Kinetics of cytochrome P450 enzymes for metabolism of sodium tanshinone IIA sulfonate in vitro

Dong-sheng Ouyang¹,², Wei-hua Huang¹, Dan Chen¹, Wei Zhang¹, Zhi-rong Tan¹, Jing-bo Peng¹, Yi-cheng Wang², Ying Guo², Dong-li Hu², Jian Xiao³ and Yao Chen¹,²*

Abstract

Background: Sodium tanshinone IIA sulfonate (STS) is a water-soluble derivative of tanshinone IIA for treating cardiovascular disorders. The roles of cytochrome P450 enzymes (CYPs) in the metabolism of STS have remained unclear. This study aims to screen the main CYPs for metabolism of STS and study their interactions in vitro.

Methods: Seven major CYPs were screened for metabolism of STS by human liver microsomes (HLMs) or recombinant CYP isoforms. Phenacetin (CYP1A2), coumarin (CYP2A6), tolbutamide (CYP2C9), metoprolol (CYP2D6), chlorzoxazone (CYP2E1), S-mephenytoin (CYP2C19), and midazolam (CYP3A4) were used as probe substrates to determine the potential of STS in affecting CYP-mediated phase I metabolism in humans. Enzyme kinetic studies were performed to investigate the modes of inhibition of the enzyme–substrate interactions by GraphPad Prism Enzyme Kinetic 5 Demo software.

Results: Sodium tanshinone IIA sulfonate inhibited the activity of CYP3A4 in a dose-dependent manner by the HLMs and CYP3A4 isoform. The $K_m$ and $V_{max}$ values of STS were $54.8 ± 14.6 \mu M$ and $0.9 ± 0.1 nmol/mg protein/min$, respectively, for the HLMs and $7.5 ± 1.4 \mu M$ and $6.8 ± 0.3 nmol/nmol P450/min$, respectively, for CYP3A4. CYP1A2, CYP2A6, CYP2C9, CYP2D6, CYP2E1, and CYP2C19 showed minimal or no effects on the metabolism of STS.

Conclusion: This in vitro study showed that STS mainly inhibited the activities of CYP3A4.

Background

Tanshinone IIA (Fig. 1a) is one of the main extracts from Salvia miltiorrhiza (Danshen) for treating cardiovascular disorders [1]. Tanshinone IIA is the most effective fat-soluble monomer extracted from Danshen, with anti-inflammatory [2], antitumor [3], antioxidative [4, 5], and antiplatelet aggregation activities [6, 7]. However, as clinical use of tanshinone IIA is limited by its poor water solubility, sodium tanshinone IIA sulfonate (STS) (Fig. 1b) has been developed through sulfonation with greater water-soluble characteristics and efficacies than tanshinone IIA [8], and has been used in injection with typical doses of 40–80 mg/d for cardioprotective [9, 10], anti-cardiomyocyte hypertrophy [11], and antiviral effects [12–14]. To date, only several studies reported the pharmacokinetics parameters of STS in rats after injection. The metabolism rate of STS in rats was fast, with $t_{1/2}$ of <0.9 h, and at 4 h after the injection, the plasma concentration of STS in rats did not exceed 20 ng/mL [15, 16].

CYPs comprise the most important phase I drug-metabolizing enzyme system, and are responsible for the metabolism of a variety of xenobiotics [17]. Yang et al. [18] reported that tanshinone (containing 20 % tanshinone IIA) could significantly increase (2–9.5-fold) the activities of CYP1A1, CYP1A2, and CYP2B1, while inhibiting (1.9-fold) the activity of CYP2E1 in rats. Ueng et al. [19] reported that tanshinone IIA could decrease 7-ethoxyresorufin O-deethylation (EROD) and 7-methoxyresorufin O-demethylation (MROD) activities in...
human liver microsomes (HLMs), and indicated that tanshinone IIA possessed the highest selectivity for inhibition of CYP1A2. The same authors further demonstrated that tanshinone IIA induced hepatic Cyp1A2 by increasing the expression levels of Cyp1A2 mRNA and protein in mice [20]. Liu et al. [21] reported that CYP2A6 was the specific isozyme responsible for hydroxyl metabolism of tanshinone IIA in HLMs. Although many experiments related to tanshinone IIA have been performed, the question of which CYP is responsible for the metabolism of STS has remained unclear.

The difference in molecular structure between STS and tanshinone IIA is only a sulfonic acid group at the C-16 position [22]. Although STS has a similar molecular structure to tanshinone IIA, the metabolism of STS and interactions with CYPs might differ from those of tanshinone IIA in HLMs. Although many experiments related to tanshinone IIA have been performed, the question of which CYP is responsible for the metabolism of STS has remained unclear.

