administration of cyclosporine A (10 mg/kg) or Danshen (400 mg/kg) are shown in Fig. 4. The relevant pharmacokinetic parameters are listed in Table 2. Co-administration of Danshen produced no significant change in the plasma profile and pharmacokinetic parameters of clopidogrel carboxylic acid metabolite, whereas cyclosporine A increased the plasma concentration of clopidogrel carboxylic acid dramatically and the AUC_{last}, $AUC_{0-\infty}$, and C_{max} of clopidogrel carboxylic acid metabolite by 148%, 140%, and 198%, respectively.

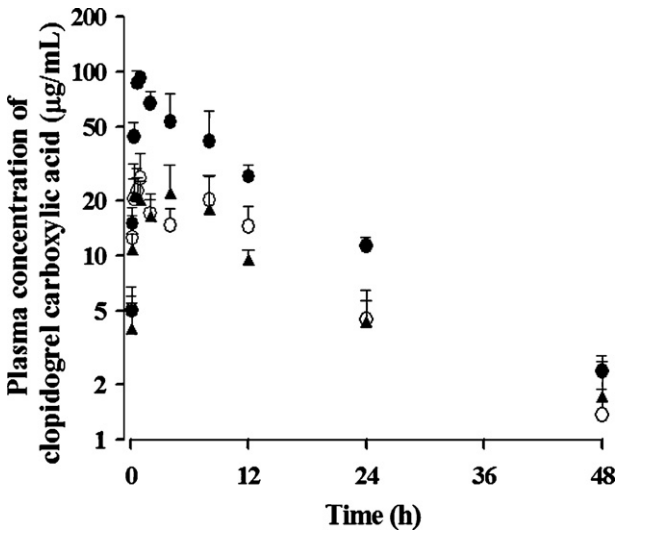

Fig. 4. Mean plasma concentration–time curves of clopidogrel carboxylic acid metabolite after oral administration of clopidogrel (30 mg/kg) with co-administration of cyclosporine A (10 mg/kg) (●) or Danshen (400 mg/kg) (▲) and without any co-administration (○). Bars represent SD.

4. Discussion

In a previous study in rats, the percentage of the dose excreted in the urine up to 24 h after intravenous administration of docetaxel was 0.311% (Choi et al., 2010). This means that intravenous docetaxel is almost completely metabolized in rats, and thus the clearance values in Table 1 could represent metabolic clearance.

After the intravenous administration of docetaxel with oral administration of Danshen, the pharmacokinetic parameters were comparable with controls (Table 1). Although Danshen is known to affect the CYP3A system, the comparable AUC and CL values of docetaxel suggested that the effect of oral Danshen (400 mg/kg, which is approximately 25-fold higher compared with the conventional dose) on docetaxel metabolism was almost negligible in vivo. After the oral administration of docetaxel with Danshen, the AUC was also comparable with controls (Table 1). This indicates that the inhibitory effects of Danshen on the intestinal metabolism of P-gp and CYP3A were also negligible. On the other hand, co-administration with cyclosporine A, a P-gp and CYP3A inhibitor, showed a much greater AUC than the control owing to inhibition of P-gp-mediated efflux and CYP3A-mediated metabolism.

Clopidogrel is rapidly, but incompletely, absorbed after oral administration and is extensively metabolized to an active metabolite (Herbert et al., 1993). It is hard to detect the parent drug in plasma due to its very low concentration. The major circulating compound, an inactive carboxylic derivative, is used to document the pharmacokinetic profile of clopidogrel (Herbert et al., 1993). Thus, in this study, we measured the concentration of clopidogrel carboxylic acid metabolite instead of clopidogrel itself.

Table 2
Pharmacokinetic parameters of clopidogrel carboxylic acid metabolite after oral administration of clopidogrel (30 mg/kg) with or without co-administration of cyclosporine A (10 mg/kg) or Danshen (400 mg/kg). Data are shown as mean ± SD.

Parameter	Control ($n=4$)	With cyclosporine A ($n=3$)	With Danshen ($n=4$)
AUC_{last} (μg/mL h)	395 ± 98	978 ± 177[a]	357 ± 95
$AUC_{0-\infty}$ (μg/mL h)	421 ± 86	1010 ± 168[a]	398 ± 79
C_{max} (μg/mL)	32.0 ± 5.1	95.4 ± 7.1[a]	25.6 ± 7.1
T_{max} (h)	2.58 ± 3.62	0.889 ± 0.193	3.33 ± 3.50

[a] $P<0.01$ *vs.* control (one-way ANOVA).

