Renal Tubular Secretion of Tanshinol: Molecular Mechanisms, Impact on Its Systemic Exposure, and Propensity for Dose-Related Nephrotoxicity and for Renal Herb-Drug Interactions

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ABSTRACT

Tanshinol has desirable antianginal and pharmacokinetic properties and is a key compound of *Salvia miltiorrhiza* roots (Danshen). It is extensively cleared by renal excretion. This study was designed to elucidate the mechanism underlying renal tubular secretion of tanshinol and to compare different ways to manipulate systemic exposure to the compound. Cellular uptake of tanshinol was mediated by human organic anion transporter 1 (OAT1) (K_m 121 µM), OAT2 (859 µM), OAT3 (1888 µM), and OAT4 (1880 µM) and rat Oat1 (117 µM), Oat2 (1207 µM), and Oat3 (1498 µM). Other renal transporters (human organic anion-transporting polypeptide 4C1 [OATP4C1], organic cation transporter 2 [OCT2], carnitine/organic cation transporter 1 [OCTN1], multidrug and toxin extrusion protein 1 [MATE1], MATE2-K, multidrug resistance-associated protein 2 [MRP2], MRP4, and breast cancer resistance protein [BCRP], and rat Oat1, Oat2, Oat3, Mdr1a, Mdr1b, Mdr1c, and Bcrp) showed either ambiguous ability to transport tanshinol or no transport activity. Rats may be a useful model, to investigate the contribution of the renal transporters on the systemic and renal exposure to tanshinol. Probencid-induced impairment of tubular secretion resulted in a 3- to 5-fold increase in the rat plasma area under the plasma concentration-time curve from 0 to infinity (AUC_0–∞) of tanshinol. Tanshinol exhibited linear plasma pharmacokinetic properties over a large intravenous dose range (2–200 mg/kg) in rats. The dosage adjustment could result in increases in the plasma AUC_0–∞ of tanshinol of about 100-fold. Tanshinol showed very little dose-related nephrotoxicity. In summary, renal tubular secretion of tanshinol consists of uptake from blood, primarily by OAT1/Oat1, and the subsequent luminal efflux into urine mainly by passive diffusion. Dosage adjustment appears to be an efficient and safe way to manipulate systemic exposure to tanshinol. Tanshinol shows low propensity to cause renal transporter-mediated herb-drug interactions.

Introduction

Herbal medicines normally contain many constituents. It is hypothesized that only a few constituents with favorable drug-like properties, rather than all the constituents present, are responsible for the pharmacologic effects of an herbal medicine (Lu et al., 2008). An herbal constituent can be defined as drug-like if it possesses the desired pharmacologic potency, a wide safety margin, appropriate pharmacokinetic (PK) properties, and adequate content in the medicine dosed. Recent multicompound PK studies have indicated that human subjects and laboratory animals are considerably exposed to only a few constituents of an herbal medicine after dosing (Lu et al., 2008; Liu et al., 2009; Li et al., 2012a; Chen et al., 2013; Cheng et al., 2013; Hu et al., 2013; Jiang et al., 2015; Li et al., 2015). Such PK studies provide information for pharmacologists regarding which herbal compounds merit further evaluation. Follow-up evaluations of PK studies should focus on the potentially important herbal compounds that exhibit the desired pharmacologic properties and have considerable body exposure after dosing. Understanding the molecular mechanisms underlying the major elimination pathways of key herbal compounds is a goal of such studies. This helps to identify the factors influencing the compound concentration after dosing and to predict the potential for compound-related herb–drug interactions.

ABBREVIATIONS: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC_0–∞, area under the plasma concentration-time curve from zero to infinity; BCRP, breast cancer resistance protein; BUN, blood urea nitrogen; CL_Intr, intrinsic clearance; CL_Tot, renal clearance; CL_Tot, renal clearance of endogenous creatinine; CL_Renal, renal clearance by the cellular efflux into urine across the apical brush border membrane; CL_Prop, total plasma clearance; C_Urin, concentration at 5 minutes after dosing; Cum_AUC, cumulative amount excreted into urine; f_unb, unbound fraction in plasma; GFR, glomerular filtration rate; HEK-293, human embryonic kidney cell line; IC50, half maximal inhibitory concentration; K_m, Michaelis constant; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MATE, multidrug and toxin extrusion protein; MC, mock cells; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; OCTN, carnitine/organic cation transporter; PK, pharmacokinetic; sCr, serum creatinine; t1/2, elimination half-life; TC, transfected cells; V_{max}, maximum velocity; V_{SS}, apparent volume of distribution at steady state.

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Salvia miltiorrhiza roots (Danshen) are used extensively in the treatment of angina pectoris in the People’s Republic of China (Zhou et al., 2005; Cheng, 2007). Emerging antianginal therapies are facilitating its long-term use as they appear to have a low incidence of side effects (Jia et al., 2012). Danshen therapies are given orally or intravenously. Polyphenols are believed to be a class of major pharmacologically relevant constituents of Danshen. A PK study of cardiotonic pills, a Danshen-based formulation, in human subjects and laboratory animals has indicated that tanshinol was the only Danshen polyphenol that exhibited considerable systemic exposure after dosing (Lu et al., 2008). The other polyphenols, including salvianolic acids A, B, and D, rosmarinic acid, lithospermic acid, and protocatechuic aldehyde, were either poorly absorbed from the gastrointestinal tract or were extensively metabolized, which resulted in their poor detection in plasma after dosing.

Tanshinol was found to be the most abundant Danshen polyphenol in clinically important Danshen-based intravenous injections (details pending publication elsewhere). In a recent PK study of DanHong injections—a Danshen-based intravenous formulation in human subjects and laboratory animals—tanshinol exhibited the most significant systemic exposure of the Danshen polyphenols after dosing (Li et al., 2015). The preceding PK studies of the Danshen polyphenols found after dosing with Danshen-based formulations suggest that tanshinol deserves additional attention and more investigation.

Materials and Methods

Tanshinol (sodium form >98.0%) was obtained from the National Institutes for Food and Drug Control (Beijing, People’s Republic of China). Para-aminobenzoic acid, prostaglandin F2α, estrone-3-sulfate, estradiol-17β-o-glucuronide, tetraethylammonium, methotrexate, probenecid, cinetidine, verapamil, indomethacin, novobiocin, creatinine, puromycin, and cisplatin were obtained from Sigma-Aldrich (St. Louis, MO). Inside-out membrane vesicles (5 mg protein/ml) prepared from insect cells expressing human multidrug resistance-associated protein (MRP) 2, human MRP4, human breast cancer resistance protein (BCRP), rat Mrp2, rat Mrp4, or rat Bcrp] were purchased from Genomembrane (Kanazawa, Japan).

