Impact of curcumin on the pharmacokinetics of rosuvastatin in rats and dogs based on the conjugated metabolites

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Abstract

1. Plasma concentrations of curcumin-O-glucuronide (COG) and curcumin-O-sulfate (COS) significantly increased after Sprague-Dawley rats dealt with the Oatp inhibitor rifampicin, with the $C_{\text{max}}$ ascending 2.9 and 6.7 times, and the AUC$_{0-\infty}$ ascending 4.4 and 10.8 times, respectively. When pretreated with the Oat inhibitor probenecid, the $C_{\text{max}}$ increased 4.4 and 20 times, and the AUC$_{0-\infty}$ increased 3.2 and 13.9 times, respectively. The results suggested that COG and COS may be the substrates of Oatp and Oat.

2. The accumulation of curcumin significantly increased in organic anion transporting polypeptide (OATP)- and organic anion transporter (OAT)-transfected human embryonic kidney (HEK) 293 systems, which suggested that curcumin was a substrate of OATP1B1, OATP1B3, OATP2B1, OAT1, and OAT3; and COG was a substrate of OATP1B1, OATP1B3, and OAT3.

3. Inhibition study using rosuvastatin as the substrate in OATP1B1- and OATP1B3-transfected cells indicated that curcumin was an OATP1B1 and 1B3 inhibitor, with IC$_{50}$ at 5.19 ± 0.05 and 3.68 ± 0.05 M, respectively; the data for COG were 1.04 ± 0.01 and 1.08 ± 0.02 M, respectively. COS was speculated to be an inhibitor of hepatic OATP1B1 as calculated using the ADMET Predictor.

4. COG and COS are substrates and inhibitors of OATP/Oatp. Co-administration of curcumin significantly increased rosuvastatin concentration in rat and dog plasma.

Keywords

Curcumin, drug–drug interaction, OAT, OATP, rosuvastatin, transporter

Introduction

Curcumin (Figure 1), a major component of turmeric (Curcuma longa L.), has been widely used as a food additive, coloring agent and medicinal agent in traditional Chinese and Indian medicines (Ammon & Wahl, 1991).

As high doses (2–8 g/day) of curcuminoids are administered to patients in clinical trials due to their limited bioavailability and negligible dose-limiting toxicity (Dhillon et al., 2008; Volak et al., 2008), most studies focused on the metabolism and potential drug-drug interactions (DDI) occurred of the parent drug. For example, curcumin was reported to be a CYP3A4 and UGT inhibitor, which resulted in a 2-fold increase of $C_{\text{max}}$ of midazolam (a CYP3A4 substrate) in rats and 100% inhibition of mycophenolic acid (a UGT substrate) glucuronidation in mouse duodenal microsomes (Basu et al., 2004; Zhang et al., 2007). Studies on transporters indicated that curcumin is also an inhibitor of P-glycoprotein (P-gp), breast cancer-resistant protein (BCRP), and multi-drug resistant associate protein (MRP). It caused a 2-fold increase of $C_{\text{max}}$ of celiprolol (a P-gp substrate) in rats after a 5-day treatment with curcumin (Zhang et al., 2007), 3-fold increase of $C_{\text{max}}$ of sulfasalazine (a BCRP substrate) in mice after co-administration of curcumin (Shukla et al., 2009), and inhibition on both MRP1- and MRP2-mediated transport in isolated membrane vesicles in MRP-expressing Sf9 cells, with IC$_{50}$ values at 15.0 and 5.0 M, respectively (Wortelboer et al., 2003).