After the oral administration of clopidogrel with oral co-administration of Danshen, the results were the same as in the case of docetaxel. The AUC of clopidogrel carboxylic acid metabolite was comparable with controls. In the positive control study with cyclosporine A, the AUC of clopidogrel carboxylic acid was increased compared with controls. This could be due to the greater AUC of parent drug, clopidogrel, through inhibition of P-gp-mediated efflux and not through inhibition of metabolism by cyclosporine A. This is because clopidogrel carboxylic acid metabolite is formed through ester hydrolysis of carboxylic acid and not through CYP-mediated metabolism. The contribution of inhibition of conversion of clopidogrel to active metabolite via CYP could not be considerable. Because approximately 85% of the administered dose is hydrolyzed by esterases to an inactive carboxylic acid derivative and, thus, only the remaining approximately 15% of the prodrug is metabolized into the active metabolite by the hepatic CYP system (Farid et al., 2007a,b; Williams et al., 2008).

In the present study, we confirmed the low potential of Danshen for herb–drug interactions. The negligible effect of orally administered Danshen on docetaxel and clopidogrel could be explained in two ways. First, the bioavailability values of Danshen and its constituents are extremely low. In rats, the absorption of CTS after oral administration at 100 mg/kg was poor, with a bioavailability of only 2.05% (Zhang et al., 2006). The corresponding values for TSA (Hao et al., 2006) and TSB (Yu et al., 2007b) were below 3.5% and about 3%, respectively; their concentrations in intestinal epithelial cells were not high enough to affect the efflux and metabolism of docetaxel and clopidogrel. Second, Danshen has limited amounts of TSA, TSB, and CTS to inhibit P-gp or CYP. For example, the amount of TSA in Danshen product according to Chinese Pharmacopoeia is 0.20% (Chinese Pharmacopoeia, 2005). The dose of TSA needed to cause a 66% inhibition of CYP1A2 activity was calculated as 40 mg/kg (Kuo et al., 2006), and this is equivalent to 20 g/kg Danshen. Moreover, weak or moderate inhibition of TSI, TSA, and CTS was observed for the CYP3A4 enzyme, whereas potent inhibition was observed for CYP1A, CYP2C9, and CYP2E1 (Wang et al., 2010a).

In conclusion, in spite of the potential herb–drug interactions of Danshen that were predicted by in vitro studies, Danshen did not affect the pharmacokinetics of docetaxel and clopidogrel in rats. This study shows that Danshen has negligible safety concern in P-gp- and CYP3A-mediated interactions because of the low in vivo exposure of its inhibitory components. The result of this study is an example of why herb–drug interaction potential should be confirmed by in vivo studies.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgement

This work was supported by a grant from the Kyung Hee University in 2010 (KHU-20100122).

References

Braunwald, E., Antman, E.M., Beasley, J.W., Califf, R.M., Cheitlin, M.D., Hochman, J.S., Jones, R.H., Kereiakes, D., Kupersmith, J., Levin, T.N., Pepine, C.J., Schaeffer, J.W., Smith 3rd, E.E., Steward, D.E., Theroux, P., Gibbons, R.J., Alpert, J.S., Faxon, D.P., Fuster, V., Gregoratos, G., Hiratzka, L.F., Jacobs, A.K., Smith Jr., S.C., 2002. ACC/AHA 2002 guideline update for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction—summary article: a report of the American College of Cardiology/American Heart Association task force on practice guidelines (Committee on the Management of Patients With Unstable Angina). J. Am. Coll. Cardiol. 40, 1366–1374.

Chan, K., Lo, A.C., Yeung, J.H., Woo, K.S., 1995. The effects of Danshen (*Salvia miltiorrhiza*) on warfarin pharmacodynamics and pharmacokinetics of warfarin enantiomers in rats. J. Pharm. Pharmacol. 47, 402–406.

China Pharmacopoeia Commission, 2005. Pharmacopoeia of the People's Republic of China (Part One). Chemical Industry Press, Beijing.

Chiou, W.L., 1978. Critical evaluation of the potential error in pharmacokinetic studies of using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. J. Pharmacokinet. Biopharm. 6, 539–546.