The difference in molecular structure between STS and tanshinone IIA is only a sulfonic acid group at the C-16 position [22]. Although STS has a similar molecular structure to tanshinone IIA, the metabolism of STS and interactions with CYPs might differ from those of tanshinone IIA in HLMs. Although many experiments related to tanshinone IIA have been performed, the question of which CYP is responsible for the metabolism of STS has remained unclear.

The difference in molecular structure between STS and tanshinone IIA is only a sulfonic acid group at the C-16 position [22]. Although STS has a similar molecular structure to tanshinone IIA, the metabolism of STS and interactions with CYPs might differ from those of tanshinone IIA in HLMs. Although many experiments related to tanshinone IIA have been performed, the question of which CYP is responsible for the metabolism of STS has remained unclear.

The difference in molecular structure between STS and tanshinone IIA is only a sulfonic acid group at the C-16 position [22]. Although STS has a similar molecular structure to tanshinone IIA, the metabolism of STS and interactions with CYPs might differ from those of tanshinone IIA in HLMs. Although many experiments related to tanshinone IIA have been performed, the question of which CYP is responsible for the metabolism of STS has remained unclear.

As CYP1A2 (13 %), CYP2A6 (4 %), CYP2D6 (2 %), CYP2E1 (7 %), CYP3A4 (30 %), CYP2C19 (8 %) and CYP2C9 (20 %) comprise 70–80 % of the hepatic CYPs in humans and are responsible for more than 95 % of clinical drug metabolism [17]. This study aims to screen the main CYPs for metabolism of STS and study their interactions in vitro.

Methods

Enzymes and chemicals

Recombinant human CYP enzymes (bactosomes), pooled human liver microsomes (HLMs) from ten individual donors (bactosomes), and β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH) were purchased from Cypex Ltd. (UK) and stored at −80 °C. STS (C_{19}H_{17}NaO_{6}S; MW: 396.39; assay: ≥98 %) was obtained from Sigma-Aldrich (China). Furafylline (FUR), trans-2-phenylcyclopropylamine hydrochloride (TRA), ketoconazole (KET), sulfaphenazole (SUL), quinidine (QUI), chlorimethiazole hydrochloride (CHL), and ticlopidine hydrochloride (TIC) were purchased from the National Institutes for Food and Drug Control (China). Phenacetin, coumarin, midazolam, tolbutamide, S-mephenytoin, metoprolol, chlorzoxazone, and standards for their metabolites, including acetaminophen, 7-hydroxyl coumarin, 1-hydroxyl midazolam, 4-hydroxyl tolbutamide, 4-hydroxyl mephenytoin, α-hydroxyl metoprolol, 6-hydroxyl chlorzoxazone, and irbesartan (internal standard) were purchased from Sigma-Aldrich (China). All other chemicals and solvents were of high-performance liquid chromatography (HPLC) grade.

Apparatus and operation conditions

The concentrations of the CYP substrates and their metabolites were quantified using a Waters 2695 Separation Module HPLC System (Waters Corp., USA) coupled to a Quattro Micro™ API Triple Quadrupole Tandem Mass Spectrometer (Waters Corp., USA) with an electrospray ionization source. The samples were separated on a HYPURITY C_{18} column (150 × 2.1 mm; internal diameter: 5 µm; Thermo, USA) with a C_{18} security guard column (4.0 × 3.0 mm; internal diameter: 5 µm). The mobile phase consisted of 20 mM ammonium formate and acetonitrile at a ratio of 60:40. Aliquots of 20 µL were injected at a mobile phase flow rate of 0.3 mL/min. Multiple reaction monitoring was performed in positive or negative modes according to different compounds. The transitions were listed in Table 1. The mass spectra of the metabolites formed in the incubations were identical to those of the corresponding authentic standards, including 7-hydroxyl coumarin, 1- hydroxyl midazolam, 4- hydroxyl tolbutamide, 4- hydroxyl mephenytoin, α- hydroxyl metoprolol, 6- hydroxyl chlorzoxazone [24].