**Cellular Transport Assays.** Human embryonic kidney 293 (HEK-293) cells (American Type Culture Collection, Manassas, VA) were grown, at 37°C and 5% CO2, in Dulbecco’s modified Eagle’s medium, which was fortified with 10% fetal bovine serum, 1% minimal essential medium nonessential amino acids, and 1% antibiotic-antimycotic solution. Full open reading frames of cDNA for human organic anion transporter (OAT) 1, human OAT2, human OAT3, human OAT4, human organic anion-transporting polypeptide (OATP) 4C1, human organic cation transporter (OCT) 2, human carnitine/organic cation transporter (OCTN) 1, human multidrug and toxin extrusion protein (MATE) 1, human MATE2-K, rat Oat1, rat Oat2, rat Oat3, rat Oct1, rat Oct2, rat Octn1, and rat Octn2, and rat Mate1 were synthesized and subcloned into pcDNA 3.1(+) expression vectors. The inserts of the pcDNA 3.1(+) constructs were sequenced and aligned according to the GenBank accession numbers NM_004790, NM_006672, NM_004254, NM_018484, NM_180991, NM_003058, NM_003059, NM_018242, NM_152908, NM_017224, NM_053537, NM_031332, NM_012697, NM_013584, NM_022770, NM_019296, and NM_001014118, respectively (www.ncbi.nlm.nih.gov/genbank/).

The pcDNA 3.1(+) transporter constructs and the empty vector were introduced separately into the HEK-293 cells with Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA). This produced transporter-expressing cells and mock-transfected cells, respectively. Before being used, the transfected cells were validated functionally using positive substrates para-aminobenzoic acid (for OAT1 and Oat1), prostaglandin F2α (OAT2 and Oat2), estrone-3-sulfate (OAT3, OAT4, OATP4C1, and Oat3), and tetraethylammonium (OCT2, OCTN1, MATE1, MATE2-K, Oct1, Oct2, Octn1, and Mate1) and using positive inhibitors probenecid (for OAT1, OAT2, OAT3, OAT4, OATP4C1, Oat1, Oat2, Oat3, and cinetidine (OCT2, MATE1, MATE2-K, Oct1, Oct2, and Mate1), and verapamil (OCTN1, Octn1, and Octn2).

Transport studies were performed in 24-well poly-lysine-coated plates with cells 48 hours after transfection. After they were washed twice with Krebs-Henseleit buffer (containing 118 mM NaCl, 4.83 mM KCl, 1.53 mM CaCl2, 0.96 mM KH2PO4, 23.8 mM NaHCO3, 1.2 mM MgSO4, 5 mM glucose, and 12.5 mM HEPES, pH 7.4; 500 μl per wash; the second wash involving 10 minutes of preincubation at 37°C, pH 7.4), the transfected cells and the mock-transfected cells were incubated with tanshinol in the presence or absence of the positive inhibitor. After incubation for 10 minutes, the transport was terminated by removing the medium from the wells and rapidly rinsing the cells 3 times with ice-cold Krebs-Henseleit buffer (500 μl per rinse). Unlike the other transporters, the transport studies with human MATE and rat Mate used a buffer (containing 145 mM NaCl, 3 mM KCl, 1 mM CaCl2, 0.5 mM MgCl2, 5 mM glucose, 5 mM HEPES, and 30 mM NH4Cl, pH 7.4) for cell washing and preincubation. The buffer (removing 30 mM NH4Cl, pH 8.4) was used for incubation. The cells were lysed with water (150 μl) using a freeze-thaw and ultrasonication. Aliquots (100 μl) of the resulting lysates were precipitated with ice-cold acetone (300 μl). After centrifugation at 21,100g for 10 minutes, the supernatants (5 μl) were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). The total amount of protein in the lysate was measured using a method first described by Bradford (1976). The transport rate in pmol/mg protein/min was calculated using the following equation:

\[ \text{Transport} = \frac{(C_t \times V_e)/T}{W_c} \]

where \( C_t \) is the concentration of the test compound in cellular lysates (μM), \( V_e \) is the volume of the lysates (μl), \( T \) is the incubation time (10 minutes), and \( W_c \) is the cellular protein amount of each well (mg). The differential uptake between the transfected cells (TC) and the mock cells (MC) was defined as the net transport ratio (TransportTC/TransportMC ratio); a net transport ratio >3 suggested a positive result.

The kinetics of cellular uptake of tanshinol as mediated by human OAT1, human OAT2, human OAT3, human OAT4, rat Oat1, rat Oat2, and rat Oat3 were assessed with respect to the Michaelis constant (\( K_m \)), maximum velocity (\( V_{max} \)), and intrinsic clearance (\( CL_{int} \)). The incubation conditions were the same.
as those for the preceding transport study except for using an incubation time of 5 minutes. The incubation time was optimized to ensure that the assessment was performed under linear uptake conditions. The concentrations of tanshinol in the incubation medium were 25–160 μM for OAT1 and Oat1, and 150–5000 μM for OAT2, OAT3, OAT4, Oat2, and Oat3. The background accumulation of tanshinol was also determined in the mock-transfected cells. The inhibitory effect of probenecid on the cellular uptake activity of tanshinol as mediated by OAT1, OAT2, OAT3, OAT4, Oat1, Oat2, and Oat3 was measured with respect to half-maximal inhibitory concentration (IC₅₀); the tanshinol concentrations were equal to the respective IC₅₀ values for the transporters. The IC₅₀ values of tanshinol against para-aminomhippuric acid (10 μM), prostaglandin F₂α (10 μM), estrone-3-sulfate (10 μM), and estrone-3-sulfate (10 μM) for OAT1, OAT2, OAT3, and OAT4, respectively, were also measured.

**Vesicular Transport Assays.** Membrane vesicles expressing one of the transporters—human MRP2, human MRP4, human BCRP, rat Mrp2, rat Mrp4, or rat Bcrp—were tested with tanshinol using a rapid filtration method. Before use, these membrane vesicles were functionally validated using estradiol-17β-glucuronide and methotrexate. To start the transport, preincubated membrane vesicle suspension (10 μl) was combined with preincubated tanshinol/ATP or tanshinol/AMP medium (50 μl). After incubation for 10 minutes, the transport was terminated by adding 200 μl of an ice-cold buffer (containing 40 mM MOPS and 70 mM KCl; adjusted to pH 7.0 with 1.7 M Tris-base) followed by immediate transfer of the mixture into a Millipore MultiScreen-FB filtration plate (0.65 μm; Billerica, MA). Five washes of the membrane vesicles with the ice-cold terminating buffer (200 μl per wash) were performed, and the filters that retained membrane vesicles were transferred to 1.5-ml polypropylene tubes. The membrane vesicles were lysed and extracted with 200 μl of 80% methanol per sample. After centrifugation at 21,100g for 10 minutes, the supernatants (5 μl) were analyzed by LC-MS/MS.

