Regular Article

**Fenofibrate Down-regulates Renal OCT2-mediated Organic Cation Transport via PPARα-independent Pathways**

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Summary: Fenofibrate, the peroxisome proliferator-activated receptor alpha (PPARα) agonists, are widely prescribed for the treatment of hyperlipidemia. The present study examined the effect of fenofibrate drugs on renal OCT2 activity in a heterologous cell system [Chinese hamster ovary (CHO-K1) cells stably transfected with rabbit (rb) OCT2], LLC-PK1, and intact mouse renal cortical slices. We found that both in the CHO-K1 cells expressing rbOCT2 and in LLC-PK1 cells, fenofibrate significantly inhibited [3H]-MPP + uptake whereas clofibrate and WY14643 had no effect. Surprisingly, the inhibitory effect of fenofibrate was not attenuated by GW6471, a PPARα antagonist, indicating that the inhibitory process observed was via a PPARα-independent pathway. Fenofibrate decreased [3H]-MPP + uptakes through a reduction of the maximal transport (Jmax) but without effect on the transporter affinity (Kt) corresponding to a decrease in membrane expression of OCT2. Since the inhibitory effect of fenofibrate was not prevented by pretreatment with cycloheximide, its inhibitory action did not involve an inhibition of protein synthesis. Similar to the effect seen in the cell-cultured system, the inhibitory effect of fenofibrate was also observed in intact renal cortical slices. Taken together, our data showed that fenofibrate decreased the activity of OCT2 by reducing the number of functional transporters on the membrane, which is likely to be a PPARα-independent pathway.

Keywords: fenofibrate; organic cation transporter; proximal tubule; drug-drug interaction; hyperlipidemia; PPARα

Introduction

Regarding the role of the kidney in the clearance of xenobiotics and therapeutic drugs from the body, several drugs such as cimetidine, procainamide, metformin, amantadine, and cisplatin are transported by organic cation transporters (OCTs). 1-5 Thus, the renal OCTs are important determinants of drug efficacy and toxicity. Renal tubular secretion of organic cations involves the basolateral uptake of organic cations from the peritubular fluid into the renal tubular cell and subsequent efflux across the apical membrane into the tubular fluid. Basolateral uptake of organic cations into the cell by OCTs is via an electrogenic-facilitated diffusion mechanism, driven by an inside-negative electrical potential difference of cell membrane. 6 Several members of the OCT family located at the basolateral membrane including OCT1, OCT2, and OCT3 have been cloned and characterized. Among these OCTs, OCT2, which has been shown to be a major player in the renal proximal tubules, is highly expressed in the basolateral membrane of the S2 segment. 7 The expression and function of OCT2 are modulated by several factors including kinase signaling pathways and sex hormones. 8-10 Since the activity of OCT2 has considerably high impact on the systemic profiles of therapeutic drugs, exploring the regulation of OCT2 is of importance.

Fibrate drugs that activate peroxisome proliferator-activated receptor alpha (PPARα) are widely used for the...
treatment of metabolic syndromes such as hyperlipidemia. Binding of fibrates to PPARα, which is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors, induces heterodimer formation with retinoid X receptor (RXR). The heterodimer complexes then translocate from cytosol to the nuclear compartment and subsequently activate its target genes that control fatty acid β-oxidation and subsequent lipolysis processes. Fibrates also reduce the serum triglyceride and cholesterol including low density lipoprotein (LDL) and very low density lipoprotein (VLDL). On the other hand, they increase serum high-density lipoprotein (HDL). In addition, fibrates have been reported to reduce kidney injury caused by hypertension, ischemia/reperfusion, and drugs such as doxorubicin.