Incubation conditions

The inhibitory effects of STS on the activities of the CYP isoforms were examined by HLMs (and the expressed CYPs, when required). The CYP isoform-specific probe reactions used were phenacetin O-deethylation (for CYP1A2), coumarin 7-hydroxylation (for CYP2A6),...
tolbutamide 4-hydroxylation (for CYP2C9), metoprolol α-hydroxylation (for CYP2D6), chlorzoxazone 6-hydroxylation (for CYP2E1), S-mephenytoin 4-hydroxylation (for CYP2C19), and midazolam 1-hydroxylation (for CYP3A4). The incubation mixtures consisted of substrate probe, HLMs (0.5 mg/mL) or CYP isoforms (10 pmol), and 0.1 M sodium phosphate buffer (pH 7.4) in a total volume of 0.2 mL that was pre-warmed for 5 min at 37 °C without (control) and with multiple concentrations of STS. Each reaction was initiated by addition of 1 mg/mL NADPH. The final incubations were performed in a shaking water bath at 37 °C for 30 min. The incubations were performed in triple, and the incubation conditions specific to each CYP isoform were within the linear range for the velocity of the reaction (incubation time as well as substrate and protein concentrations). STS, probe substrates, and inhibitors were dissolved in methanol, and the final solvent concentration in all incubations (including controls) was 1 %. The reactions were stopped by adding 0.2 mL of ice-cold acetonitrile containing irbesartan (114.9 ng/mL) as the internal standard. The samples were vortexed for 5 min. After centrifugation (5415D centrifuge, Eppendorf, Germany) (12,000 × g for 10 min), the supernatants were transferred and aliquots of 20 µL were injected into the HPLC–MS/MS system for analysis.

Kinetic analysis of STS
Kinetic analyses were performed for STS, and the data generated were used as a guide for selecting the appropriate concentrations of STS in the subsequent inhibition experiments. Thus, the kinetic parameters for the metabolism of STS were determined by incubating increasing concentrations of STS (1–100 µM) (without inhibitor) at 37 °C with HLMs (0.5 mg/mL) or CYP isoforms (10 pmol), and 0.1 M sodium phosphate buffer (pH 7.4) in a total volume of 0.2 mL. The incubation conditions consisted of substrate probe, HLMs (0.5 mg/mL) or CYP isoforms (10 pmol), and 0.1 M sodium phosphate buffer (pH 7.4) in a total volume of 0.2 mL. The equation for STS reaction velocity (V) by the HLMs or CYP isoforms was expressed as $V = \frac{C_0 - C_f}{T/C_p}$, where $C_0$ and $C_f$ represent the initial and final concentrations of STS in the incubation solution, respectively, T is the incubation time (min), and $C_p$ is the protein concentration (mg/mL or nmol). All values were expressed as the mean ± standard deviation (SD). The mean intrinsic clearance rate ($CL_{int}$) for the in vitro incubation was estimated by $V_{max}/K_m$.

Specific CYP isoforms screened for metabolism of STS
We determined the inhibitory effects of specific inhibitors on the metabolism of STS by HLMs to screen for the specific CYP isoform responsible for STS metabolism. Inhibitors including FUR (CYP1A2 inhibitor; 1 µM), TRA (CYP2A6 inhibitor; 1 µM), SUL (CYP2C9 inhibitor; 1 µM), QUI (CYP2D6 inhibitor; 1 µM), CHL (CYP2E1 inhibitor; 5 µM), TIC (CYP2C19 inhibitor; 1 µM), and KET (CYP3A4 inhibitor; 1 µM) were separately incubated with STS (10 µM), HLMs, and NADPH under the same incubation conditions as mentioned above. The concentrations of the inhibitors used were approximately the same as their respective $IC_{50}$ values from a previous report [25]. The inhibitory effects of the specific inhibitors on the metabolic clearance rate (MCR) of STS were evaluated separately to screen for the CYP isoforms responsible for STS metabolism. The relative activity of each CYP isoform was calculated by dividing the peak area of STS incubated with the inhibitor by the peak area of STS in the negative control.

Inhibition studies for $IC_{50}$ determination
A pilot inhibitory analysis of each CYP isoform was performed to determine the potency of inhibition and to select CYP isoforms for further detailed study of their
inhibitory activities. Various concentrations of STS (1–100 µM) and a single CYP isoform-specific substrate concentration (the $K_i$ value) were used to determine the inhibitory effects of STS on specific CYP isoforms. The substrates comprising phenacetin, coumarin, tolbutamide, metoprolol, chlorzoxazone, S-mephenytoin, and midazolam were employed at concentrations of 10, 5, 100, 7.5, 40, 100, and 5 µM, respectively [25]. All incubation conditions were the same as those described above. The inhibitory effects on the CYP isoforms were investigated individually by incubating HLMs in the absence or presence of STS. An incubation solution containing only the solvent used to dissolve STS was regarded as the negative control, while solutions containing only the specific inhibitors were regarded as positive controls. The $IC_{50}$ values for STS were determined and compared with those of the specific inhibitors mentioned above (Table 2).