The transport rate in pmol/mg protein/min was calculated using the following equation:

\[
\text{Transport} = \frac{(C_V \times V_c)}{W_v} \times T/W_V
\]

where \(C_V\) is the concentration of the compound in vesicular lysates supernant (μM), \(V_c\) is the volume of the lysates (μl), \(T\) is the incubation time (10 minutes), and \(W_v\) is the amount of vesicle protein amount per well (0.05 mg). Positive results for ATP-dependent transport were defined as a net transport proportion (100% solvent B), elution proportion segment (1.5 minutes), and elution proportion segment (2 minutes) for creatinine, with H.E. The histopathologic examinations of the tissue sections were conducted by a veterinary pathologist and subjected to peer review.

**Quantification of Tanshinol and Other Test Compounds in Biologic Samples.** Validated LC/MS/MS-based bioanalytic methods were used to measure the concentrations of tanshinol, para-aminomhippuric acid, prostaglan-
din F₂α, estrone-3-sulfate, estradiol-17β-glucuronide, methotrexate, tetrax-
thylammonium, probenecid, and creatinine in biologic samples. A TSQ Quantum mass spectrometer (Thermo Fisher, San Jose, CA) was interfaced via an electrospray ionization-probe with an Agilent 1100 series liquid chromatograph (Waldbronn, Germany). Chromatographic separation was achieved on a 5 μm Gemini C₁₈ column (50 mm × 2.0 mm i.d.; Phenomenex, Torrance, CA). Tetraethylammonium and creatinine levels were analyzed using a 3-μm Luna Hilic column (100 mm × 3.0 mm i.d.; Phenomenex). The mobile phase, which consisted of solvent A (water/acetonitrile, 98:2, v/v, containing 1 mM formic acid) and solvent B (water/acetonitrile, 2:98, v/v, containing 1 mM formic acid), was delivered at 0.35 ml/min, except for in the analysis of creatinine at 0.3 ml/min. A pulse gradient elution method was used in the measurement of the compounds (except for creatinine), with an analyte-dependent start proportion (0–50% solvent B) and analyte-independent elution proportion (100% solvent B), elution proportion segment (1.5 minutes), and column equilibrium segment (3.5 minutes) (Wang et al., 2007); for creatinine, the gradient parameters were 100% solvent B and 80% solvent B, 1 minute and 7 minutes, respectively.
The renal clearance (CLR) was calculated by dividing the cumulative amount of transport activity by the Kinetica software package (version 5.0; Thermo Scientific, Philadelphia, PA). The glomerular filtration rate (GFR) of the rats was estimated in terms of renal transport ratios as a function of tanshinol concentration. The IC50 for inhibition was determined using noncompartmental analysis with GraFit software (version 5; Surrey, United Kingdom) was constructed using weighted (1/\(V^2\)) nonlinear regression analysis of initial transport rates as a function of tanshinol concentration. The IC50 for inhibition of transport activity was obtained from a plot of percentage activity remaining curve from 0 to infinity (AUC0\(\rightarrow\)\(\infty\)) by the area under the plasma concentration-time curve from 0 to infinity (AUC0\(\rightarrow\)\(\infty\)) by the kidney homogenate AUC0\(\rightarrow\)\(\infty\) values shown in Table 2. OAT2, OAT3, and OAT4 exhibited lower affinity for tanshinol than OAT1 (Table 2). The uptake of tanshinol mediated by OAT1, OAT2, OAT3, and OAT4 was considerably inhibited by probenecid, and the IC50 values are shown in Table 2. Human OATP4C1, OCT2, OCTN1, MATE1, and MATE2-K did not exhibit any transport activities for tanshinol (Table 1).

### Results

In Vitro Interactions between Tanshinol and Human Renal Transporters.

There was significantly more uptake of tanshinol into human OAT1-expressing HEK-293 cells than into the mock-transfected cells, suggesting that tanshinol was a substrate of OAT1. Tanshinol was also taken up by cells expressing human OAT2, OAT3, and OAT4. The relevant net transport ratios in Table 1. OAT1-mediated uptake of tanshinol was saturable with \(K_{max}\) and \(V_{max}\) values shown in Table 2. OAT2, OAT3, and OAT4 exhibited lower affinity for tanshinol than OAT1 (Table 2). The uptake of tanshinol mediated by OAT1, OAT2, OAT3, and OAT4 was considerably inhibited by probenecid, and the IC50 values are shown in Table 2. Human OATP4C1, OCT2, OCTN1, MATE1, and MATE2-K did not exhibit any transport activities for tanshinol (Table 1).

Human MRP2 and MRP4 had low in vitro transport activities for tanshinol; reliable kinetic parameters were difficult to find. Human BCRP had no statistically significant transport activity. Tanshinol exhibited low inhibition potency toward the OAT transporters; its IC50 values against para-aminobiphenyluric acid for OAT1, against prosta-glandin F2α for OAT2, against estrone-3-sulfate for OAT3, and against estrone-3-sulfate for OAT4 are shown in Table 2. Tanshinol (1 mM) did not exhibit any statistically significant inhibitory activity toward OATP4C1, OCT2, OCTN1, MRP2, MRP4, BCRP, MATE1, or MATE2-K (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Human renal SLC transporters</th>
<th>Human renal ABC transporters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not Treated with Any Inhibitor</td>
<td>Treated with 1 mM Positive Inhibitor</td>
</tr>
<tr>
<td><strong>Transporter</strong></td>
<td><strong>Positve Substrate (10 (\mu M))</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Human renal SLC transporters</strong></td>
<td><strong>OAT1</strong></td>
<td>146.9 ± 22.0</td>
</tr>
<tr>
<td></td>
<td><strong>OAT2</strong></td>
<td>68.0 ± 4.6</td>
</tr>
<tr>
<td></td>
<td><strong>OAT3</strong></td>
<td>23.9 ± 1.8</td>
</tr>
<tr>
<td></td>
<td><strong>OAT4</strong></td>
<td>14.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td><strong>OATP4C1</strong></td>
<td>5.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td><strong>OCT2</strong></td>
<td>103.2 ± 10.7</td>
</tr>
<tr>
<td></td>
<td><strong>OCTN1</strong></td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td><strong>MATE1</strong></td>
<td>60.9 ± 2.0</td>
</tr>
<tr>
<td></td>
<td><strong>MATE2-K</strong></td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td><strong>Human renal ABC transporters</strong></td>
<td><strong>MRP2</strong></td>
<td>24.2 ± 0.5</td>
</tr>
<tr>
<td></td>
<td><strong>MRP4</strong></td>
<td>20.2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td><strong>BCRP</strong></td>
<td>17.7 ± 0.1</td>
</tr>
</tbody>
</table>

