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Effects of the aqueous extract from *Salvia miltiorrhiza Bunge* on caffeine pharmacokinetics and liver microsomal CYP1A2 activity in humans and rats

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Abstract

Objectives The effects of the aqueous extract of *Salvia miltiorrhiza Bunge* (Danshen) on metabolism/pharmacokinetics of caffeine and on liver microsomal CYP1A2 activity in humans and rats have been investigated.

Methods The effects of Danshen aqueous extract on CYP1A2 activity were determined by metabolism of model substrates in the rat *in vivo* and in humans and rats *in vitro*. HPLC was used to determine model substrates and metabolites.

Key findings In the rat, single dose Danshen aqueous extract treatment (100 or 200 mg/kg, i.p.) decreased metabolism of caffeine to paraxanthine, with overall decrease in caffeine clearance (6–20%), increase in area under the curve (AUC; 7–24%) and plasma half-life ($t^1/_2$ 14–16%). Fourteen-day Danshen aqueous extract treatment (100 mg/kg/day, i.p. or 200 mg/kg/day, p.o.) decreased caffeine clearance (16–26%), increased AUC (18–31%) and prolonged plasma $t^1/_2$ (8–10%). Aqueous extract of Danshen (125–2000 μg/ml) competitively inhibited human and rat liver microsomal CYP1A2 activity with inhibition constant (K_i) values at 190 and 360 μg/ml, respectively.

Conclusions These studies demonstrated that Danshen aqueous extract affected the metabolism of CYP1A2 substrates through competitive inhibition and altered their clearance.

Keywords caffeine; competitive inhibition; CYP1A2; Danshen

Introduction

The use of herbs and their products and nutraceuticals, commonly known as dietary supplements in the United States of America, natural health products in Canada, phytomedicines in Europe or traditional medicines in other developing countries, has increased substantially in recent years.[1] Potential interaction of these herbs with therapeutic agents represents a safety concern in patients receiving drug treatment for chronic illness/disease, since the concomitant use of these natural products with therapeutic agents may lead to serious clinical consequences. The importance and the diversity of such interactions have been reviewed recently.[2] Induction or inhibition of hepatic and intestinal drug metabolising enzymes such as cytochrome P450 (CYP) or drug transporters such as P-glycoprotein represent the major mechanism for the enhanced or reduced bioavailability of drugs when herbal/botanical products are co-administered.[3]

Salvia miltiorrhiza Bunge (Danshen) as a Traditional Chinese medicine has been used widely in China, Japan, the United States of America and European countries for the treatment of cardiovascular and cerebrovascular diseases.[4] Initial evidence that Danshen may affect P450-mediated metabolism came from our previous studies in which Danshen reduced the clearance of warfarin in the rat i*n vivo*.[5,6] Previous in-vitro studies have shown that tanshinones (tanshinone I, tanshinone IIA, dihydrotanshinone and cryptotanshinone) isolated from Danshen may play an important role in the induction or inhibition of CYP1A2 in mice and humans.[7–11] On the other hand, a Danshen extract rich in hydrophilic components (salvianolic acid B and danshensu) did not affect the pharmacokinetics of theophylline in healthy volunteers and sodium tanshinone IIA sulfonate, a derivative of tanshinone IIA, induced the activity of CYP1A2 in healthy volunteers.[12,13] The different results and potential mechanisms require further exploration. In Chinese folk medicine, the commonly used

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preparation method for Danshen root for human consumption is the hot water extract method; thus for this study, interest is on the aqueous extract of Danshen.

The effects of the aqueous extract from Danshen root on caffeine pharmacokinetics and metabolism have been studied after a single dose or daily dosing for 14 days in rats. To further elucidate the in-vivo results, the effect of Danshen aqueous extract on liver microsomal CYP1A2 activity has been studied in humans and rats.

Materials and Methods

Animals

Male Sprague-Dawley rats (250–300 g) were supplied by the Laboratory Animal Service Center, The Chinese University of Hong Kong (CUHK). The rats were kept in a holding room under standard conditions with a 12-h light–dark cycle, and with free access to rodent cubes (Glen Forrest Stockfeeders, Australia) and tap water. All the experimental procedures had been approved by the Animal Experimentation Ethics Committee (CUHK) in accordance to the Department of Health (HKSAR) guidelines in the Care and Use of Animals.