**Determination of $K_i$**

In pilot experiments ($IC_{50}$ determination), STS inhibited CYP3A4, while its effects on the remaining CYPs (CYP1A2, CYP2A6, CYP2C9, CYP2D6, CYP2E1, and CYP2C19) were minimal. Therefore, Dixon plots for the inhibition of CYP3A4 were determined by incubating the substrate probe at multiple concentrations with or without the test inhibitor at multiple concentrations with the HLMs and cofactors. The inhibition data obtained from the pilot experiments were used as a guide to generate appropriate probe substrate and test inhibitor concentrations for determination of the $K_i$ values for each CYP isoform. The isoform-specific probe substrate concentrations used were 5–50 µM midazolam for CYP3A4. The STS concentrations used were 0–100 µM.

**Calculation of enzyme kinetics and statistical analysis**

The MCR of the incubation solution without any specific inhibitor and STS were added to the incubation assay. The $IC_{50}$ values were determined by analyzing the plot of the logarithm of the inhibitor concentration versus the percentage of activity remaining after inhibition, using the SPSS for Windows Version 11.5 (SPSS Inc., USA). The inhibition data were fitted to different models of enzyme inhibition (competitive, noncompetitive, and uncompetitive) by nonlinear least-squares regression analysis using GraphPad Prism 5 software (GraphPad Co. Ltd., USA) to calculate the $K_i$ values.

**Results**

**Kinetic analysis of STS**

The metabolism of STS after incubation with HLMs and CYP3A4 was shown in Fig. 2a, b. The $K_m$ and $V_{max}$

---

**Table 2 $IC_{50}$ and $K_i$ values of STS against human CYP isoforms compared with that of specific inhibitors reported in literature**

| CYP         | Activity                  | $IC_{50}$ (µM) | Specific inhibitor/ reported values $^a$ | $K_i$ (µM) | Specific inhibitor/ reported values $^a$ |
|-------------|----------------------------|----------------|------------------------------------------|------------|------------------------------------------|
| CYP1A2      | Phenacetin O-deethylation  | >100           | FUR/1.4 [23]                             | –          | FUR/3 [21]                                |
| CYP2A6      | Coumarin 7-hydroxylation   | >100           | TRA/0.42 ± 0.07 [24]                     | –          | TRA/0.17 [24]                             |
| CYP2C9      | Tolbutamide 4-hydroxylation| >100           | SUL/0.3–1.5 [23, 25]                     | –          | SUL/0.3 [21]                              |
| CYP2D6      | Metoprolol α-hydroxylation | >100           | QUI/0.02–0.68 [23, 25]                   | –          | SUL/0.027–0.4 [21, 26, 27]                |
| CYP2E1      | Chlorzoxazone 6-hydroxylation| >100           | DIE/21, 30 [23]                          | –          | CHL/12 [28]                              |
| CYP2C19     | S-Mephenytoin 4-hydroxylation| >100           | TCL/0.52–1.6 [25]                        | –          | TCL/1.2 ± 0.5 [21]                        |
| CYP3A4      | Midazolam 1-hydroxylation  | 6.377 (5.536, 7.347)$^b$ | KET/0.08–0.24 [21]                     | 3.183 (0.184, 6.95)$^b$ | KET/0.015 [21] |

$^a$–$^b$ Represents the data that is not determined

STS sodium tanshinone II A sulfonate; FUR furafylline; TRA trans-2-phenylcyclopropylamine hydrochloride; SUL sulfaphenazole; QUI quinidine; CHL chlorothiazide hydrochloride; TCL ticlopidine hydrochloride; KET ketocozolnazo; DIE diethyldithiocarbamate

$^a$ $IC_{50}$ and $K_i$ values of specific inhibitors were regarded as positive controls.

$^b$ Represents 95 % confidence interval
values for HLMs and CYP3A4 were 54.8 ± 14.6 µM and 0.9 ± 0.1 nmol/mg protein/min, respectively, and 7.5 ± 1.4 µM and 6.8 ± 0.3 nmol/nmol P450/min, respectively. The in vitro $CL_{int}$ values for STS with HLMs and CYP3A4 were 0.016 mL/mg protein/min and 0.902 mL/nmol P450/min, respectively.