### Human renal SLC transporters

- **OAT1**: *Para*-aminobiphenyluric acid
- **OAT2**: Prostaglandin F2α
- **OAT3**: Estrone-3-sulfate
- **OAT4**: Estrone-3-sulfate
- **OATP4C1**: Estrone-3-sulfate
- **OCT2**: Tetracyclammonium
- **OCTN1**: Tetracyclammonium
- **MATE1**: Tetracyclammonium
- **MATE2-K**: Tetracyclammonium

### Human ren.nl ABC transporters

- **MRP2**: Estradiol-17β-D-glucuronide
- **MRP4**: Estradiol-17β-D-glucuronide
- **BCRP**: Methotrexate

### Rat renal SLC transporters

- **Oat1**: *Para*-aminobiphenyluric acid
- **Oat2**: Prostaglandin F2α
- **Oat3**: Estrone-3-sulfate
- **Oat4**: Estrone-3-sulfate
- **Oct1**: Tetracyclammonium
- **Octn1**: Tetracyclammonium
- **Octn2**: Tetracyclammonium
- **Oct2**: Tetracyclammonium
- **Mate1**: Tetracyclammonium

### Rat renal ABC transporters

- **Mrp2**: Estradiol-17β-D-glucuronide
- **Mrp4**: Estradiol-17β-D-glucuronide
- **Bcrp**: Methotrexate

The concentrations of methotrexate for BCRP and Bcrp were 100 \(\mu M\). The concentration of probenecid to inhibit OAT2- and Oat2-mediated transport of prostaglandin F2α was 10 \(\mu M\). Net transport ratios represent the mean ± S.D. (n = 3). When the net transport ratio was >3 (for the solute carrier family transporters) or >2 (for the ABC transporters), there were statistically significant differences between Transport\(_{tc}\) and Transport\(_{tc}\) or between Transport\(_{tc}\) and Transport\(_{tc}\); reliable kinetic parameters were difficult to find. Human BCRP had no statistically significant transport activity. Tanshinol exhibited low inhibition potency toward the OAT transporters; its IC50 values against *para*-aminobiphenyluric acid for OAT1, against prostaglandin F2α for OAT2, against estrone-3-sulfate for OAT3, and against estrone-3-sulfate for OAT4 are shown in Table 2. Tanshinol (1 mM) did not exhibit any statistically significant inhibitory activity toward OATP4C1, OCT2, OCTN1, MRP2, MRP4, BCRP, MATE1, or MATE2-K (Table 1).
mediated tubular secretion of tanshinol in rats. Probenecid had a total data suggest that probenecid treatment could impair Oat1/Oat3-
60 and 9 times, respectively, as much as the IC50 for rat Oat3. These V
urine (comparable to that of human OAT2. Probenecid inhibited the Oat1-
Oat3 was considerably greater than that of human OAT3 but
m concentration-dependent binding to rat plasma protein; its unbound
of probenecid (100 mg/kg) was used to impair the rat tubular secretion
termine the impact of the renal transporter-mediated tubular secretion
Pharmacokinetics and Disposition of Tanshinol in Rats.
interaction profiles with tanshinol similar to the human transporters.
transport activity. Taken together, the rat renal transporters exhibited
in vitro transport activities for tanshinol, and rat Bcrp had no significant
transport activities for tanshinol (Table 1). Rat Mrp2 and Mrp4 had low
in Table 2. Rat Oct1, Oct2, Octn1, Octn2, and Mate1 exhibited no
14 times, respectively, as much as its IC50 against tanshinol for rat Oat1,
poor, with a CLR of 0.001 l/h/kg; the fraction of the dose excreted into
in vivo regulatory role of the human renal transporters in the plasma
OAT1, OAT2, and OAT3, respectively. To delineate and extrapolate the
in vivo transport activities for tanshinol, and rat Bcrp had no significant
were examined with respect to saturability and affinity. Tanshinol was
taken up by cells expressing rat Oat1, Oat2, and Oat3; the relevant Km,
Km, Vmax, and CLint values are shown in Table 2. The results indicated that
rat Oat3 was considerably greater than that of human OAT3 but only 20%
changes in the CLR of tanshinol may be caused predominantly by
tanshinol (2 mg/kg), the systemic exposure to tanshinol in probenecid-treated rats was significantly enhanced as compared with that in the same rats when they were not given pro-
benecid treatment (Fig. 1). As shown in Table 3, probenecid treatment
resulted in 3- to 5-fold increases in plasma AUCCL of tanshinol (P = 0.0001), 1.6- to 2.3-fold elevations in Cmin (P = 0.00001), and 1.7- to 2.3-fold increases in elimination half-life (t1/2) (P = 0.00001).
Probenecid treatment led to a notable decrease in CLR of tanshinol, only 20%–34% of that in the same rats when given no probenecid (P = 0.00004).
To rule out differences in glomerular function as a confounding factor, endogenous creatinine excretion was measured in the rats during both the probenecid treatment period and the probenecid-free period. Probenecid treatment was not found to significantly change the renal clearances of endogenous creatinine (P = 0.158) or the f0 values of tanshinol (P = 0.795) (Table 3). Accordingly, the probenecid-induced changes in the CLR of tanshinol may be caused predominantly by decreases in Oat-mediated tubular secretion. Noncompartmental PK analysis also revealed probenecid-induced abnormalities in total plasma

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Km (µM)</th>
<th>Vmax (pmol/mg protein/min)</th>
<th>CLint (µl/min/kg)</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human OAT1 (Para-aminomethylphenolic acid)</td>
<td>60.3 ± 5.5</td>
<td>1379 ± 39</td>
<td>22.9</td>
<td>–</td>
</tr>
<tr>
<td>Human OAT2 (Prostaglandin F2α)</td>
<td>45.5 ± 12.4</td>
<td>360 ± 54</td>
<td>7.91</td>
<td>1528 ± 51</td>
</tr>
<tr>
<td>Human OAT3 (Estrone-3-sulfate)</td>
<td>28.1 ± 3.7</td>
<td>73.1 ± 4.4</td>
<td>2.60</td>
<td>2803 ± 150</td>
</tr>
<tr>
<td>Human OAT4 (Estrone-3-sulfate)</td>
<td>49.9 ± 5.3</td>
<td>232 ± 9</td>
<td>4.64</td>
<td>4079 ± 410</td>
</tr>
<tr>
<td>Rat Oat1 (Para-aminomethylphenolic acid)</td>
<td>102 ± 17</td>
<td>2299 ± 138</td>
<td>22.5</td>
<td>–</td>
</tr>
<tr>
<td>Rat Oat2 (Prostaglandin F2α)</td>
<td>27.0 ± 10.3</td>
<td>511 ± 113</td>
<td>18.9</td>
<td>–</td>
</tr>
<tr>
<td>Rat Oat3 (Estrone-3-sulfate)</td>
<td>17.1 ± 4.1</td>
<td>161 ± 11</td>
<td>9.41</td>
<td>–</td>
</tr>
</tbody>
</table>

**TABLE 2**

Comparative kinetic parameters for human OAT and rat Oat transporters mediating in vitro transport of tanshinol.