Materials

Pooled human liver microsomes were obtained from GenTest Corporation (Woburn, MA, USA) and stored at −80°C until use. All the experimental procedures involving humans were carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) guidelines. Dried Danshen root was purchased from Eu Yan Sang Ltd (Hong Kong). Caffeine, paraxanthine, phenacetin, paracetamol, metacetamol, β-nicotinamide adenine dinucleotide phosphate (NADP), D-glucose 6-phosphate (G6P), glucose 6-phosphate dehydrogenase (G6PDH), heparin sodium and urethane were from Sigma Chemical Co. (St Louis, MO, USA). Acetonitrile (HPLC Grade) was purchased from Labscan Analytical Sciences (Bangkok, Thailand). Methanol (HPLC Grade) was from BDH Laboratory Supplies (Poole, UK), ethyl acetate (HPLC grade) was from Fisher Chemicals (Leicester, UK). Acetic acid, glacial, (HPLC grade) was from Scharlau Chemie (Barcelona, Spain).

Preparation of Danshen aqueous extract

Dried Danshen root (600 g) was cut into small pieces and boiled with 1000 ml distilled water under reflux conditions. After 1 h the mixture was filtered and the filtrate was collected. A further 1000 ml water was added to the Danshen residue and boiled for 1 h. Finally, all filtrate was collected and combined, and allowed to cool at room temperature. The powder form of the aqueous extract was obtained by freeze-drying. Approximately 105 g Danshen aqueous extract was obtained with a yield of 17.5%.

Identification and quantification of Danshen aqueous extract

HPLC was performed to quantify and confirm the presence of its well known constituents in Danshen aqueous extract, which included the lipophilic components crytotanshinone, dihydrotanshinone, tanshinone I and tanshinone IIA, and also the water-soluble danshensu and salvianolic acid B. All standards were purchased from Chengdu Congon Bio-tech Co., Ltd (China). For determination of the lipid soluble constituents, Danshen extract was dissolved in methanol at a concentration of 100 μg/ml. HPLC analysis was performed in triplicate; each with 50 μl of the sample. The four lipid soluble components (crytotanshinone, dihydrotanshinone, tanshinone I and tanshinone IIA) were separated on an Agilent Zorbax Eclipse XDB-C18 (4.6×150 mm, 5 μm) with XDB-C18 guard column. A gradient elution of water (A) and acetonitrile (B) was used at a flow rate of 1.0 ml/min, commencing with 45% A and 55% B for 16 min, then to reach 80% B at 20 min and maintained to 24 min. Detection was by Agilent 1100 HPLC with a multiple wavelength detector at 245 nm. Standard curves for tanshinone I, tanshinone IIA, crytotanshinone and dihydrotanshinone were linear between 5 and 100 μM. The intra-assay and inter-assay coefficients of variation were 2.8 and 4.1%, respectively. The detection limit was 35 ng/ml. The contents of the lipophilic components in Danshen extract found were: dihydrotanshinone (12.7 ± 0.5 μg/g), crytotanshinone (34.6 ± 0.9 μg/g), tanshinone I (10.4 ± 0.4 μg/g) and tanshinone IIA (22.6 ± 0.5 μg/g).

For determination of the water-soluble constituents, aqueous extract from Danshen root was dissolved in water at a concentration of 10 mg/ml, and then diluted with methanol–water (50 : 50) to 1 mg/ml. HPLC analysis was performed in triplicate; each with 50 μl of the sample. Danshensu and salvianolic acid B were separated on an Alltech Alltima C18 (4.6×250 mm, 5 μm) with a Supelco Peliguard LC-18 guard column. A gradient elution of A (water–acetonitrile–formic acid, 90 : 10 : 0.4) and B (acetonitrile) was used at a flow rate of 0.7 ml/min, commencing with 0% B, rising to 30% B at 10 min, then to 50% B at 20 min. Detection was by an Agilent 1100 Series HPLC with diode-array detector at 280 nm. Standard curves for danshensu, salvianolic acid B, protocatechuic acid, protocatechuic aldehyde, caffeic acid and rosmarinic acid were linear between 5 and 100 μM. The intra-assay and inter-assay coefficients of variation were 1.3 and 2.2%, respectively. The detection limit was 90 ng/ml. The contents of the major hydrophilic components were: danshensu (3.1 ± 0.03 mg/g), salvianolic acid B (37.3 ± 0.5 mg/g), rosmarinic acid (1.8 ± 0.02 mg/g) and protocatechuic aldehyde (0.16 ± 0.001 mg/g).