Specific CYP isoforms for the metabolism of STS

The inhibitory effects of the CYP specific inhibitors on the MCR of STS in HLMs were shown in Fig. 3. The concentrations of FUR, TRA, SUL, QUI, TIC, and KET were 1 µM, while that of CHL was 5 µM. The concentrations were selected on the basis of previously reported $IC_{50}$ or $K_i$ values for the CYP isoforms to ensure adequate inhibitory selectivity, and maximal inhibitory potency [26–29]. In the presence of KET (1 µM), the MCR of STS decreased to 37.4 % of that of the control. However, the other inhibitors had no obvious inhibitory effects on the metabolism of STS. The effects of the screened enzymes were further confirmed with human recombinant CYPs using specific inhibitors, and the MCR of STS was decreased to 58.4 % (STS, 10 µM) and 29.4 % (STS, 50 µM) of the control value for CYP3A4 (Fig. 4), indicating that CYP3A4 was the major enzyme responsible for the metabolism of STS in vitro.
Estimation of IC50 and Ki values
The inhibitory effects of multiple concentrations of STS (1–100 µM) on the activity of each CYP isoform were determined by metabolism of a single concentration of isoform-specific probe with HLMs (or expressed CYPs, when needed). STS showed potent inhibition of CYP3A4 (midazolam 1-hydroxylation), with an IC50 of 6.4 µM. The inhibitory effects of STS on the activities of CYP1A2, CYP2A6, CYP2C9, CYP2D6, CYP2E1, and CYP2C19 were negligible (Fig. 5); therefore, we did not calculate the IC50 or Ki values for these enzymes related to STS.

Discussion
According to Jeong et al. [30], IC50 values are qualitatively informative and helpful for addressing whether inhibition has occurred, but are of limited quantitative use because they can be influenced by the substrate concentration selected. Hence, it might not be accurate to use these parameters for quantitative prediction of drug interactions in vivo. Therefore, we performed additional experiments designed to estimate the Ki values. The preliminary inhibition data generated from a single probe substrate reaction were used to simulate the appropriate range of substrate and inhibitor concentrations for use in the construction of Dixon plots for inhibition of the CYP isoforms by STS in HLMs, from which precise Ki values were estimated.

For CYP3A4, the Ki values were determined by midazolam as the probe substrate. Among all of the CYPs tested, CYP3A4 was the most sensitive to inhibition by STS (Table 2). Representative Dixon plots for the inhibition of CYP3A4 in HLMs were shown in Fig. 6. Visual inspection of the Dixon plots and further analysis of the parameters of the enzyme inhibition models suggested that the inhibition data fitted well to a competitive type of inhibition (Fig. 7). The Ki values estimated by a non-linear regression model for competitive enzyme inhibition of CYP3A4-catalyzed midazolam 1-hydroxylation in HLMs were less than 5 µM (Table 2, Fig. 7).

According to Kong et al. [31], the potency of a test compound could be classified according to its IC50 values, as follows: potent, if IC50 is ≤20 µg/mL or ≤10 µM; moderate, if IC50 is 20–100 µg/mL or 10–50 µM; and weak, if IC50 is ≥100 µg/mL or ≥50 µM. Thus, STS was a potent inhibitor for CYP3A4 and a weak inhibitor for the other six CYPs tested in this study.

Although STS was a potent inhibitor of CYP3A4, the IC50 value of CYP3A4 for midazolam 1-hydroxylation was 26.6–79.7-fold higher and the Ki value was approximately 212-fold higher than that of KET in HLMs compared with a previous study [26] (Table 2), indicating that the inhibitory effect of STS on CYP3A4 was much less than that of KET.
Although the molecular structural difference between STS and tanshinone IIA is only the presence of a sulfonic acid group bond at the C-16 position [22], it resulted in a difference in inhibition of CYP activity. Wang et al. [32] reported that tanshinone IIA was a potent competitive CYP1A2 inhibitor with $K_i$ values of 1.45 $\mu$M for pooled HLMs and 0.05 $\mu$M for a specific human CYP1A2 isoform, and a medium competitive inhibitor of CYP2C9. In this study, STS was a potent competitive CYP3A4 inhibitor with a $K_i$ value of 3.2 $\mu$M for pooled HLMs, but was not an inhibitor of CYP1A2.