Choi, Y.H., Suh, J.H., Lee, J.H., Cho, I.H., Lee, M.G., 2010. Effects of tesmilifene, a substrate of CYP3A and an inhibitor of P-glycoprotein, on the pharmacokinetics of intravenous and oral docetaxel in rats. J. Pharm. Pharmacol. 62, 1084–1088.

Farid, N.A., McIntosh, M., Garofolo, F., Wong, E., Shwajch, A., Kennedy, M., Young, M., Sarkar, P., Kawabata, K., Takahashi, M., Pang, H., 2007a. Determination of the active and inactive metabolites of prasugrel in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. 21, 169–179.

Farid, N.A., Payne, C.D., Small, D.S., Winters, K.J., Ernest 2nd, C.S., Brandt, J.T., Darstein, C., Jakubowski, J.A., Salazar, D.E., 2007b. Cytochrome P450 3A inhibition by ketoconazole affects prasugrel and clopidogrel pharmacokinetics and pharmacodynamics differently. Clin. Pharmacol. Ther. 81, 735–741.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics, 2nd ed. Marcel-Dekker, New York.

Hao, H., Wang, G., Cui, N., Li, J., Xie, L., Ding, Z., 2006. Pharmacokinetics, absorption and tissue distribution of tanshinone IIA solid dispersion. Planta Med. 72, 1311–1317.

Herbert, J.M., Frehel, D., Vallee, E., Kieffer, G., Gouy, D., Berger, Y., Necciari, J., Defreyn, G., Maffrand, J.P., 1993. Clopidogrel, a novel antiplatelet and antithrombotic agent. Cardiovasc. Drug Rev. 11, 180–198.

Holbrook, A.M., Pereira, J.A., Labiris, R., McDonald, H., Douketis, J.D., Crowther, M., Wells, P.S., 2005. Systematic overview of warfarin and its drug and food interactions. Arch. Intern. Med. 165, 1095–1106.

Hu, Z., Yang, X., Ho, P.C., Chan, S.Y., Heng, P.W., Chan, E., Duan, W., Koh, H.L., Zhou, S., 2005. Herb–drug interactions: a literature review. Drugs 65, 1239–1282.

Izzo, A.A., Di Carlo, G., Borrelli, F., Ernst, E., 2005. Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction. Int. J. Cardiol. 98, 1–14.

Kuo, Y.H., Lin, Y.L., Don, M.J., Chen, R.M., Ueng, Y.F., 2006. Induction of cytochrome P450-dependent monooxygenase by extracts of the medicinal herb *Salvia miltiorrhiza*. J. Pharm. Pharmacol. 58, 521–527.

Li, X.X., Zhou, Z.W., Zhou, S.F., 2008. Role of P-glycoprotein in the transport of tanshinone I, one active triterpenoid from *Salvia miltiorrhiza*. Drug Metab. Lett. 2, 223–230.

Lu, T., Yang, J., Gao, X., Chen, P., Du, F., Sun, Y., Wang, F., Xu, F., Shang, H., Huang, Y., Wang, Y., Wan, R., Liu, C., Zhang, B., Li, C., 2008. Plasma and urinary tanshinol from *Salvia miltiorrhiza* (Danshen) can be used as pharmacokinetic markers for cardiotonic pills, a cardiovascular herbal medicine. Drug Metab. Dispos.: Biol. Fate Chem. 36, 1578–1586.

Marre, F., Sanderink, G.J., de Sousa, G., Gaillard, C., Martinet, M., Rahmani, R., 1996. Hepatic biotransformation of docetaxel (Taxotere) in vitro: involvement of the CYP3A subfamily in humans. Cancer Res. 56, 1296–1302.

McEntee, M., Silverman, J.A., Rassnick, K., Zgola, M., Chan, A.O., Tau, P.T., Page, R.L., 2003. Enhanced bioavailability of oral docetaxel by co-administration of cyclosporin A in dogs and rats. Vet. Comp. Oncol. 1, 105–112.

Qiu, F., Zhang, R., Sun, J.A.J., Hao, H., Peng, Y., Ai, H., Wang, G., 2008. Inhibitory effects of seven components of danshen extract on catalytic activity of cytochrome P450 enzyme in human liver microsomes. Drug Metab. Dispos. 36, 1308–1314.

Ringel, I., Horwitz, S.B., 1991. Studies with RP 56976 (taxotere): a semisynthetic analogue of taxol. J. Natl. Cancer Inst. 83, 288–291.