Nevertheless, metabolic studies demonstrated that orally ingested curcumin is extensively transformed to curcumin-O-glucuronide (COG, Figure 1) and curcumin-O-sulfate (COS, Figure 1) in both rodents and humans, with negligible amount of the parent form remained in plasma (Asai & Miyazawa, 2000; Chen et al., 2012; Hoehle et al., 2007; Ireson et al., 2002; Pan et al., 1999; Wang & Qiu, 2013). According to the FDA’s guidance (CDER, 2012), the DDI for metabolites present at >25% of parent drug AUC should be investigated. However, no studies were conducted on the disposition of the predominant conjugated metabolites of curcumin, especially on the DDI between the conjugated metabolites and other potentially co-administered drugs.
Conjugated metabolites are often considered as substrates of organic anion transporting polypeptide (OATP) and organic anion transporter (OAT) because of their properties as organic anions (DeGorter et al., 2012). Hence, we hypothesized that the conjugated metabolites, COG and COS, may be substrates of OATP and OAT, and may lead to competitive DDI with other substrates. The objectives of this study were (1) to clarify the contribution of hepatic uptake transporters on the disposition of the conjugated metabolites after oral administration of curcumin; (2) to evaluate the impact of the conjugated metabolites on the pharmacokinetics of rosuvastatin (a substrate of OATP), and elucidate the potential mechanisms.

Materials and methods

Chemicals and reagents

Curcumin, COG, rifampicin, probenecid, and rosuvastatin were purchased from Melone Pharmaceutical Co., Ltd. (Dalian, China). Fetal bovine serum, Dulbecco’s modified Eagle’s medium, 0.05% trypsin-EDTA, penicillin G, and streptomycin were purchased from Invitrogen (Carlsbad, CA).

Methanol and acetonitrile of HPLC grade were purchased from Sigma (St Louis, MO). Formic acid of HPLC grade was purchased from Tedia (Fairfield, OH). Deionized water was obtained from a Millipore Milli-Q gradient water purification system (Molsheim, France).

Animals

Male Sprague-Dawley (SD) rats (200–300 g, 7–8 weeks) and male adult beagle dogs (10–15 kg, 1-year-old) were purchased from Hubei Provincial Center for Disease Control and Prevention (Wuhan, China). All the animals had been acclimated for 7 days before the experiments were conducted. The experiments were conducted in accordance with the Guidelines for Animal Experimentation of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China) and approved by the Animal Ethics Committee of the institution.

DDI between rifampicin/probenecid and curcumin in rats

Eighteen SD rats were fasted overnight and randomized into three groups. Curcumin prepared in 0.5% carboxymethylcellulose sodium (CMC-Na) was orally administered to three groups at the dose of 100 mg/kg (Asai & Miyazawa, 2000). The first group was set as the control and received no additional treatments. The second group was intravenously administered with rifampicin from tail at the dose of 20.0 mg/kg (Takeuchi et al., 2011), 15 min before the oral administration of curcumin. Likewise, probenecid was intravenously administered from tail to Group 3 at the dose of 100 mg/kg (Wang et al., 2014), 15 min before the oral administration of curcumin. Approximately 200 μl of blood samples were collected from the orbit at pre-dosing and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h post-dosing. Blood samples were placed in heparin-containing tubes and immediately centrifuged at 13 000 × g for 5 min at 4 °C to prepare plasma.

Cell culture

Human embryonic kidney (HEK) 293 cells transfected with human OATP1B1, OATP1B3, OATP2B1, OAT1, OAT3, and empty vector-transfected control cells (mock) were used. Briefly, the cell lines were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal bovine serum, 100 mg·ml⁻¹ of streptomycin, 100 U·ml⁻¹ of penicillin, and 100 g·ml⁻¹ of hygromycin B at 37 °C in an atmosphere with 95% relative humidity and 5% (v/v) CO₂. 0.5 ml of the cells were seeded into the 24-well BD Biocoat poly-D-lysine-coated plates (BD Biosciences, Bedford, MA).
at an initial density of $2.0 \times 10^5$ cells/well. The cell culture medium was replaced with fresh culture medium containing 5 mM sodium butyrate 24 h before the transport studies to induce the expression of the transporters. Transporting experiments were performed on the third day when the cells grew to confluence (Zhong et al., 2014).