Effects of a single dose Danshen aqueous extract treatment on the pharmacokinetics of caffeine

For the single dose treatment, rats were treated with Danshen aqueous extract (100 or 200 mg/kg, i.p.) or saline (control). One day after pretreatment, the rats were anaesthetised with urethane (20% w/v, 6 ml/kg, i.p.) and the carotid artery was cannulated for collection of blood samples, and the jugular vein for injection of caffeine and replacing saline after blood sampling. A single dose of caffeine (60 mg/kg, i.v.) was given via the jugular vein approximately 90 min after anaesthesia. Serial blood samples (0.3 ml) were collected via the carotid artery at 0, 10, 20, 40, 60, 90, 120, 180, 240, 300 and 360 min after caffeine administration. Plasma was separated and stored

at −20°C before analysis by HPLC. Cimetidine (60 mg/kg, i.p.) was used as the positive control for the enzyme inhibition studies.

Effects of 14-day Danshen aqueous extract treatment on the pharmacokinetics of caffeine

Rats were pretreated with Danshen aqueous extract (100 mg/kg/day, i.p. or 200 mg/kg/day. p.o.) for 14 days. The control rats received an equal volume of saline. On the morning of day 15, the rats were anaesthetised and pharmacokinetic experiments were performed as described in the previous section.

HPLC analysis of caffeine and metabolite (paraxanthine)

The HPLC analysis of caffeine and paraxanthine was as described, with modifications by Zhu *et al*.[14] The HPLC system consisted of a Hewlett Packard (HP) 1050 series pumping system and a multiple wavelength detector (set at 313 nM). The eluates were analysed by passing through a guard column (Supelco Pelliguard LC-18) and a reversed-phase C18 (Supelco Spherisorb S5/ODS2, 250 × 4.6 mm, 5 μm) column and flow rate of 0.6 ml/min. The experiment was performed in ambient temperature. A gradient condition of solvent A (water) and solvent B (acetonitrile) was programmed as follows: 0 min, 15% B; 3 min, 15% B; 9 min, 85% B; 12 min, 85% B; 13 min, 15% B. The detection wavelength was set at 272 nm. Under the experimental conditions, paraxanthine, caffeine and phenacetin (internal standard) were eluted at 7.6, 10.4 and 12.5 min, respectively.

Preparation of rat liver microsomes

Male Sprague-Dawley rats (250–300 g) were killed by exsanguination. The liver was excised, rinsed with ice-cold 0.9% NaCl solution, weighed and homogenised in a 0.1 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose. The homogenate was centrifuged at 10 000*g* at 4°C for 30 min. The supernatant was then centrifuged at 105 000*g* at 4°C for 60 min. The pellet was reconstituted with 0.1 mM phosphate buffer (pH 7.4) and the protein concentration of the liver microsomes was determined by a protein assay.[15]

Assays of CYP1A2 activity in human and rat liver microsomes

CYP1A2 activity was assessed by formation of paracetamol from phenacetin by the method reported previously.[16] The oxidative metabolism of phenacetin was measured in a system consisting of an NADPH-generating system and microsomes according to the method specified below. The incubation mixture (final volume of 500 μl in 0.05 M Tris/KCl buffer, pH 7.4) consisted of an NADPH-regenerating system for human (1.3 mM NADP, 3.3 mM G6P, 0.4 U/ml G6PDH, 3.3 mM magnesium chloride and 0.8 mg/ml pooled human liver microsomes), and rat (10 mM NADP, 5 mM G6P, 2 U/ml G6PDH, 5 mM magnesium chloride and 1 mg/ml rat liver microsomes). For the inhibition study, 50 μM phenacetin was used. For kinetic studies, phenacetin concentrations ranged from 10 to 100 μM. The concentrations of Danshen extract used were from 125 to 2000 μg/ml. Furafylline, a selective CYP1A2 inhibitor, was used as positive control. The tubes were incubated in Eppendorf Themomixer at 800 rev/min, 37°C. The reaction was initiated by adding protein to the incubation mixture. After 30 min, incubations were terminated by adding 500 μl ice-cold acetonitrile. The tubes were then centrifuged in a microcentrifuge at 13 000*g* for 12 min to precipitate protein. The supernatant was collected and metacetamol (10 μl, 50 μg/ml; 3.31 nmol) was added as an internal standard. The whole mixture was then extracted with 500 μl ethyl acetate at 1400 rev/min in a Thermomixer for 30 min at 25°C. The tubes were then centrifuged at 8000*g* for 8 min. The organic layer was transferred to a glass conical tube and evaporated at a heat block at 40°C under a gentle stream of nitrogen gas. The residue was dissolved in 120 μl mobile phase, and then 50 μl was used for HPLC analysis. HPLC analysis of phenacetin and paracetamol was performed as described by Wang *et al*.[10]