Values represent the mean ± S.D. (n = 3).

- **In Vitro Interactions between Tanshinol and Rat Renal Transporters.** Rat Oat1, Oat2, and Oat3 are orthologs of human OAT1, OAT2, and OAT3, respectively. To delineate and extrapolate the in vivo regulatory role of the human renal transporters in the plasma pharmacokinetics and renal disposition of tanshinol, the rat transporters were examined with respect to saturability and affinity. Tanshinol was taken up by cells expressing rat Oat1, Oat2, and Oat3; the relevant Km, Vmax, and CLint values are shown in Table 2. The results indicated that Oat1, Oat2, and Oat3 exhibited in vitro saturability and affinity for tanshinol similar to their human counterparts, except that the Vmax of rat Oat3 was considerably greater than that of human OAT3 but comparable to that of human OAT2. Probenecid inhibited the Oat1-, Oat2-, and Oat3-mediated transport of tanshinol with IC50 values shown in Table 2. Rat Oct1, Oct2, Octn1, Octn2, and MATE1 exhibited no transport activities for tanshinol (Table 1). Rat Mrp2 and Mrp4 had low in vitro transport activities for tanshinol, and rat Bcrp had no significant transport activity. Taken together, the rat renal transporters exhibited interaction profiles with tanshinol similar to the human transporters.

- **Impact of Probenecid-Impaired Tubular Secretion on Plasma Pharmacokinetics and Disposition of Tanshinol in Rats.** To determine the impact of the renal transporter-mediated tubular secretion on systemic exposure to and renal disposition of tanshinol, an i.v. bolus of probenecid (100 mg/kg) was used to impair the rat tubular secretion of tanshinol by inhibiting Oat1 and Oat3. Probenecid exhibited concentration-dependent binding to rat plasma protein; its unbound fraction in plasma (fu) increased from 28% to 60% as the plasma concentrations increased from 200 µM to 2000 µM. Probenecid exhibited unbound plasma concentrations at 5 minutes (unbound Cmin) and 4 hours after dosing (unbound C4h) that were 100 and 14 times, respectively, as much as its IC50 against tanshinol for rat Oat1, respectively (Supplemental Table 1). The unbound Cmin and C4h were 60 and 9 times, respectively, as much as the IC50 for rat Oat3. These data suggest that probenecid treatment could impair Oat1/Oat3-mediated tubular secretion of tanshinol in rats. Probenecid had a total plasma clearance (CLRat/Oat3) of 0.09 l/h/kg in rats. Its renal excretion was poor, with a CLR of 0.001 l/h/kg; the fraction of the dose excreted into urine (fuU) was only 1.5%.

After an i.v. bolus of tanshinol (2 mg/kg), the systemic exposure to tanshinol in probenecid-treated rats was significantly enhanced as compared with that in the same rats when they were not given probenecid treatment (Fig. 1). As shown in Table 3, probenecid treatment resulted in 3- to 5-fold increases in plasma AUCCL of tanshinol (P = 0.0001), 1.6- to 2.3-fold elevations in Cmin (P = 0.00001), and 1.7- to 2.3-fold increases in elimination half-life (t1/2) (P = 0.00001). Probenecid treatment led to a notable decrease in CLR of tanshinol, only 20%–34% of that in the same rats when given no probenecid (P = 0.00004).

To rule out differences in glomerular function as a confounding factor, endogenous creatinine excretion was measured in the rats during both the probenecid treatment period and the probenecid-free period. Probenecid treatment was not found to significantly change the renal clearances of endogenous creatinine (P = 0.158) or the fu values of tanshinol (P = 0.795) (Table 3). Accordingly, the probenecid-induced changes in the CLR of tanshinol may be caused predominantly by decreases in Oat-mediated tubular secretion. Noncompartmental PK analysis also revealed probenecid-induced abnormalities in total plasma
clearance (CL\textsubscript{tot,p}) of tanshinol, demonstrating 61%–78% reductions ($P = 0.00004$), and in apparent volume of distribution at steady state ($VSS$), demonstrating 5%–38% reductions ($P = 0.006$).

The effects of probenecid on tissue distribution of tanshinol were further determined by measuring tanshinol concentrations in the tissue homogenates of rats after dosing. As with the systemic exposure, probenecid treatment led to heart, lung, brain, and liver $C_{\text{max}}$ and $AUC_{0-\infty}$ levels of tanshinol that were higher than those in the normal rats not treated with probenecid ($P = 0.000005$–0.005) (Table 4). However, probenecid treatment did not cause a significant change in the average kidney $AUC_{0-\infty}$ level ($P = 0.309$); the average maximum kidney concentration after dosing ($C_{\text{max}}$) in the probenecid-treated rats was markedly lower than that in the normal rats ($P = 0.001$). These data suggest that the reduced $VSS$ of tanshinol by probenecid treatment resulted, at least in part, from the change in kidney exposure to the compound.

**Dose-Dependent Changes in Levels of Systemic and Renal Exposure to Tanshinol in Rats.** Changes in systemic and renal exposure to tanshinol were evaluated in a single ascending dose study in rats. As shown in Table 5 and Fig. 2A–C, systemic exposure to tanshinol increased as a function of the dose (i.v.; 2–200 mg/kg). Plasma $C_{\text{min}}$ of tanshinol exhibited a dose-proportional increase; the slope of ln(plasma $AUC_{0-\infty}$) versus ln(dose) was 1.04 (Table 6). There were dose-independent trends in plasma $t_{1/2}$ ($P = 0.280–0.542$), $CL_{\text{tot,p}}$ ($P = 0.193–0.842$), and $VSS$ ($P = 0.196–0.568$) of tanshinol (Table 5).

Meanwhile, kidney $C_{\text{min}}$ and $AUC_{0-\infty}$ of tanshinol also increased as the dose increased (Table 5 and Fig. 2D–F). The slopes of ln(kidney $C_{\text{min}}$) and ln(kidney $AUC_{0-\infty}$) versus ln(dose) were 0.98 and 1.07, respectively. Over the dose range, the kidney $t_{1/2}$ of tanshinol was also dose-independent ($P = 0.066–0.417$). The kidney $C_{\text{min}}$ and $AUC_{0-\infty}$ of tanshinol at each dose level were 6.9–11.0 and 5.1–6.7 times, respectively, as high as the corresponding plasma data.