**In vitro uptake experiments**

In order to examine the possible uptake transporter-mediated DDI, the following experiments were conducted: (1) the intracellular accumulation of curcumin (1.0 or 10.0 $\mu$M) and COG (1.0 or 10.0 $\mu$M) in mock-control, OATP1B1, OATP1B3, OATP2B1, OAT1, and OAT3-transfected cells after 10-min incubation to determine if curcumin and COG were the substrates of the above-mentioned transporters; (2) the intracellular accumulation of rosuvastatin (5.0 $\mu$M) in mock-control, OATP1B1 and OATP1B3-transfected cells after 2-min incubation with curcumin (50.0 $\mu$M) or COG (50.0 $\mu$M) to determine if the inhibitions occurred; (3) the intracellular accumulation of rosuvastatin (5.0 $\mu$M) in OATP1B1 and OATP1B3-transfected cells after 2-min incubation with curcumin or COG at a range of concentrations (0–100 $\mu$M) to determine the IC$_{50}$ values of curcumin and COG. The uptake experiments were terminated at the designated times by aspirating the incubation solution and washing the cells three times with ice-cold HBSS. The cells were lysed by the addition of 300 $\mu$l of deionized water and multigelation. After cell lysis, 50.0 $\mu$l of the solubilized cell samples, 25.0 $\mu$l of the internal standard (IS, tolbutamide at 20.0 ng/ml$^{-1}$), and 150 $\mu$l of acetonitrile were sequentially added to centrifuge tubes. After vortex mixing and centrifugation at 13 000 $\times$ g for 5 min, 10.0 $\mu$l of the supernatants was injected for LC-MS/MS analysis. The protein content of the solubilized cells was measured using a BCA protein assay kit.

**DDI between curcumin and rosuvastatin in rats**

Twelve SD rats were fasted overnight and randomized into two groups. Rosuvastatin prepared with 0.5% CMC-Na was orally administered to the first group at the dose of 5.0 mg/kg (Basu et al., 2013) as the control, while rosuvastatin (5.0 mg/kg) and curcumin (500 mg/kg) were co-administered to the second group to examine the DDI. Blood samples of approximately 200-$\mu$l volume were collected from the oibtal vein at pre-dosing and 0.08, 0.25, 0.5, 1.2, 3, 4, 6, 8, 12, and 24 h post-dosing. Blood samples were placed in heparin-containing tubes and immediately centrifuged at 13 000 $\times$ g for 5 min at 4$^\circ$C.

**DDI between curcumin and rosuvastatin in dogs**

Six beagle dogs received single doses of rosuvastatin (prepared in 0.5% CMC-Na) at 5.0 mg/kg via gavages after an overnight fast on the first day. Blood samples of approximately 1 ml were collected from the jugular vein at pre-dosing and 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 h post-dosing. Blood samples were placed in heparin-containing tubes and immediately centrifuged at 13 000 $\times$ g for 5 min at 4$^\circ$C. After a three-day wash-out period, the dogs received an oral dose of 100 mg/kg curcumin 30 min before rosuvastatin administration, and blood was sampled up to 12 h as on the first day. All samples were stored at –70$^\circ$C until analysis.

**Quantifications by liquid chromatography with tandem mass spectrometry**

For curcumin COG and COS, chromatographic separations were performed on a XB-C18 column (50 mm × 2.1 mm, 5 $\mu$m; Welch Ultimate, Shanghai, China). The mobile phase was a mixture of 0.1% formic acid in water (A) and methanol (B). The following gradient elution was used: 0–0.2 min, 50% B; 0.2–0.8 min, 50–95% B; 0.8–1.7 min 95% B; 1.7–1.8 min, 95–50% B; 1.8–2.8 min, 50% B. The flow rate was set at 0.8 ml/min, and the injection volume was 10.0 $\mu$l. The autosampler and the column temperature were set at 4$^\circ$C and 40$^\circ$C, respectively. The optimized MS parameters were set as follows: ion spray voltage, –4500 V; source temperature, 550$^\circ$C; nebulizer gas (Gas 1), 70 psi; heater gas (Gas 2), 80 psi; curtain gas, 30 psi. The optimized multiple reaction monitoring (MRM) transitions and related parameters were as follows: $m/z$ 367 → 217 with a declustering potential (DP) of –70 V and a collision energy (CE) of –15 eV for curcumin; $m/z$ 543 → 217 with a DP of –90 V and a CE of –28 eV for COG; $m/z$ 447 → 217 with a DP of –90 V and a CE of –28 eV for COS; $m/z$ 294 → 250 with a DP of –30 V and a CE of –18 eV for diclofenac (IS). The dwell time for each transition was 50 ms. As the unavailability of the standard of COS, the MRM transition used was the same as the previous study reported (Cao et al., 2014), and validated using Q-Trap MS. The relative concentration of COS calculated using the COG standard.