Pharmacokinetic and statistical analysis

Pharmacokinetic parameter calculation was by standard non-compartmental methods by the PK Solutions 2.0 (Summit Research Services, USA). $C_{initial}$ is the initial caffeine concentration extrapolated to time zero. Area under the curve, AUC, from time 0 to 360 min was calculated by the trapezoidal rule. Vd is the apparent volume of distribution defined as the volume of fluid required to contain the total amount of drug in the body, calculated by Vd = D/(AUC x k), where D is the dose of caffeine and k is the elimination rate constant. The elimination half-life ($t^1/_2$) was calculated as 0.693/k, where k, the elimination rate constant, was calculated from semilog regression on the terminal phase of the plasma concentration–time curve. CL is the total clearance calculated by CL = D/AUC as the volume of plasma from which the drug is totally removed in unit time by all elimination processes in the body.

Enzyme kinetics data were fitted by nonlinear regression analysis using GraphPad Prism 5 (GraphPad Software Inc., CA, USA). The data were fitted to the Michaelis–Menten model and further analysed using Dixon plots. The coefficient of determination (R^2) and the Dixon plots were used to determine the quality of fit to a specific model. The inhibition constant (K_i) was obtained by the Prism program and Dixon plots. IC50 values (concentration of inhibitor to cause 50% inhibition of original enzyme activity) were calculated from plots of the percentage of the product formed vs the concentration of the inhibitor.

One-way analysis of variance was used to estimate the significance of differences. A P-value less than 0.05 was considered to indicate statistical significance.

Results

Effects of a single dose Danshen aqueous extract treatment on the pharmacokinetics of caffeine

Caffeine is metabolised via CYP1A2 to form theobromine (11%), paraxanthine (80%) and theophylline (4%). The concentration of paraxanthine divided by the concentration of caffeine in the plasma sample at 6 h is commonly used to

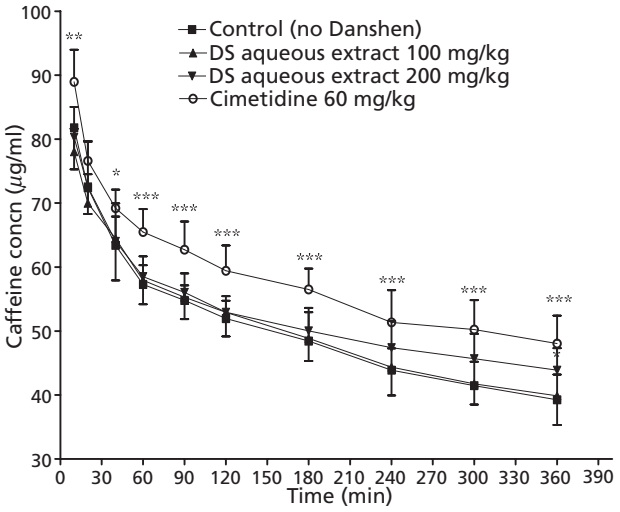

Figure 1 Concentration–time profiles of caffeine after single dose treatment with saline control or Danshen aqueous extract after a standard dose of caffeine. Danshen aqueous extract, DS (i.p.): 100 or 200 mg/kg. Caffeine dose: 60 mg/kg (i.v.). Results were mean ± SD of six rats. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ when compared with control.

assess CYP1A2 activity.[13] Figure 1 shows the plasma concentration–time profiles of caffeine after a single dose treatment with Danshen (100 or 200 mg/kg, i.p.). As summarised in Table 1, Danshen aqueous extract (200 mg/kg, i.p.) treatment decreased caffeine clearance by 20% and the paraxanthine/caffeine ratio by 33%, with increases in the AUC (24%) and plasma half-life (14%). The enzyme inhibitor cimetidine significantly delayed the clearance of caffeine starting from 10 min (Figure 1) by increasing the $C_{initial}$, $t^1/_2$, and AUC, and decreased the Vd and CL (Table 1).