The CYP3A4 enzyme, one of the dominant CYP enzymes in both the liver and extrahepatic tissues such as the intestine, plays an important role in the oxidation of xenobiotics and contributes to the biotransformation of about 60 % of currently used therapeutic drugs [33]. Human CYP3A4 is one of the most abundant drug-metabolizing P450 isoforms in HLMs, and accounts for approximately 40 % of the total P450 activity [32]. Because STS was a potent inhibitor of CYP3A4 and given that CYP3A4 is responsible for the metabolism and disposition of a large number of currently used drugs, the potential herb–drug interactions of STS with drugs that were substrates of CYP3A4 or drugs with a narrow therapeutic index could not be negligible in the clinic. The present study supports the notion that STS is a substrate of CYP3A4 alone, while the issue of whether STS may be metabolized by other enzyme systems, such as more complex in vitro systems (hepatocytes for instance) that are not present (or functional in microsomes) remains unclear. Therefore, the present data do not support that STS would be a victim of drug–drug interactions (DDIs) by other CYP3A4 inhibitors, and further studies are required to elucidate this point.

Although STS in the form of injections is often used in clinical settings, pharmacokinetics studies in humans were lacking. Meanwhile, (Additional file 1) pharmacokinetics studies [15, 16] of STS in rats indicated that STS was widely distributed in most tissues after intravenous administration (2 mg/kg), and that it was mainly cleared via both the liver and kidney. The maximal STS concentration (>10 $\mu$g/mL) was found in the liver at 5 min after drug administration, subsequently declined progressively during 30 min, and then decreased quickly over time thereafter. STS could be determined at 12 and 4 h after drug administration in the liver and kidney, respectively. STS was hardly detected at 2 h after drug administration in other tissues [15, 16]. Therefore, co-administered medicines were suggested to be given at least 2 h after STS administration to avoid the risk of DDIs with STS.

Genetic variations in the CYP3A4 gene may influence the level or function of the CYP3A4 protein, and more than 30 single nucleotide polymorphisms (SNPs) have been identified in the CYP3A4 gene [34]. In previous studies, allelic variants in the gene encoding CYP3A4 were shown to affect enzyme activities, such as observations that variant CYP3A4 forms T363 M (<40 %) and T185S (<60 %) reduced testosterone 6β- and 2β-hydroxylase activities compared with the wild-type enzyme [35], while some SNPs in the CYP3A4 gene such as CYP3A4*1B and CYP3A4*22 were reported to affect the pharmacokinetics of tacrolimus [36]. However, the issue of whether genetic polymorphisms of CYP3A4 can affect the DDIs of STS requires further study for clarification in the future.

Conclusion
This in vitro study showed that STS mainly inhibited the activities of CYP3A4.

Additional file

Additional file 1. The post-hoc test for the effects of the specific inhibitors on the MCR of STS.

Abbreviations
STS: sodium tanshinone IIA sulfonate; CYPs: cytochrome P450 enzymes; HLMs: human liver microsomes; NADPH: $\beta$-nicotinamide adenine dinucleotide 2$^\prime$-phosphate reduced tetrasodium salt hydrate; FUR: furafylline; TRA: trans-2-phenylcyclopropylamine hydrochloride; KET: ketoconazole; SUL: sulfaphenazole; QUI: quinidine; CHL: chlorothiazole hydrochloride; TIC: ticlopidine hydrochloride; MCR: metabolic clearance rate.

Authors’ contributions
YC and DSOY designed the study and performed the data analysis. YC, WHH, DC, WZ, ZRT and JX conducted the acquisition of data. YC, JBP, YCW, YG,
References