The analysis of rosuvastatin was performed on a Gemini C18 column (50 mm × 2.1 mm, 5 $\mu$m; Phenomenex, Torrance, CA). The mobile phase was a mixture of 0.1% formic acid in water (A) and acetonitrile (B). The following gradient elution was used: 0–0.2 min, 20% B; 0.2–1.2 min, 20%–95% B; 1.2–1.5 min 95% B; 1.5–1.6 min, 95%–20% B; 1.6–2.5 min, 20% B. The flow rate was set at 1.0 ml/min, and the injection volume was 10.0 $\mu$l. The autosampler and the column temperature were set at 4$^\circ$C and 40$^\circ$C, respectively. The optimized MS parameters were set as follows: ion spray voltage, –4500 V; source temperature, 550$^\circ$C; nebulizer gas (Gas 1), 70 psi; heater gas (Gas 2), 80 psi; curtain gas, 30 psi. The optimized MRM transitions and related parameters used were as follows: $m/z$ 480 → 418 with a DP of –70 V and a CE of –15 eV for rosuvastatin; $m/z$ 269 → 170 with a DP of –38 V and a CE of –27 eV for tolbutamide (IS). The dwell time for each transition was 50 ms.

**Data analysis**

The LC-MS/MS data were analyzed by Analyst software (version 1.6.1, AB SCIEX, Foster City, CA). The pharmacokinetic data analysis was performed according to a non-compartmental model using Phoenix WinNonlin (version 6.3, Pharsight Inc., Cary, NC). All data were expressed as the mean ± SD. Treatment effects were evaluated by one-way analysis of covariance. $p$ Values less than 0.05 were considered as statistically significant.
Results

DDI between rifampicin/probenecid and curcumin in rats

The effect of rifampicin or probenecid on the pharmacokinetics of curcumin and its major conjugated metabolites was investigated by comparing the concentrations and kinetic parameters of curcumin and the conjugated metabolites after single administration of curcumin with or without rifampicin/probenecid, as shown in Table 1 and Figure 2. Although the concentrations of curcumin in the plasma were below 1.0 ng/ml, the lower limit of quantification (Figure 2A), the plasma concentrations of COG and COS were significantly increased after co-administration with rifampicin and probenecid at a dose of 20.0 mg/kg and 100 mg/kg, respectively (Figure 2B and C). The results indicated that COG and COS were probably the substrates of Oatps and Oats.

Pretreatment of rifampicin resulted in a 2.9-fold increase in $C_{\text{max}}$ (164 ± 22 ng/ml versus 477 ± 112 ng/ml, $p < 0.001$), 3.9-fold increase in AUC$_{0-24}$ (680 ± 86 ng·h·ml$^{-1}$ versus 2676 ± 725 ng·h·ml$^{-1}$, $p < 0.001$), and 4.4-fold increase in AUC$_{0-\infty}$ (682 ± 725 ng·h·ml$^{-1}$ versus 2685 ± 723 ng·h·ml$^{-1}$, $p < 0.001$) for COG; and a 6.7-fold increase in $C_{\text{max}}$ (11.6 ± 2.7 ng/ml versus 77.9 ± 9.0 ng/ml, $p < 0.001$), 11.1-fold increase in AUC$_{0-24}$ (57.0 ± 10.9 ng·h·ml$^{-1}$ versus 634 ± 57 ng·h·ml$^{-1}$, $p < 0.001$), and 10.8-fold increase in AUC$_{0-\infty}$ (60.9 ± 11.8 ng·h·ml$^{-1}$ versus 656 ± 54 ng·h·ml$^{-1}$, $p < 0.001$) for COS.