Effects of daily Danshen aqueous extract treatment for 14 days on pharmacokinetics of caffeine

The plasma concentration–time profiles of caffeine after daily Danshen aqueous extract treatment (100 mg/kg/day, i.p.) for 14 days are shown in Figure 2. Figure 3 shows the plasma concentration–time profiles of caffeine after daily oral treatment with Danshen aqueous extract 200 mg/kg/day (p.o.) for 14 days. Treatment of Danshen aqueous extract 100 mg/kg/day (i.p.) for 14 days decreased caffeine clearance, the Vd, and the paraxanthine/caffeine ratio, with increases in the $t^1/_2$ and AUC (Table 2). Treatment of Danshen aqueous extract 200 mg/kg/day (p.o.) for 14 days also decreased caffeine clearance and the paraxanthine/caffeine ratio, increased the $t^1/_2$ and AUC, but had no effect on the Vd, which indicated 14 days Danshen oral treatment had little or no effect on the distribution of caffeine (Table 2).

Effects of Danshen aqueous extract on liver microsomal CYP1A2 activity in humans and rats

The aqueous extract of Danshen root inhibited the formation of paracetamol in a concentration-dependent manner, with a decrease in the paracetamol / phenacetin ratio in the human and rat liver microsomes. The IC50 values for Danshen aqueous extract in humans and rats were 840 and 1110 μg/ml, respectively. Enzyme kinetic studies of the phenacetin O-deethylation (CYP1A2) were determined with various phenacetin concentrations, in the presence or absence of Danshen aqueous extract (Figures 4 and 5). The apparent enzyme kinetic parameters for phenacetin O-deethylation were estimated by the Prism program, which were the goodness of the fit in the competitive model. The Dixon plot transformation of the enzyme velocities vs inhibitor concentrations also showed that Danshen aqueous extract acted as a competitive inhibitor. In addition the K_i was 190 μg/ml for humans and 360 μg/ml for rats. The inhibitory effects of Danshen aqueous extract on phenacetin O-deethylation were comparable with furafylline, a specific human CYP1A2 inhibitor.

Discussion

Danshen hot water extract has been reported to have many types of pharmacological action, such as promoting blood circulation, clearing blood from the heart, relaxing the coronary artery, antioxidant activity and antifibrotic activity.[17,18] Since the commonly used preparation method for Danshen root for human consumption in Chinese medicine is the hot water extract method, Danshen aqueous extract was selected as the test preparation to study the interaction with other drugs. This study has demonstrated that the aqueous extract of Danshen dried root inhibited the pharmacokinetics of caffeine in rats.

We evaluated the effects of Danshen aqueous extract on CYP1A2 activity *in vivo* in rats by measuring the plasma concentrations of caffeine and its metabolite paraxanthine. Since caffeine is metabolised via CYP1A2 to form approximately 80% paraxanthine, caffeine is a typical probe substrate for evaluating CYP1A2 activity *in vivo* and the paraxanthine / caffeine ratio in the plasma sample at 6 h is commonly used to assess CYP1A2 activity in man.[13,19] Additionally, P-glycoprotein cannot affect the hepatic metabolism of caffeine because caffeine is not a P-glycoprotein substrate.[20] In this study, the intraperitoneal route was chosen to compare with the oral route (the usual route of administration in humans) in rats. In addition, the dosage selection of Danshen extract was based on similar studies with warfarin and the clinical dosages used in previous studies.[6,21] Fourteen-day Danshen aqueous extract treatment (100 mg/kg/day, i.p.) significantly decreased the value for Vd, which may have been due to the changes in the plasma protein binding. Further study is required to confirm the result and to explain it.