Shirakawa, K., Takara, K., Tanigawara, Y., Aoyama, N., Kasuga, M., Komada, F., Sakaeda, T., Okumura, K., 1999. Interaction of docetaxel ("Taxotere") with human P-glycoprotein. Jpn. J. Cancer Res. 90, 1380–1386.

Simon, T., Verstuyft, C., Mary-Krause, M., Quteineh, L., Drouet, E., Méneveau, N., Steg, P.G., Ferrières, J., Danchin, N., Becquemont, L., 2009. Genetic determinants of response to clopidogrel and cardiovascular events. N. Engl. J. Med. 360, 363–375.

Sparreboom, A., van Tellingen, O., Nooijen, W.J., Beijnen, J.H., 1998. Preclinical pharmacokinetics of paclitaxel and docetaxel. Anticancer Drugs 9, 1–17.

Tang, M., Mukundan, M., Yang, J., Charpentier, N., LeCluyse, E.L., Black, C., Yang, D., Shi, D., Yan, B., 2006. Antiplatelet agents aspirin and clopidogrel are hydrolyzed by distinct carboxylesterases, and clopidogrel is transesterificated in the presence of ethyl alcohol. J. Pharmacol. Exp. Ther. 319, 1467–1476.

Taubert, D., Kastrati, A., Harlfinger, S., Gorchakova, O., Lazar, A., von Beckerath, N., Schomig, A., Schomig, E., 2004. Pharmacokinetics of clopidogrel after administration of a high loading dose. Thromb. Haemost. 92, 311–316.

Taubert, D., von Beckerath, N., Grimberg, G., Lazar, A., Jung, N., Goeser, T., Kastrati, A., Schomig, A., Schomig, E., 2006. Impact of P-glycoprotein on clopidogrel absorption. Clin. Pharmacol. Ther. 80, 486–501.

Wang, X., Cheung, C.M., Lee, W.Y., Or, P.M., Yeung, J.H., 2010a. Major tanshinones of Danshen (*Salvia miltiorrhiza*) exhibit different modes of inhibition on human CYP1A2, CYP2C9, CYP2E1 and CYP3A4 activities in vitro. Phytomedicine 17, 868–875.

Wang, X., Lee, W.Y., Or, P.M., Yeung, J.H., 2010b. Pharmacokinetic interaction studies of tanshinones with tolbutamide, a model CYP2C11 probe substrate, using liver microsomes, primary hepatocytes and in vivo in the rat. Phytomedicine 17, 203–211.

Williams, E.T., Jones, K.O., Ponsler, G.D., Lowery, S.M., Perkins, E.J., Wrighton, S.A., Ruterbories, K.J., Kazui, M., Farid, N.A., 2008. The biotransformation of prasugrel, a new thienopyridine prodrug, by the human carboxylesterases 1 and 2. Drug Metab. Dispos.: Biol. Fate Chem. 36, 1227–1232.

Yu, C.M., Chan, J.C., Sanderson, J.E., 1997. Chinese herbs and warfarin potentiation by 'danshen'. J. Intern. Med. 241, 337–339.

Yu, X.Y., Lin, S.G., Zhou, Z.W., Chen, X., Liang, J., Liu, P.Q., Duan, W., Chowbay, B., Wen, J.Y., Li, C.G., Zhou, S.F., 2007a. Role of P-glycoprotein in the intestinal absorption of tanshinone IIA, a major active ingredient in the root of *Salvia miltiorrhiza* Bunge. Curr. Drug Metab. 8, 325–340.

Yu, X.Y., Zhou, Z.W., Lin, S.G., Chen, X., Yu, X.Q., Liang, J., Duan, W., Wen, J.Y., Li, X.T., Zhou, S.F., 2007b. Role of ATP-binding cassette drug transporters in the intestinal absorption of tanshinone IIB, one of the major active diterpenoids from the root of *Salvia miltiorrhiza*. Xenobiotica 37, 375–415.

Zhang, J., Huang, M., Guan, S., Bi, H.C., Pan, Y., Duan, W., Chan, S.Y., Chen, X., Hong, Y.H., Bian, J.S., Yang, H.Y., Zhou, S., 2006. A mechanistic study of the intestinal absorption of cryptotanshinone, the major active constituent of *Salvia miltiorrhiza*. J. Pharmacol. Exp. Ther. 317, 1285–1294.

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.