1. Gao S, Liu Z, Li H, Little PJ, Liu P, Xu S. Cardiovascular actions and thera‑
peutic potential of tanshinone II A. Atherosclerosis. 2012;220:3–10.
2. Li W, Zhang Y, Xing C, Zhang M. Tanshinone II A represses inflammatory
response and reduces radiuclopathic pain by inhibiting IRAK-1 and
NF-κB/p38/JNK signaling. Int J Pharm. 2015;482:382–9.
3. Liu F, Yu G, Wang G, Liu H, Wu X, Wang Q, Liu M, Liao K, Wu M, Cheng
X, Hao H. An NQO1-initiated and p33-independent apoptotic pathway
determines the anti-tumor effect of tanshinone II A against non-small cell
lung cancer. F1000Res. 2012;2:e42138.
4. Hu H, Zhai C, Qian G, Gu A, Liu J, Yang F, Xu W, Jin D, Wang H, Hu Y,
Zhang Y, Tang G. Protective effects of tanshinone II A on myocardial ischemia
reperfusion injury by reducing oxidative stress, HMGB1 expression, and
inflammatory reaction. Pharm Biol. 2015;53:1752–61.
5. Wang P, Zhou S, Liu L, Yu Y, Yuan X, Zhang H, Li R, Fang J, Liu P. Hydro‑
gen peroxide-mediated oxidative stress and collagen synthesis in
cardiac fibroblasts: blockade by tanshinone II A. J Ethnopharmacol.
2013;145:152–61.
6. Maione F, De Feo V, Caiazzo E, De Martino L, Cicala C, Mascolo N.
Tanshinone II A, a major component of Salvia miltiorrhiza Bunge, inhibits
platelet activation via Erk-2 signaling pathway. J Ethnopharmacol.
2014;155:1236–42.
7. Maione F, Cantone V, Chini MG, De Feo V, Mascolo N, Bifulco G. Molecular
mechanism of tanshinone II A and cryptotanshinone in platelet anti‑
aggregating effects: an integrated study of pharmacology and computa‑
tional analysis. Fitoterapia. 2015;100:174–8.
8. Yu H, Subedi RK, Nepal PR, Kim YG, Choi HK. Enhancement of solubility
and dissolution rate of cryptotanshinone, tanshinone I and tanshinone
IIA extracted from Salvia miltiorrhiza. Arch Pharm Res. 2012;35:1457–64.
9. Long R, You Y, Li W, Jin N, Huang S, Li T, Liu K, Wang Z. Sodium tanshinone
IIA sulfonate ameliorates experimental coronary no-reflow phenomenon
through down-regulation of FGL2. Life Sci. 2015;142:8–18.
10. Mao S, Wang L, Zhao X, Shang H, Zhang M, Hinek A. Sodium tanshinone
IIA sulfonate for reduction of periprocedural myocardial injury during
percutaneous coronary intervention (STAMP trial): rationale and design.
Int J Cardiol. 2015;182:329–33.
11. Yang L, Hu J, Hao HZ, Yin Z, Liu G, Zou XJ. Sodium tanshinone II A sulfonate attenuates the transforming growth factor-β1-induced dif‑
f erentiation of atrial fibroblasts into myofibroblasts in vitro. Int J Mol Med.
2015;35:1026–32.
12. Zhang JQ, Gu YL, Wu CH, Ma HL, He JP, Bai YS, Li HQ. Effects of sodium
tanshinone IIA sulfonate against Marek’s disease virus in experimentally
infected chickens. Int J Biol Macromol. 2013;58:258–62.
13. Wang J, Sun P, Feng Y, Guo J, Sun Y, Lei H, Xu J, Li H. Sodium tanshinone
IIA sulfonate affects Marek’s disease virus replication by inhibiting gb
expression. Pharm Biol. 2016;54:701–4.
14. Sun N, Cong XM, Jiang JB, Zhao JX, Wang WK, Duan ZB, Hu YL, Lei HM,
Li HQ. Sodium tanshinone IIA sulfonate inhibits the meq, u49 and VP22
expression of Marek’s disease virus. Antivir Ther. 2014;19:793–8.
15. Mao S, Jin H, Bi Y, Li Z, Li H, Hou S. Ion-pair reversed-phase HPLC
method for determination of sodium tanshinone II A sulfonate in biological
samples and its pharmacokinetics and biodistribution in mice. Chem
Pharm Bull. 2007;55:753–6.
16. Hao H, Wang G, Cui N, Li J, Ding Z. Determination of sodium tanshinone
II A sulfonate in plasma by liquid chromatography-electrospray ionisation‑
tandem mass spectrometry. Biomed Chromatogr. 2007;21:1172–9.
17. Zhou S, Gao Y, Jiang W, Huang M, Xu A, Parrot JW. Interactions of herbs
with cytochrome P450. Drug Metab Rev. 2003;35:35–98.
18. Yang XF, Wang NP, Lu WH, Zeng DF. Effects of Ginkgo biloba extract and
tanshinone on cytochrome P450 isozymes and glutathione transferase
in rats. Acta Pharmacol Sin. 2003;24:1033–8.