Pretreatment of probenecid resulted in a 4.4-fold increase in $C_{\text{max}}$ (164 ± 22 ng/ml versus 718 ± 51 ng/ml, $p < 0.001$), 3.2-fold increase in AUC$_{0-24}$ (680 ± 86 ng·h·ml$^{-1}$ versus 2180 ± 116 ng·h·ml$^{-1}$, $p < 0.001$), and 3.2-fold increase in AUC$_{0-\infty}$ (60.9 ± 11.8 ng·h·ml$^{-1}$ versus 2197 ± 122 ng·h·ml$^{-1}$, $p < 0.001$) for COG; and a 20-fold increase in $C_{\text{max}}$ (11.6 ± 2.7 ng/ml versus 232 ± 20 ng/ml, $p < 0.001$), 4.6-fold increase in AUC$_{0-24}$ (57.0 ± 10.9 ng·h·ml$^{-1}$ versus 835 ± 63 ng·h·ml$^{-1}$, $p < 0.001$), and 4.6-fold increase in AUC$_{0-\infty}$ (60.9 ± 11.8 ng·h·ml$^{-1}$ versus 844 ± 51 ng·h·ml$^{-1}$, $p < 0.001$) for COS.

Table 1. Pharmacokinetic parameters of COG and COS (relative) in rats after oral administration of 100 mg/kg curcumin following intravenous treatment with 20.0 mg/kg rifampicin and 100 mg/kg probenecid ($n = 6$).

| Treatment | $C_{\text{max}}$(ng ml$^{-1}$) | $T_{\text{max}}$(h) | AUC$_{0-24}$(ng h·ml$^{-1}$) | AUC$_{0-\infty}$(ng h·ml$^{-1}$) | $t_{1/2}$(h) |
|-----------|------------------|------------------|-----------------|-----------------|-----------|
| COG (control) | 164 ± 22 | 2.00 ± 0.00 | 680 ± 86 | 682 ± 87 | 2.87 ± 0.28 |
| COG + rifampicin | 477 ± 112* | 2.00 ± 0.00 | 2676 ± 725* | 2685 ± 723* | 2.71 ± 0.43 |
| COG + probenecid | 718 ± 51* | 2.00 ± 0.00 | 2180 ± 116* | 2197 ± 122* | 4.09 ± 0.77* |
| COS (control) | 11.6 ± 2.7 | 1.00 ± 0.00 | 57.0 ± 10.9 | 60.9 ± 11.8 | 3.08 ± 1.42 |
| COS + rifampicin | 77.9 ± 9.0* | 2.80 ± 0.84* | 634 ± 57* | 656 ± 54* | 4.65 ± 0.73 |
| COS + probenecid | 232 ± 20* | 2.00 ± 0.00 | 835 ± 63* | 844 ± 51* | 2.66 ± 0.96 |

*Significant difference compared with the control.

Figure 2. Plasma concentrations of curcumin (A), COG (B), and relative plasma concentration of COS (C) in rats ($n = 6$) following administration of 100 mg/kg curcumin, before (●) and after treatment with 20 mg/kg rifampicin (■) or 100 mg/kg probenecid (▲). Mean ± SD.
in $C_{\text{max}}$ (11.6 ± 2.7 ng·mL⁻¹ versus 232 ± 20 ng·mL⁻¹, $p < 0.001$), 14.6-fold increase in AUC₀₋₂₄ (57.0 ± 10.9 ng·h·mL⁻¹ versus 835 ± 63 ng·h·mL⁻¹, $p < 0.001$), and 13.9-fold increase in AUC₀₋∞ (60.9 ± 11.8 ng·h·mL⁻¹ versus 844 ± 51 ng·h·mL⁻¹, $p < 0.001$) for COS.