It is worth mentioning that the rat Oat1/Oat3-mediated basolateral uptake is expected to result in the real concentration of tanshinol in the tubular epithelium being considerably higher than the associated kidney homogenate concentration. Tanshinol exhibited concentration-independent renal clearance by luminal efflux into urine ($CL_{R,c-u}$; $P = 0.548–0.956$); no evidence of saturation of $CL_{R,c-u}$ suggested that the luminal efflux of tanshinol into urine probably did not involve transporter-mediated mechanism (Table 5).

Taken together, tanshinol exhibited a linear plasma pharmacokinetics over a wide range of i.v. doses in rats, and the change in systemic exposure to tanshinol by dosage adjustment (about 100 times) was substantially greater than that by probenecid-impaired tubular secretion (about 3–5 times). A similar scenario is expected to take place in humans. Oat1/Oat3-mediated tubular uptake resulted in a level of kidney exposure to tanshinol considerably higher than the level of systemic exposure. This raised concerns regarding the risk of dose-related nephrotoxicity of tanshinol.

**Lack of Nephrotoxicity after Tanshinol Overdose in Rats.** As with saline-treated rats (the negative controls), both the renal tubules and glomeruli of rats given 14 consecutive days of subchronic treatment with tanshinol at an i.v. dose of 200 mg/kg per day (equivalent to 100 times the clinical daily dose) were histologically normal on day 15 (Fig. 3). No evidence of toxicity to the liver was observed in tanshinol-treated rats (data not shown). Consistent with these histopathological observations, the rats undergoing multiple-dose treatment with tanshinol showed serum markers of renal function (BUN, 4.6–6.3 mM; sCr, 20–24 µM) within the normal ranges (4.2–7.8 mM for BUN and 16–31 µM for sCr) (Fig. 3).

### Table 3

Comparative plasma pharmacokinetics and renal excretion of tanshinol after an i.v. bolus dose of tanshinol at 2 mg/kg in rats not treated with probenecid and in rats treated with probenecid.

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Not Treated</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{min}}$ (µM)</td>
<td>11.8 ± 1.9</td>
<td>23.2 ± 3.6$^a$</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (µM·h)</td>
<td>3.19 ± 0.57</td>
<td>10.6 ± 3.1$^a$</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>0.196 ± 0.055</td>
<td>0.441 ± 0.062$^a$</td>
</tr>
<tr>
<td>$MRT$ (h)</td>
<td>0.173 ± 0.015</td>
<td>0.430 ± 0.052$^a$</td>
</tr>
<tr>
<td>$CL_{\text{min}}$ (l/h/kg)</td>
<td>3.26 ± 0.64</td>
<td>1.01 ± 0.24$^a$</td>
</tr>
<tr>
<td>$VSS$ (l/kg)</td>
<td>0.538 ± 0.087</td>
<td>0.428 ± 0.077$^a$</td>
</tr>
</tbody>
</table>

| Urine data | | |
| $Cum.A_1$ (µmol) | 1.51 ± 0.09 | 1.28 ± 0.14$^a$ |
| $CL_{\text{U}}$ (l/h/kg) | 1.94 ± 0.38 | 0.530 ± 0.096$^a$ |
| $f_{U}$ (%) | 59.7 ± 3.8 | 50.7 ± 5.7$^a$ |
| $CL_{\text{U}}$/GFR$\times f_{U}$ | 6.56 | 1.72 |

| Renal plasma protein binding data | | |
| $f_{P}$ (%, at 0.5–50 µM) | 98.6 ± 1.1 | 96.5 ± 2.5 |
| $CL_{R,c-u}$ (l/h/kg) (Before dosing) | 0.295 ± 0.043 | 0.315 ± 0.036 |
| $CL_{R,c-u}$ (l/h/kg) (6 h after dosing) | 0.314 ± 0.060 | 0.288 ± 0.037 |

$^a$ $P < 0.05$ when compared with the rat group not treated with probenecid.
The rat blood and kidney tissue samples were collected at 5 and 30 minutes and 1 and 2 hours after dosing. Urine samples were collected 0–24 hours after dosing. The details of the rat PK study are described in Materials and Methods (the third rat PK study).

### Table 5.
Comparative plasma and kidney pharmacokinetics of tanshinol in rats receiving an i.v. bolus dose of tanshinol solution (2–200 mg/kg)

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Dosage (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Plasma data</td>
<td></td>
</tr>
<tr>
<td>C_{max} (μM)</td>
<td>15.5 ± 1.5</td>
</tr>
<tr>
<td>AUC_{0→∞} (μM-h)</td>
<td>4.33 ± 0.45</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>0.278 ± 0.017</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.272 ± 0.030</td>
</tr>
<tr>
<td>VSS (l/kg)</td>
<td>0.635 ± 0.023</td>
</tr>
<tr>
<td>CL_{tot,p} (l/h/kg)</td>
<td>2.35 ± 0.25</td>
</tr>
<tr>
<td>Urine data</td>
<td></td>
</tr>
<tr>
<td>Cum. A_{e} (μmol)</td>
<td>1.48 ± 0.06</td>
</tr>
<tr>
<td>f_{u} (%)</td>
<td>61.5 ± 3.6</td>
</tr>
<tr>
<td>CL_{u} (l/h/kg)</td>
<td>1.44 ± 0.10</td>
</tr>
<tr>
<td>Kidney data</td>
<td></td>
</tr>
<tr>
<td>C_{max} (μM)</td>
<td>118 ± 37</td>
</tr>
<tr>
<td>AUC_{0→∞} (μM-h)</td>
<td>22.7 ± 6.9</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>0.277 ± 0.010</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.232 ± 0.011</td>
</tr>
<tr>
<td>CL_{u} (l/h/kg)</td>
<td>0.289 ± 0.075</td>
</tr>
</tbody>
</table>

AUC_{0→∞}, area under the plasma concentration-time curve from 0 to infinity; CL_{u}, renal clearance by the cellular efflux into urine across the apical brush border membrane; CL_{u}, renal clearance; CL_{tot,p}, total plasma clearance; C_{max}, concentration at 5 min after dosing; Cum. A_{e}, cumulative amount excreted; MRT, mean residence time; t_{1/2}, elimination half-life; VSS, apparent volume of distribution at steady state.