19. Ueng YF, Kuo YH, Peng HC, Chen TL, Jan WC, Peter Guengerich F, Lin YL.
Diterpene quinone tanshinone II A selectively inhibits mouse and human
cytochrome P4501A2. Xenobiotica. 2003;33:603–13.
20. Ueng YF, Kuo YH, Wang SY, Lin YL, Chen CF. Induction of CYP1A by a
diterpene quinone tanshinone II A isolated from a medicinal herb Salvia
miltiorrhiza in C57BL/6 J but not in DBA/2J mice. Life Sci. 2004;74:885–96.
21. Liu HY, Hu Y, Liu Y, He YQ, Li W, Yang L. Hydroxylation of tanshinone
IIA in human liver microsomes is specifically catalysed by cytochrome
P4502A6. Xenobiotica. 2009;39:382–90.
22. Chen S, Zhao X, Li Y, Yang D, Zhou T, Fan G. Impurities preparation of
sodium tanshinone II A sulfonate by high-speed counter-current chroma‑
matography and identification by liquid chromatography/multitarget
tandem mass spectrometry. J Chromatogr A. 2013;1288:28–34.
23. Chen Y, Tu JH, He YJ, Zhang W, Wang G, Tan ZR, Zhou G, Fan L, Zhou HH.
Effect of sodium tanshinone II A sulfonate on the activity of CYP1A2 in
healthy volunteers. Xenobiotica. 2009;39:508–13.
24. Huang X, Guo Y, Huang WH, Zhang W, Tan ZR, Peng JB, Wang YC, Hu DL,
Ouyang DS, Xiao J, Wang Y, Luo M, Chen Y. Searching the cytochrome
P450 enzymes for the metabolism of meranzin hydrate: a prospec‑
tive antidepressant originating from Chaihu-Shugan-San. Plos One.
2014;9:e113819.
25. Xu C, Desta Z. In vitro analysis and quantitative prediction of efa‑
virenz inhibition of eight cytochrome P450 (CYP) enzymes: major
effects on CYPs 2B6, 2C8, 2C9 and 2C19. Drug Metab Pharmacokinet.
2013;28:362–71.
26. Bourrie M, Meunier V, Berger Y, Fabre G. Cytochrome P450 P450 isoforms
inhibitors as a tool for the investigation of metabolic reactions catalyzed
by human liver microsomes. J Pharmacol Exp Ther. 1996;277:321–32.
27. Li X, Wang K, Wei W, Liu YY, Gong L. In vitro metabolism of brucine by
human liver microsomes. J Pharmacol Exp Ther. 1996;277:321–32.
28. Taavitsainen P, Juvonen R, Pelkonen O. In vitro inhibition of cytochrome
P450 enzymes using an in vitro cocktail and liquid chromatography‑tandem
mass spectrometry. Xenobiotica. 2013;35:1987–93.
29. Qin CZ, Ren X, Tan ZR, Chen Y, Yin JY, Yu J, Qu J, Zhou HH, Liu ZQ. A
study on the effect of sodium tanshinone IIA sulfonate on the differentiation
of atrial fibroblasts into myofibroblasts in vitro. Int J Cardiol. 2015;182:329–33.
30. Zhou S, Gao Y, Jiang W, Huang M, Xu A, Parrot JW. Interactions of herbs
with cytochrome P450. Drug Metab Rev. 2003;35:35–98.
31. Le Page M, Mohamed Z. Evaluation of the effects of Mitragyna speciosa alkaloid
mitragynine inhibition of eight cytochrome P450 (CYP) enzymes: major
effects on CYPs 2B6, 2C9, 2C19, and 3A. Antimicrob Agents Chemother.
2003;47:3623–9.
32. Ouyang DS, Xiao J, Wang Y, Luo M, Chen Y. Searching the cytochrome
P450 enzymes for the metabolism of meranzin hydrate: a prospec‑
tive antidepressant originating from Chaihu-Shugan-San. Plos One.
2014;9:e113819.
human CYP1A2, CYP2C9, CYP2E1 and CYP3A4 activities in vitro. Phytomedicine. 2010;17:868–75.

33. Haddad A, Davis M, Lagman R. The pharmacological importance of cytochrome CYP3A4 in the palliation of symptoms: review and recommendations for avoiding adverse drug interactions. Support Care Cancer. 2007;15:251–7.

34. Zhou LP, Yao F, Luan H, Wang YL, Dong XH, Zhou WW, Wang QH. CYP3A4*1B polymorphism and cancer risk: a HuGE review and meta-analysis. Tumour Biol. 2013;34:4649–60.

35. Murayama N, Nakamura T, Saeki M, Saito Y, Sai K, Ishida S, Nakajima O, Itoda M, Ohno Y, Ozawa S, Sawada J. CYP3A4 gene polymorphisms influence testosterone 6β-hydroxylation. Drug Metab Pharmacokinet. 2002;17:150–6.

36. Gervasini G, Garcia M, Macias RM, Cubero JJ, Caravaca F, Benitez J. Impact of genetic polymorphisms on tacrolimus pharmacokinetics and the clinical outcome of renal transplantation. Transplant Int. 2012;25:471–80.