**Accumulation of curcumin and COG in OATP- and OAT-transfected HEK293 systems**

The uptake of curcumin and COG in OATP-, and OAT-transfected HEK293 systems was investigated to further verify whether they were the substrates of OATP and OAT (Supplementary Table S1 and Figure 3). The cell function was validated with the recommended substrates as the positive controls (CDER, 2012). The uptake rates were significantly increased in functional systems compared with the Mock systems, and inhibited by OATP/OAT inhibitors (Supplementary Table S2).

As depicted in Figure 3(A), the accumulation of curcumin increased significantly in HEK293-OATP1B1, -1B3, -2B1, -OAT1, and -OAT3 cells compared with HEK293-mock cells. The uptake of curcumin increased 12.8- to 6.5-fold in HEK293-OATP1B1 cells, 8.5- to 9.0-fold in HEK293-OATP1B3 cells, 15.2- to 6.4-fold in HEK293-OAT2B1 cells, 9.9-to 10.0-fold in HEK293-OAT1 cells, and 6.9- to 3.8-fold in HEK293-OAT3 cells compared with HEK293-mock cells, suggesting that curcumin was a substrate of OATP1B1, OATP1B3, OATP2B1, OAT1, and OAT3.

Similarly, as shown in Figure 3(B), the accumulation of COG significantly increased in HEK293-OATP1B1, -1B3, and -OAT3 cells compared with HEK293-mock cells. Specifically, the uptake of COG increased 2.4- to 4.4-fold in HEK293-OATP1B1 cells, 5.8- to 8.4-fold in HEK293-OATP1B3 cells and 2.3- to 4.6-fold in HEK293-OAT3 cells compared with HEK293-mock cells, while the accumulation of COG in HEK293-OAT2B1 and HEK293-OAT1 cells increased by <2-fold compared with HEK293-mock cells. This suggested that COG was a substrate for OATP1B1, OATP1B3, and OAT3, but not a substrate of OATP2B1 and OAT1.

**Inhibitory effects of curcumin and COG on the uptake of rosuvastatin in OATP1B1- and OATP1B3-transfected HEK293 cells**

As shown in Figure 4, the accumulation of rosuvastatin was significantly suppressed in the presence of 200 μM rifampicin, a specific OATP inhibitor (CDER, 2012; International Transporter Consortium et al., 2010). Similarly, the uptake of rosuvastatin was significantly reduced by curcumin and COG at 50.0 μM, respectively. This indicated that potential DDI occurred between rosuvastatin and curcumin/COG mediated by OATP. The accumulation of rosuvastatin (5.0 μM) was reduced in a concentration-dependent manner by curcumin (IC₅₀ = 5.19 ± 0.05 and 3.68 ± 0.05 μM, respectively) and COG (IC₅₀ = 1.04 ± 0.01 and 1.08 ± 0.02 μM, respectively) in OATP1B1- and 1B3-transfected HEK 293 cells after a 2-minute incubation (Figure 5).

**DDI between curcumin and rosuvastatin in rats and dogs**

To determine whether DDI occurred between curcumin and OATP/Oatps substrates, the animal models of rats and dogs were used in the experiments. As shown in Table 2...
and Figure 6, the plasma concentrations of rosuvastatin significantly increased after co-administration of curcumin at the dose of 500 mg/kg in rats and 100 mg/kg in dogs. In rats (Figure 6A), pretreatment with curcumin resulted in a 1.3-fold increase in C\text{max}, 2.0-fold increase in AUC\text{0–24}, and 2.2-fold increase in AUC\text{0–1} of rosuvastatin; in dogs (Figure 6B), a 1.4-fold increase in C\text{max}, 1.6-fold increase in AUC\text{0–24}, and 1.7-fold increase in AUC\text{0–1} of rosuvastatin were observed.

