Interaction of curcumin with hepatic and renal organic cation transporter

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Introduction

Organic cation transporters (OCTs) play an important role in the elimination of endogenous and exogenous compounds. Change in transport function of transporters affect membrane transport, pharmacological action and toxicity of drugs and xenobiotics.¹ In human, OCT1 and OCT2 expression has been found in hepatocytes and kidney, respectively.¹ OCT1 and OCT2 located at basolateral membrane of hepatocytes and renal proximal tubular cells are responsible for transport of organic cations into the cells. Substrates of OCTs cover a wide range of chemical structures including pharmacological agents (e.g. morphine, tamoxifen, and metformin), environmental toxins/pollutants, and active components found in herbal preparation (e.g. lithospermic acid, rhein).¹ Several studies have been reported that herbal medicinal products inhibit several drug transporters including OCT1, OCT2, and p-glycoprotein (P-gp).

Curcumin is a main bioactive polyphenolic compound of Curcuma longa L. Rhizomes. A wide range of bioactive properties have been reported.² It is used in traditional Asian medicine and in nutritional supplement.³ Many studies have been reported that curcumin has a biological and pharmacological activity, including antioxidation, anti-inflammation, antimicrobial, antiproliferation, and cancer prevention.³⁴ The increasing use of curcumin products as health supplement or therapeutic purposes could affect transport function of drug transporters and lead to the potential of herb-drug interaction. The present study investigated whether curcumin interacts with OCT1 and OCT2 in cells stably transfected with OCT1 or OCT2. In addition, the effect of curcumin was confirmed in human hepatocytes and human renal proximal tubular cells of kidney that endogenously express hOCT1 and hOCT2, respectively.

Methods

Chemicals: ³H-1-methyl-4-phenylpyridinium (³H-MPP⁺; 80 μCi/mol) was purchased from PerkinElmer (MA, USA). Curcumin, tetrapentyl-ammonium (TPeA), transferrin, selenium, and epidermal growth factor were purchased from Sigma-Aldrich (MO, USA). All other chemicals are analytical grade and purchased from commercial sources.

Cell culture: Chinese hamster ovary (CHO-K1) cells stably transfected with rbOCT1 or rbOCT2 were cultured in F12K medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin under 5% CO₂/ 95% air at 37°C. The hepatocellular carcinoma cells (HepG2) and human renal proximal tubular cells (RPTEC/TERT1), cell lines expressing human OCT1 and OCT2, respectively, were obtained from American Type Culture Collection (ATCC) and the cultured conditions for these cell lines were conducted as recommended by ATCC.
Uptake studies: In this study, $^3$H-MPP$^+$ was used as an OCTs substrate for determination of OCTs transport function. The cells were seeded in 24-well plates and maintained in culture at 37 °C under a humidified 95% air atmosphere, containing 5%CO$_2$. After confluent, cells were washed twice with warm Waymouth buffer (WB) pH 7.40 (WB: 135 mM NaCl, 5 mM KCl, 13mM HEPES, 2.5mM CaCl$_2$.2H$_2$O, 1.2 mM MgCl$_2$.0.8 mM MgSO$_4$.7H$_2$O, and 28 mM D-glucose) and incubated with WB for a further 15 minutes. Cells were incubated with WB containing $^3$H-MPP$^+$ with or without curcumin for 5 minutes. Uptakes were stopped by removing the transport buffer and rinsing the cells with three successive washed of 1 ml ice-cold WB. Cells were solubilized with 200 µl of 0.4 N NaOH in 10% SDS overnight, followed by neutralizing with 100 µl of 1 N HCl. Cell lysate was transferred into scintillation vials to measure accumulated radioactivity with liquid scintillation β counter (1214 Rackbeta, Wallac). The uptake of $^3$H-MPP$^+$ was calculated as fmol/min/cm$^2$ and then expressed as a mean percentage of the control.

Inhibitory potency (IC$_{50}$) of curcumin: The confluence cell monolayers were incubated WB containing $^3$H-MPP$^+$ plus varying concentration of curcumin (0 – 40 µg/ml) for 5 min. After the end of incubation, the cell monolayers were washed and lysed by the same method as mention above. IC$_{50}$ values were estimated using nonlinear regression analysis.

Statistical analysis: Results were presented as mean±SD. Statistical differences between control and treatment groups were determined using one-way ANOVA followed by Tukey's test, with $P<0.05$ considered to be statistically significant.

Results
Interaction of curcumin with single expressing OCT1 or OCT2 cells
To determine the interactions of curcumin with OCT1 and OCT2, the effect of curcumin on OCT1 and OCT2 transport function was determined in rbOCT1-CHO-K1, the single expressing OCT1 cell, and rbOCT2-CHO-K1, the single expressing OCT2 cell. The results showed that curcumin at concentration of 10 and 40 µg/ml significantly inhibited OCT1- and OCT2-mediated $^3$H-MPP$^+$ uptake in rbOCTs-CHO-K1 cells (Figure 1).