Our previous studies have demonstrated that the extract from a Danshen capsule (supplied by Winsor Health Products Limited, Hong Kong) rich in tanshinones inhibited the metabolism of caffeine without affecting CYP1A2 expression in rats.[10] The present in-vivo results showed that Danshen aqueous extract inhibited CYP1A2 activity in the rat, as indicated by the paraxanthine / caffeine ratio and the plasma concentration–time profiles and pharmacokinetic parameters of caffeine. However, Qiu *et al*.[12] reported that Danshen extract tablets rich in hydrophilic components (salvianolic

Table 1 Pharmacokinetics of caffeine after single dose treatment of Danshen aqueous extract

Parameter	Control saline	Danshen aqueous extract (100 mg/kg, i.p.)	Danshen aqueous extract (200 mg/kg, i.p.)	Cimetidine (60 mg/kg)
$t^1/_2$ (min)	239 ± 31.2	277 ± 40.6	272 ± 44.0*	312 ± 44.3*
$C_{initial}$ (μg/ml)	69.6 ± 4.0	69.1 ± 2.1	69.1 ± 4.2	75.5 ± 3.7***
AUC (mg min/ml)	38.0 ± 5.1	40.5 ± 5.1	47.2 ± 5.8**	49.9 ± 5.3**
Vd (ml/kg)	835 ± 43.6	843 ± 31.7	838 ± 49.0	772 ± 38.9***
CL (ml/min/kg)	1.55 ± 0.23	1.46 ± 0.20	1.24 ± 0.14*	1.11 ± 0.22***
PX / CF ratio	0.21 ± 0.04	0.18 ± 0.04	0.14 ± 0.02**	0.10 ± 0.03***

Caffeine dose was 60 mg/kg (i.v.). $t^1/_2$, elimination half-life; $C_{initial}$, initial caffeine concentration extrapolated to time zero; AUC, area under the curve; Vd, apparent volume of distribution; CL, total clearance; PX / CF ratio, paraxanthine / caffeine ratio at 6 h. Results were mean ± SD of six animals. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with corresponding controls (saline).

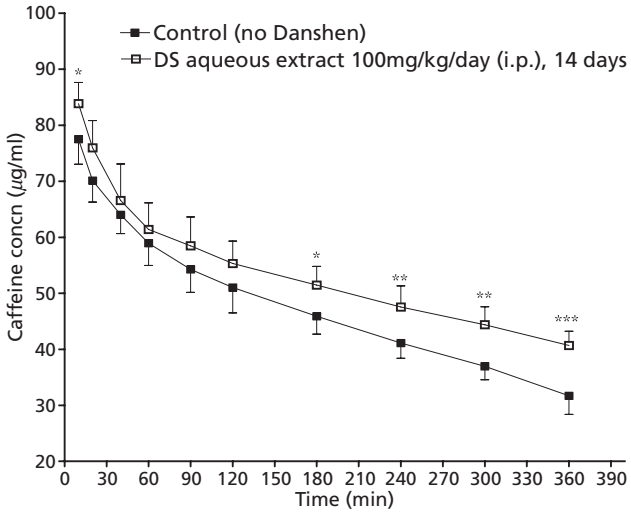

Figure 2 Concentration–time profiles of caffeine after a 14-day treatment with Danshen aqueous extract after a standard dose of caffeine. DS, Danshen aqueous extract. Caffeine dose: 60 mg/kg (i.v.). Results were mean ± SD of six rats. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ when compared with control.