**Discussion**

In the present study, we evaluated the impact of orally administrated curcumin on the pharmacokinetics of rosuvastatin and discussed the potential mechanisms.

Glucuronide and sulfate conjugates are first speculated as OATP or OAT substrates because of their properties as organic anions. The logarithm of the partition coefficient between n-octanol and water (clog D) values (at pH 7.4) of COG and COS were calculated to be −1.48 and −0.34, respectively, using ADMET Predictor (version 7.2.0001, Shanghai PharmoGo Co., Ltd., Shanghai, China). This indicated that the two conjugated metabolites are hydrophilic compounds, which do not readily cross the plasma membrane via passive diffusion. Therefore, we speculated that COG and COS were substrates of both OATP/Oatp and OAT/Oat. To verify the hypothesis, curcumin was administered to SD rats after treatment with rifampicin (an OATP/Oatp inhibitor) and probenecid (an OAT/Oat inhibitor). As OATP1B1, OATP1B3, OATP2B1, and OAT2 are predominantly expressed on the basolateral membrane of hepatocytes (DeGorter et al., 2012), co-administration of rifampicin or probenecid significantly increased the plasma concentrations of COG and COS. Furthermore, in vitro transporter studies in HEK293 systems revealed that curcumin was a substrate of OATP1B1, OATP1B3, OATP2B1, OAT1, and OAT3, and COG was a substrate of OATP1B1, OATP1B3, and OAT3.
Inhibition studies were then conducted to test whether the conjugated metabolites could cause competitive DDI with OATP substrates. Rosuvastatin was chosen as a probe to investigate the potential DDI, as it is a substrate of OATP/Oatp with limited metabolism, and OATP1B1 predominantly contributed to hepatic uptake of rosuvastatin (Kitamura et al., 2008; Nezasa et al., 2002; Sheng et al., 2015). The in vitro inhibition study indicated that curcumin reduced the accumulation of rosuvastatin in OATP1B1- and 1B3-transfected HEK293 systems with IC₅₀ values at 5.19 ± 0.05 and 3.68 ± 0.05 μM, respectively; and COG with IC₅₀ values at 1.04 ± 0.01 and 1.08 ± 0.02 μM, respectively. COS was speculated to be (80% confidence) an inhibitor of hepatic OATP1B1, as calculated using the ADMET Predictor due to unavailability of the reference standard of COS. These results are consistent with the in vivo observations that the Cₘₐₓ and AUC of rosuvastatin significantly increased after co-administration with curcumin. Although the distribution and function of transporters in animals are different from humans in some way, rat is still a popular animal model in the study of transporter-mediated DDI (DeGorter et al., 2012).

Since the concentrations of curcumin in rat and dog plasma samples were below 1.0 ng/ml, and the major metabolites were the conjugated metabolites COG and COS, the DDI occurred probably between the conjugated metabolites and rosuvastatin via competition over Oatp, but not between curcumin and rosuvastatin. Furthermore, the relative concentration of COG is much lower than COS, which is consistent with the previous enzymatic hydrolysis study (Asai & Miyazawa, 2000), so the DDI occurred was probably mediated by COG. COG/COS was considered as a moderate inhibitor of Oatp with the AUC or Cₘₐₓ of rosuvastatin increased only or below 2-fold in rats or dogs, respectively. The t₁/₂ of rosuvastatin was significantly influenced by co-administration of curcumin, which is also indicated that the DDI occurred at the elimination phase via Oatp.