![Figure 1](image-url) Effect of curcumin on OCT1- and OCT2-mEDIATE uptake of $^3$H-MPP$^+$ in CHO-K1 cells stably OCT1 and OCT2. The uptake measurements were carried out in the presence of $^3$H-MPP$^+$ plus curcumin at 10 and 50 µg/ml for 5 min. TPEA (100 µM) was used as a positive control for OCT inhibition. The uptake is expressed as a mean percentage of control (mean±SD) form 3 experiments. *$P<0.05$ compared with control.

Inhibitory potency (IC$_{50}$) of curcumin on OCT1 and OCT2 transport function
The potency of curcumin to inhibit OCT1- or OCT2-mediated $^3$H-MPP$^+$ uptake was examined in rbOCT1-CHO-K1 or rbOCT2-CHO-K1 cells. As shown in figure 2A and 2B, curcumin inhibited rbOCT1-mediated $^3$H-MPP$^+$ uptake and rbOCT2-mediated $^3$H-MPP$^+$ uptake in a dose-dependent manner. The IC$_{50}$ of curcumin inhibiting OCT1 was 2.88±2.77 µg/ml and OCT2 was 4.78±1.97 µg/ml, respectively.
Effect of curcumin on hOCT1-mediated ³H-MPP⁺ uptake in human hepatocyte and hOCT2-mediated ³H-MPP⁺ uptake in human renal proximal tubular cells

The inhibitory effect of curcumin on OCT1 transport function in human hepatic cells endogenously express hOCT1 was further examined using HepG2 cells. HepG2 cells were incubated with medium containing ³H-MPP⁺ alone or ³H-MPP⁺ plus curcumin at the concentration range of 0-40 µg/ml for 5 min. After the incubation period, the cellular accumulations of ³H-MPP⁺ were measured. As shown in Fig. 3A, curcumin did not significantly alter hOCT1-mediated ³H-MPP⁺ uptake, indicating curcumin might not interact with OCT1 in HepG2 cells. Next, we investigated the effect of curcumin on OCT2 transport function in human renal proximal tubular cells using RPTEC/TERT1 cells. The results showed that curcumin interacted with hOCT2 at the IC₅₀ of 3.24±2.19 µg/ml (Figure 3B).

Discussion

The uses of curcumin as health supplement and therapeutic purposes become increasing. Therefore, the present study investigated the potential interaction of curcumin with OCT1 and OCT2 which play a crucial role in elimination of cationic therapeutic drugs. Our data revealed that curcumin inhibited OCT1- and OCT2-mediated uptake of ³H-MPP⁺ in OCT transfected cells models in concentration-dependent manner with low IC₅₀. It seemed unlikely that the inhibitory effect of curcumin was via reduction of cell viability as evidence showed that exposing the cells with curcumin for 5 min did not affect cell viability (unpublished data). Curcumin might be competitively transported into the cells and subsequently inhibits ³H-MPP⁺ uptake. Another possible mechanism responsible for the inhibition was that curcumin might behave as inhibitor of the transporters. These hypotheses could be revealed in further study.

Since, OCT2 plays an important role in renal clearance of cationic drugs, we then determined whether the inhibitory effect of curcumin found in transfected CHO-K1 cells was revealed in human renal proximal tubular cells. The present study, RPTEC/TERT1 cells that express hOCT2 were
selected as a model to study hOCT2 transport function. We found that curcumin inhibited \( ^{3} \text{H}\)-MPP\(^{+}\) uptake with same range of IC\(_{50}\) with transfected cells. These data confirmed that curcumin could inhibit hOCT2 of human kidney. The clinical relevance of curcumin on herb-drug interaction might be concerned because the high inhibitory potency of curcumin on OCT2 (low IC\(_{50}\)). Data obtained from the pharmacokinetic showed that curcumin was distributed in liver, and kidney.\(^{10}\) Our finding results showed that curcumin inhibited OCT1 and OCT2, suggesting administration of curcumin might interfere hepatic and renal clearance of cationic drugs that mediated by OCT1 and OCT2. Although, curcumin showed an inhibition on OCT1 in transfected cells (OCT1-CHO-K1 cells), it produced no effect on \( ^{3} \text{H}\)-MPP\(^{+}\) uptake in HepG2 cells. These results implied that HepG2 cells which are derived from cancer cells might express other cationic transporters. It has been reported that HepG2 cells express OCT3\(^{11}\) that might be not inhibited by curcumin. In addition, curcumin has been reported to inhibit P-glycoprotein that is responsible for transport cationic drug out of the cell.\(^{4}\) The effect of curcumin on OCT1 transport function in human hepatocyte should be confirmed in normal primary hepatocyte. Therefore, curcumin might affect the renal total clearance of cationic drugs. This is notion should be proved in animal model.

**Conclusion**

In conclusions, we report the inhibitory effect of on renal human OCT2 which might interfere renal cationic drug disposition. Therefore, co-administration of curcumin with other cationic drugs might have herb-drug interaction.

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**References**