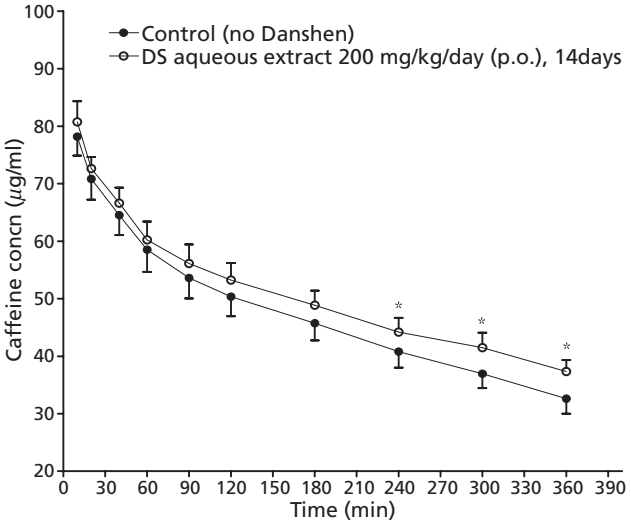

Figure 3 Concentration–time profiles of caffeine after a 14-day treatment with Danshen aqueous extract after a standard dose of caffeine. DS, Danshen aqueous extract. Caffeine dose: 60 mg/kg (i.v.). Results were mean ± SD of six rats. *$P < 0.05$ when compared with control.

acid B and danshensu) did not affect the pharmacokinetics of theophylline (a probe substrate for evaluating CYP1A2 activity) in healthy volunteers. The discrepancies may have been due to species differences in CYP1A2 expression and the probe substrates used in the study. In addition, the different composition of Danshen extracts used, that is, the different proportions of hydrophilic and hydrophobic components present, may have affected the outcome of the study. Therefore, the potential mechanism of interaction of Danshen with CYP1A2 needs further study.

To clarify the basis of drug–drug interactions and to avoid and/or predict potential interactions of Danshen, in-vitro studies on human and rat hepatic microsomes were performed to examine the inhibitory potency of Danshen aqueous extract towards the activity of CYP1A2. Murine and human CYP1A2 showed approximately 72% amino acid sequence homology with common catalytic activities.[22] The water extract of Danshen root showed similar inhibitory effects on human and rat CYP1A2 activity with similar IC50 and K_i values seen in this study. Therefore the Sprague-Dawley rat was a good metabolism model for CYP1A2, which has similar typical substrates and metabolism activities as humans. Previously, we reported that the K_i values of tanshinones (pure compounds) were very small, e.g. dihydrotanshinone (3.6 μM), cryptotanshinone (4.1 μM), tanshinone I (22.6 μM) and tanshinone IIA (23.8 μM).[10] In this study, the K_i value was much larger than those of tanshinones, because the aqueous extract of Danshen contained a variety of ingredients and was relatively rich in hydrophilic components (salvianolic acid B and danshensu) with low levels of tanshinones. In addition, the hydrophilic components of Danshen, including salvianolic acid B and danshensu, did not affect the metabolism of the CYP1A2 probe substrates.[10]

Characterisation of the cytochrome P450 isoforms responsible for the metabolismof drugs and herbs is important for the identification of potential drug–drug or drug–herb interactions.[23,24] In clinical practice in China, it is very common to prescribe principal synthetic or biotechnological drugs

Table 2 Pharmacokinetics of caffeine after a 14-day treatment with Danshen aqueous extract

Parameter	Control saline (i.p.)	Danshen aqueous extract (100 mg/kg/day, i.p., 14 days)	Control saline (p.o)	Danshen aqueous extract (200 mg/kg/day, p.o., 14 days)
$t^1/_2$ (min)	239 ± 25.8	264 ± 32.4	231 ± 26.2	250 ± 27.9
$C_{initial}$ (μg/ml)	71.0 ± 3.8	73.6 ± 5.2	70.8 ± 3.3	72.1 ± 3.1
AUC (mg min/ml)	31.3 ± 2.8	41.1 ± 4.0***	31.3 ± 2.6	37.1 ± 3.6**
Vd (ml/kg)	844 ± 48.8	787 ± 37.9*	839 ± 37.2	819 ± 29.5
CL (ml/min/kg)	1.94 ± 0.19	1.43 ± 0.12***	1.90 ± 0.15	1.60 ± 0.15**
PX / CF ratio	0.20 ± 0.03	0.12 ± 0.02***	0.19 ± 0.03	0.14 ± 0.01**

Caffeine dose was 60 mg/kg (i.v.). $t^1/_2$, elimination half-life; $C_{initial}$, initial caffeine concentration extrapolated to time zero; AUC, area under the curve; Vd, apparent volume of distribution; CL, total clearance; PX / CF ratio, paraxanthine / caffeine ratio at 6 h. Results were mean ± SD of six animals. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with corresponding controls (saline).