In contrast, the positive controls of cisplatin and puromycin caused considerable renal injury in rats. The lesions observed in cisplatin-treated rats were characterized by dilated tubules filled with necrotic tubular epithelial cells, cellular debris, and proteinaceous casts, but the glomeruli were histologically normal. The histopathologic evaluation of the kidneys from the puromycin-treated rats demonstrated renal cellular degeneration, necrosis, and sloughing of proximal tubule epithelium and vacuolation of glomerular podocytes. In rats, cisplatin treatment led to the BUN (11–33 mM) and sCr levels (47–108 μM) that exceeded the normal ranges. Abnormally elevated BUN and sCr levels were also observed in the puromycin-treated rats: 103–131 mM and 179–266 μM, respectively.

Collectively, tanshinol exhibited very little dose-related nephrotoxicity in rats. A similar scenario is expected to take place in humans.

### Discussion

Tanshinol is a carboxyl acid and cleared predominantly by renal excretion. Many organic anions are substrates of renal organic anion transporters (Masereeuw and Russel, 2010). Renal excretion of tanshinol mainly involves active tubular secretion. This suggests that the transporters influence the systemic exposure to and renal disposition of tanshinol via mediating the tubular secretion. To test this hypothesis, a comprehensive investigation of interactions between renal transporters...
and tanshinol was undertaken. This resulted in an understanding of the mechanistic tubular secretion of tanshinol and enabled us to subsequently explore the impact of tubular secretion on systemic exposure to tanshinol and propensity of the compound for dose-related nephrotoxicity and for renal transporter-mediated herb-drug interactions.

Cellular uptake of tanshinol could be mediated by human OAT1, OAT2, OAT4, and OAT3 (in decreasing order of affinity for tanshinol), rather than human OATP4C1, OCT2, OCTN1, MATE1, and MATE2-K. The transporters OAT1, OAT2, and OAT3 are expressed at the basolateral membrane of renal proximal tubules and play roles in uptake of tanshinol from blood in tubular secretion (Enomoto et al., 2002; Motohashi et al., 2002). OAT1 and OAT3 are major renal transporters, and OAT2 probably expresses at a lower level. OAT2 and OAT3 exhibited an in vitro CLint that was 39% and 1%, respectively, of the OAT1 efficiency.

OAT4 has a major role in the tubular reabsorption of organic anions from urine (Ekaratanawong et al., 2004). OAT4 exhibited a CLint that was only 1% of the OAT1 value. 1) The low affinity of OAT4 for tanshinol, 2) its relatively low expression in the kidney, and 3) the short residence time of tanshinol in luminal filtrate (a couple of seconds) indicate that OAT4 had limited contribution to renal excretion of tanshinol.

The apically located MRP2, MRP4, BCRP, MATE1, MATE2-K, and OCTN1 transporters that support luminal efflux from proximal renal tubules (Masereeuw and Russel, 2010) exhibited ambiguous or no in vitro ability to transport tanshinol. A similar scenario was observed with the rat orthologs at the apical membrane. Rats exhibited dose-independent trends in kidney t1/2 and CLR,c-u of tanshinol over the i.v. dose range 2–200 mg/kg; its C5min in kidney homogenate increased from 118 to 971 μM as the dose increased (Table 5).

A Caco-2 cell-based study revealed that tanshinol had favorable membrane permeability for intestinal absorption (Lu et al., 2008). The concentration of tanshinol in the rat epithelia of proximal tubules at the dose 200 mg/kg should be much higher than 10 mM. Such a high intracellular concentration is expected to exceed the Km and results in the saturation of possible transporter-mediated efflux. According to the Michaelis-Menten equation for membrane permeation, when the saturation of possible transporter-mediated efflux can be the dominating mechanism (Sugano et al., 2010). Accordingly, the luminal efflux of tanshinol into urine was most likely based on a passive diffusion mechanism.

In rats, Oat1 and Oat3 are highly expressed at the basolateral membrane of renal proximal tubules, and Oat2 is at the apical membrane (Kojima et al., 2002). The roles of Oat1 and Oat3 are to support the basolateral uptake of organic anions from blood. Rat Oat2 has a major role in tubular reabsorption of organic anions from urine. Tanshinol was a substrate of Oat1, Oat2, and Oat3 but not of Oct1 and Oct2. The CLint values of Oat2 and Oat3 were 29% and 14% that of Oat1, respectively. Rat MRP2, MRP4, Bcrp, and Mate1 are expressed at the apical membrane. The apical membrane efflux transporters exhibited limited (MRP2 and MRP4) or no (Bcrp, Octn1, Octn2, and Mate1) affinity for tanshinol.

Based on these results, rats were used to investigate the impact of renal transporters on systemic and renal exposure to tanshinol. For this purpose, probenicid was used to impair Oat1/Oat3-mediated tubular secretion of tanshinol. However, it also exhibits inhibitory activity against rat Oatp1a1/Oatp1a4, MRP2/Mrp3, and UDP-glucuronosyltransferases (Sugiyama et al., 2001; Horikawa et al., 2002; Uchaipichat et al., 2004). For tanshinol, transport mediated by rat Mrp2 and human MRP3 is very poor, and metabolism via glucuronidation is limited (details pending publication elsewhere). Tanshinol (molecular weight 198 Da) is not a substrate of rat

For the systemic exposure data of tanshinol from the single ascending dose study in rats receiving an i.v. bolus dose of tanshinol solution (2–200 mg/kg), the i.v. dose range 2

| Summary of results from dose proportionality assessment of a single ascending dose study in rats receiving an i.v. bolus dose of tanshinol solution (2–200 mg/kg) |
|---------------------------|--------------------------|-----------------------|
| PK Parameter | r | P | Slope (90% CI) | Conclusion |
| Plasma Cmax | 0.998 | 2.31 × 10−16 | 0.989 (0.955–1.023) | Linear |
| AUC0–∞ | 0.997 | 2.57 × 10−16 | 1.038 (1.002–1.075) | Inconclusive |
| Kidney Cmax | 0.985 | 2.73 × 10−11 | 0.978 (0.893–1.062) | Inconclusive |
| AUC0–∞ | 0.990 | 1.66 × 10−12 | 1.070 (0.996–1.144) | Inconclusive |

Fig. 3. Comparative kidney histology (A–F) and serum biochemistry (G and H) in rats receiving subchronic treatment of 14 consecutive days of saline (negative control), tanshinol (i.v., 200 mg/kg/d), puromycin (i.p., 40 mg/kg/d; positive control), and cisplatin (i.p., 1 mg/kg/d; positive control). The rat blood samples were collected before (open bars) and after (solid bars) 15 days of treatment for assessment of blood urea nitrogen (BUN) and serum creatinine (sCr). The rat kidney tissues were sampled and processed for H&E staining to evaluate tubular damage, glomerular damage, and histology. *P < 0.05 versus the negative control. Stain: H&E; original magnification, 200×.
Oat1 or Oat1b2. Therefore, inhibition of Oatp1a1/Oatp1a4, Mrp2/Mrp3, and UDP-glucuronosyltransferases by probenecid probably had negligible effect on the rat pharmacokinetics of tanshinol.