Based on the above-mentioned metabolism and transporter studies, the absorption and elimination of curcumin have much in common with scutellarein and baicalein, namely: (1) all of them go through extensively conjugated metabolism (Gao et al., 2011; Xing et al., 2005); and (2) after an oral dose, their parent forms and metabolites are mainly excreted through bile (Abe et al., 1990; Gao et al., 2011). So, we hypothesized that the distribution of curcumin is as follows (Figure 1): after oral administration, curcumin goes through extensively conjugating metabolism in enterocytes to form COG (mainly by UGT1A8 and UGT1A10) and COS (mainly by SULT1A1 and SULT1A3), which leads to low bioavailability of curcumin (Hoehle et al., 2007; Ireson et al., 2002; Metzler et al., 2013). Similar to scutellarin (Gao et al., 2012), COG and COS are probably pumped into the mesenteric blood by MRP3 as the major circulating metabolites. Then, the conjugated metabolites pass through the liver via the uptake transporters OAT and OATP, as shown in the in vitro uptake experiments. Finally, COG and COS are excreted from bile and hydrolyzed in gastrointestinal tract by colonic microflora, making the parent form as the major constituent in feces. This is consistent with the mass balance studies, which indicated that 50–60% of the oral dose of curcumin was recovered from bile, among which 95% presented as glucuronides (Holder et al., 1978; Ravindranath & Chandrasekhar, 1981). Food–drug interactions, particularly those between herbs and drugs, have become a major concern in recent years due to the growing popularity of herbs as complementary medicines (Tindle et al., 2005; Zhang et al., 2007). Drug interactions with some herbal medicines and supplements, such as St. John’s wort, garlic and grapefruit juice, have been reported (Dresser et al., 2005; John et al., 1999; Piscitelli et al., 2002). For example, oral administration of grapefruit or orange juice at low concentrations potentially and selectively inhibited in vivo OATP2B1-mediated uptake, which led to a decrease of fexofenadine bioavailability (Akamine et al., 2015).

DDI are often thought to be harmful, and great attention should be paid when an enzyme or a transporter modulator was co-administered with its substrate (Zhou et al., 2013). For example, co-administration of ketoconazole led to a 35-fold increase in plasma exposure of terfenadine, resulting in heart QT prolongation and torsade de pointes arrhythmia (Boxenbaum, 1999). For this reason, terfenadine was therefore withdrawn from the market (Honig et al., 1993; Von Moltke et al., 1994).

On the other hand, increased plasma concentration of drugs or herb constituents may intensify the pharmacological activities (Zhu et al., 2014). The concept of herb–herb or herb–drug combination to enhance therapeutic benefits has been utilized and practiced in Traditional Chinese Medicine for more than 2000 years (Che et al., 2013; Lee et al., 2011). Ginseng, a well-known herb, was often used in combination with anticancer drugs to enhance the chemotherapeutic outcomes. In this situation, ginseng was not only used to reduce the drug-induced toxicity, but also as P-gp and CYP inhibitors to increase the chemotherapeutic drug exposure, such as 5-fluorouracil, irinotecan, docetaxel, and cisplatin (Chen et al., 2014).

Curcumin is considered as a safe drug, and is widely used as food additives. As abovementioned, its parent form was reported to be an inhibitor of CYP3A4, P-gp, BCRP, and MRP, while its major conjugated metabolites were identified as OATP/Oatp and OAT/Oat substrates and inhibitors in this study. Hence, the combinatorial use of curcumin with other substrates of CYP3A4, P-gp, BCRP, MRP, OAT, and OATP should be considered, especially for the drugs with narrow therapeutic windows, such as digoxin, aminophylline, warfarin, and so on. On the other hand, some expensive antineoplastic agents, such as vincristine, taxol, etoposide, and carboplatin, are substrates of BCRP or P-gp with low therapeutic plasma concentrations. Pretreatment with curcumin may increase the exposure of these substrates and lower the treatment cost (Sreenivasan & Krishnakumar, 2015).

**Conclusions**

In summary, our study confirmed that the glucuronide and sulfated conjugates of curcumin are substrates and inhibitors of OATP/Oatp and OAT/Oat. Besides, the DDI between curcumin and statins may actually occur through its major conjugated metabolites, COG and COS. Understanding the metabolism and excretion of curcumin involving the
transporters may help chemist conduct structural optimization of curcumin to improve its bioavailability, and enable clinicians to predict possible transporter-based DDIs. However, follow-up clinical studies are required to verify the clinical significance of these findings.

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Declaration of interest

We declare that we have no conflicts of interest.

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Supplementary material available online