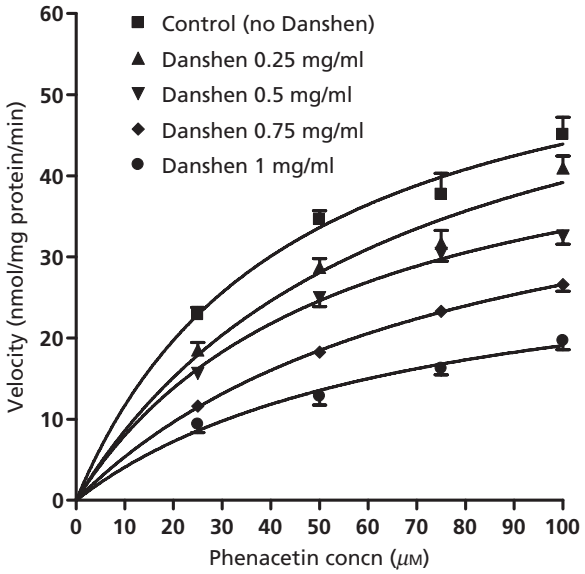

Figure 4 Kinetics of paracetamol formation in pooled human liver microsomes. Reactions were performed in the presence of phenacetin (25, 50, 75, 100 μM) at various concentrations of Danshen aqueous extract (0, 0.25, 0.5, 0.75, 1 mg/ml). Data are expressed as mean ± SD of three determinations.

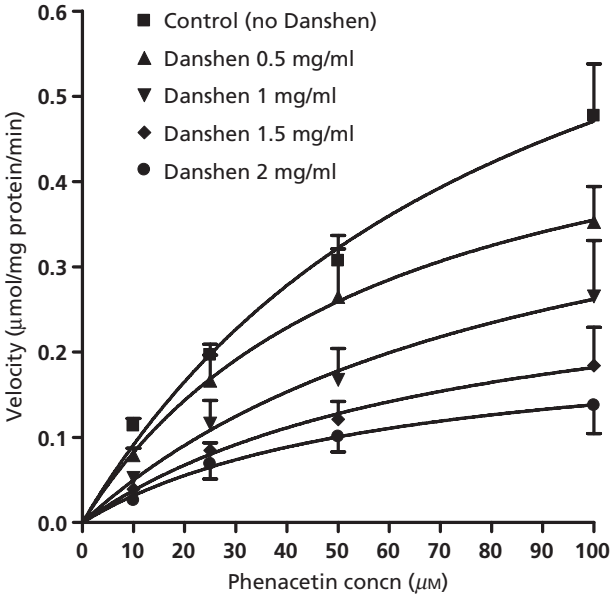

Figure 5 Kinetics of paracetamol formation in rat liver microsomes. Reactions were performed in the presence of phenacetin (10, 25, 50, 100 μM) at various concentrations of Danshen aqueous extract (0, 0.5, 1, 1.5, 2 mg/ml). Each data point represents the mean ± SD of six rats.

together with Danshen for supplementary purposes. However, exaggerated anticoagulation and bleeding complications have been observed during concurrent use of Danshen and warfarin in patients. Previous studies in our laboratory have shown that Danshen increased the prothrombin time of warfarin in the rat and decreased the clearance of *R*- and *S*-warfarin in rats *in vivo*.[5,6] Since warfarin metabolism involves CYP1A2, CYP2C11 (rat) / CYP2C9 (human) and CYP3A isoforms in both the rat and human,[25] it was not possible to confirm which of the CYP isoforms was more affected with respect to Danshen–warfarin interactions. To assess the potential interactions of Danshen with drugs that utilise cytochrome P450 in their metabolism, it is important to determine the effects of Danshen on the P450 activity of individual isoforms. This study confirmed Danshen aqueous extract inhibited human and rat CYP1A2 activity. Since warfarin metabolism involves CYP1A2 in human and rats, inhibition of CYP1A2 activity by Danshen may be a reason for Danshen–warfarin interactions, although it is not as important compared with the inhibition of CYP2C9/11.[25]

Conclusions

Co-administration of Danshen aqueous extract may affect the metabolism of CYP1A2 substrates through competitive inhibition and alter their clearance. This finding provided some safe information on how to use Danshen preparations in clinical practice. Further systematic study *in vivo* is needed to identify the interactions of Danshen and its components with cytochrome P450s in humans.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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