Probenecid-induced impairment of tubular secretion resulted in 61%–78% reductions in the CLun,p of tanshinol, and the CLun/(GFR × f) ratio of tanshinol was reduced from 6.6 to 1.7. Although probenecid treatment led to 3 to 5 times the enhancement of systemic exposure to the compound (AUCun,x), the kidney exposure to tanshinol was reduced. It is worth mentioning that probenecid treatment also resulted in a decrease in nonrenal clearance (CLnon,p – CLun,p; from 1.32 to 0.51 L/h/kg). This probably resulted, at least in part, from inhibition of hepatic Oat2 by probenecid, unbound plasma C5min and C0, which were 6 and 0.8 times, respectively, its IC50 against tanshinol.

Tanshinol exhibited a linear plasma pharmacokinetics over the i.v. dose range 2–200 mg/kg in rats; dosage adjustment could cause approximately 100-fold increases in the plasma AUCun,x and C5min of tanshinol. For tanshinol, Oat1 exhibited a higher affinity and CLun than Oat3. When the doses were 2–15 mg/kg, all the unbound plasma C5min of tanshinol (15–97 μM) was lower than the KM for Oat1. This suggested that the basolateral uptake for tubular secretion of tanshinol was mainly mediated by Oat1. As the dose increased, the initial unbound plasma concentrations of tanshinol, particularly at 200 mg/kg, exceeded the KM for Oat1 (Fig. 2A). Tanshinol pharmacokinetics remained linear with the increasing dose, and the CLR was not markedly saturated over 2–200 mg/kg, suggesting that, at a higher concentration, Oat3 supplements Oat1 in mediating renal uptake. This was evidenced by the KM for Oat3, which was higher than the unbound plasma concentrations of tanshinol at the 200 mg/kg dose.

The preceding linear pharmacokinetics mainly depended on Oat1/3-mediated tubular secretion. For matching levels of systemic exposure to tanshinol after dosing to its effective concentrations for the antiangiogenic activities, dosage adjustment was a more effective way to manipulate exposure, because the change in the systemic exposure to tanshinol via dosage adjustment (about 100 times) was statistically significantly greater than that via probenecid-induced drug interaction (about 3 to 5 times). Despite the Oat-mediated tubular secretion mechanism, tanshinol exhibited very little dose-related nephrotoxicity. This suggests that dosage adjustment probably is also a safe way to manipulate exposure to tanshinol, and it did not need to be used in concert with drug combination.

Gao et al. (2009) and Li et al. (2009) reported that tanshinol exhibited very little dose-related toxicity in rats, mice, or dogs. Tanshinol is expected to have a similar linear plasma PK property over a large i.v. dose range in humans. This is because, like the rat Oat transporters, the human OAT transporters also exhibit low affinities and high transport capacities for tanshinol. Similar to rat Oat1, human OAT1 played a key role in mediating the renal uptake of tanshinol at low concentrations; like rat Oat3, human OAT2 and OAT3 supplemented OAT1, to mediate the tanshinol uptake at high concentration.

Herb-drug interactions are an important safety concern (Li et al., 2012b). Rat Oat transporters was found to influence systemic exposure to tanshinol; likewise, human OAT transporters are expected to be clinically important. Human OAT1, OAT3, and OCT2 are major renal transporters with a broad range of substrates; renal drug interactions often occurred in relation to their actions (Giacomini et al., 2010). Both the KM and IC50 data shown in Table 2 indicated that tanshinol had low affinity for OAT1. After an i.v. infusion daily dose of DanHong injection (40 mL containing around 55 mg of tanshinol) in human subjects, the average maximum plasma concentration of tanshinol was measured as about 2.5 μM (Li et al., 2015). Tanshinol exhibits a f0 of 85% in human plasma and a short t1/2 of 1.1–1.3 hours (Lu et al., 2008). According to the equation (drug-drug interaction index = unbound Cmax/IC50), the OAT1-mediated drug-drug interaction index was calculated for tanshinol as 0.02. This suggests that tanshinol has a low propensity to act as an inhibitory perpetrator in OAT1-mediated drug interactions when DanHong injection is used at a clinically relevant dose. Compared with OAT1, the renal transporters OAT2, OAT3, and OAT4 exhibited higher KM and IC50 values for tanshinol (Table 2), suggesting a lower potential for these transporter-mediated herb-drug interactions. In addition, tanshinol had no inhibition potency toward human OATP4C1, OCT2, OCTN1, MRPs, MRP4, BCRP, MATE1, or MATE2-K. In rats, the probenecid-impaired tubular secretion resulted in 1.6- to 4.5-fold elevations in systemic exposure to tanshinol, suggesting that tanshinol could be a substrate victim on Oat transporters. A similar scenario is expected to take place in humans.

However, the change in systemic exposure is probably not clinically relevant, because tanshinol exhibits very little dose-related toxicity. Wang and Sweet (2013) reported that the Danshen polyphenols rosmarinic acid, lithospermic acid, and salvianolic acid A exhibited strong inhibitory activities against human OAT1 or OAT3 (KM, 0.16–0.59 μM). Like probenecid, these Danshen polyphenols, concurrently present in Danshen-based i.v. injections, may influence systemic and renal exposure to tanshinol after dosing. Studying the PK matrix effects will help more accurately define and predict the exposure level and pharmacokinetics of tanshinol.

Understanding the mechanisms governing systemic exposure to tanshinol helps with matching the exposure levels after dosing to the effective concentrations for its antiangiogenic activities; this most likely results in enhanced efficacy of Danshen-based therapy. In summary, renal tubular secretion of tanshinol involves the basolateral uptake from blood primarily by human OAT1 and rat Oat1, and the subsequent luminal efflux into urine, mainly by passive diffusion (Fig. 4). Human OAT2/OAT3 and rat Oat3 are also important for the basolateral uptake at high tanshinol concentrations in blood. Human OAT4- and rat Oat2-mediated tubular reabsorption of tanshinol may have limited contribution to renal excretion. Tanshinol shows low propensity to cause renal transporter-mediated herb-drug interactions. Tanshinol exhibits linear pharmacokinetics properties over a large i.v. dose range and very little dose-related nephrotoxicity in rats. Dosage adjustment appears to be an efficient, safe way to manipulate its systemic exposure. Additional safety studies are under way to define the risk of hyperhomocysteinemia related to dose-dependent tanshinol methylation.

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Authorship Contributions

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Performed data analysis: C. Li, Jia, Yang, L. Li.
Wrote or contributed to the writing of the manuscript: C. Li, Jia.
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