Nowadays, treatment of metabolic diseases often involves administration of a drug regimen for several conditions such as hypertension, diabetes and hyperlipidemia, which increases the chance of drug-drug interaction. Since several drug transporters such as organic anion transporting polypeptides (OATPs), P-glycoprotein and hepatic OCT1 are differentially regulated by PPARα, it is possible that antihyperlipidemic fibrate drugs which are ligands of PPARα may also have effects on the renal OCT2 activity. The present study, thus, aimed to evaluate the effect of fibrate drugs on the activity of OCT2-mediated organic cation transport using CHO-K1 cells transfected with rabbit (rb) OCT2 (OCT2-CHO-K1), LLC-PK1 (cells that endogenously expressed OCT2), and intact mouse renal cortical slices.

Materials and Methods

Materials: [3H]-MPP⁺ (methyl-4-phenylpyridinium acetate) (80 Ci/mmol) was purchased from American Radio Labeled Chemical Inc (MO, USA). Feno-ibrate and clofibrate were purchased from Sigma Aldrich (MO, USA) whereas GW6471 and WY14643 were purchased from Tocris Bioscience (MN, USA). mOCT2 antibody was obtained from LifeSpan BioSciences (WA, USA). Other chemicals were obtained from various sources with highest purity available.

Cell culture: OCT2-CHO-K1 cells, provided by Prof. Stephen H. Wright (Dept. of Physiology, Univ. of Arizona), were cultured in F12 Ham Kaighn’s Modification (F12K) medium supplemented with 10% FBS and 100 µl/ml penicillin and 100 µg/ml streptomycin. LLC-PK1 cells (the epithelial cell line derived from porcine renal cortex), purchased from American Type Culture Collection (ATCC) were cultured in low glucose Dulbecco’s modified Eagle medium supplemented with 10% FBS, 100 µl/ml penicillin and 100 µg/ml streptomycin. Both types of cells were incubated at 37°C in a humidified 5% CO₂ and 95% air atmosphere.

Transport assay in cell culture studies: The OCT2-CHO-K1 cells or LLC-PK1 were seeded and grown in 24-well plates for 48 h. The confluent cells were washed twice with Dulbecco’s modified phosphate-buffered saline (D-PBS) (NaCl 137 mM, KCl 3 mM, Na₂HPO₄,7H₂O 0.5 mM, KH₂PO₄ 1 mM, MgCl₂,6H₂O 0.5 mM, CaCl₂,2H₂O 1 mM, d-glucose 5.6 mM at pH 7.4) and then preincubated at 37°C for 30 min. The transport assay was performed by incubating the cells in D-PBS containing [3H]-MPP⁺ (~10 nM), a prototypic substrate of OCT2 at 37°C for 1 min uptake. The uptake was stopped by removing the transport buffer and washed three times with ice-cold D-PBS. Then, the cells were solubilized overnight by adding 10% SDS in 0.4 N NaOH, neutralized with HCl. The accumulated radioactivity was measured by liquid scintillation β counter (LKB Wallac, Sweden). The transport of [3H]-MPP⁺ was calculated as mol/min/cm² of the confluent monolayer surface and expressed as percentage of control.

Cell viability assay: Cell viability was evaluated using MTT assay as previously described. In brief, OCT2-CHO-K1 cells were seeded on 96-wells plate for 24 h and subsequently incubated with MTT reagent (5 mg/ml) at 37°C for 4 h. At the end of the incubation period, the medium was replaced with DMSO before measuring the absorbance at 540 nm (EL 312 spectrophotometer, Bio-Kinetics Reader; Bio-Tek Instruments Inc., Helsinki, Finland). Data were calculated as percent viability compared to that of vehicle treatment.

Kinetics analysis of OCT2 activity: The OCT2-CHO-K1 cells were preincubated in medium containing 100 µM fenofibrate or vehicle for 24 h, then washed twice with D-PBS and incubated in D-PBS for 30 min. At the end of the incubation period, the uptake study was performed by incubating the cells in D-PBS containing 10 nM [3H]-MPP⁺ at various concentrations of unlabeled MPP⁺ for 1 min. The uptake was stopped by removing the transport buffer and the cells were washed three times with 1 ml of ice-cold D-PBS. The accumulated radioactivity was measured as mentioned above. Cellular uptake of [3H]-MPP⁺ was calculated as fmol/cm²/min of the confluent monolayer surface. The hyperbolic inhibition of [3H]-MPP⁺ transport was described by Michaelis-Menten kinetics of competitive interaction of unlabeled and labeled MPP⁺ as defined by the following equation:

\[ J = \frac{J_{max}[T]}{K_t + [T] + [T]} + C \]

where \( J \) is the rate of [3H]-MPP⁺ transport at concentration equal to [\( ^*T \)]; \( J_{max} \) is the maximum rate of MPP⁺ uptake; \( K_t \) is the MPP⁺ concentration that results in half-maximum transport (Michaelis constant); \( [T] \) is the concentration of unlabeled MPP⁺ in the transport reaction; and \( C \) is a constant that represents the component of total MPP⁺ uptake that is not saturated (over the range of substrate concentration tested) and presumably reflects the combined influence of diffusive flux, nonspecific binding, and/or incomplete rinsing of the cell layer.
Animals: CD1-ICR-outbred mice were obtained from the National Laboratory Animal Center (NLAC) at Salaya Campus, Mahidol University, Thailand. The animals were cared for by the Animal Center, Faculty of Science, Mahidol University. All of the animal experiments were approved by the Animal Ethical Committee of Mahidol University, Bangkok, Thailand. Male ICR mice (8–12 weeks old, 26–32 g) were fed with a normal diet or diet supplemented with 0.2% (w/w) fenofibrate for 14 days. This procedure has been reported to successfully activate PPARα in mice.

Renal slice preparation and uptake study: Mice were sacrificed by intraperitoneal sodium pentobarbital injection. Kidneys were removed and renal cortical slices (≤0.5 mm) were cut with the Stadie-Riggs microtome on ice and were incubated in modified Cross and Taggart solution (NaCl 95 mM, KCl 5 mM, Na2HPO4.7H2O 9.5 mM, mannitol 80 mM, Tris-ultra pure 20 mM, CaCl2.2H2O 0.74 mM at pH 7.4) for 15 min. The renal cortical slice uptake study was performed as previously described. Briefly, the renal slices were incubated with modified Cross and Taggart solution containing [3H]-MPP⁺ for 30 min. The uptake was stopped by transferring the renal slices to an ice-cold transport solution containing 1 mM unlabelled-MPP⁺. Then, they were washed three times in ice-cold modified Cross and Taggart solution with 1 mM unlabelled-MPP⁺. The renal slices were blotted, weighed and solubilized in 1 N NaOH for 24 h and neutralized with 1 N HCl, prior to the measurement of [3H]-MPP⁺ accumulation. The uptake of [3H]-MPP⁺ was calculated as tissue per medium ratio (T/M) (dpm/mg of tissue per dpm/µl transport buffer). The results were presented as a mean percentage of the control (vehicle treatment) ± S.E.

Immunoblotting: The mouse renal cortex was dissected and homogenized in ice-cold lysis buffer solution (250 mM sucrose, 10 mM KCl, 1.5 mM MgCl2, 0.684 mM EDTA, 2 mM phenylmethylsulfonyl fluoride and complete protease inhibitor at pH 7.2). The homogenate solution was centrifuged at 800 g at 4°C for 20 min and the supernatant was collected and stored at −80°C. The whole cell lysate was taken and centrifuged at 100,000 g at 4°C for 20 min. The pellet was a membrane fraction and was resuspended with buffer containing 10 mM Tris-HCl, 125 mM sucrose, 0.684 mM EDTA, 2 mM PMSF and complete protease inhibitor. An equal amount of the whole tissue protein and membrane fraction were separated by 10% SDS-PAGE, and transferred to nitrocellulose membranes. The blots were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS) for 1 h, followed by incubation overnight with rabbit polyclonal antibodies against mouse mOCT2. The blots were washed with TBS followed by incubation with goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody for mOCT2. The immunoreactive bands were visualized with a chemiluminescence reagent (ECL) kit. The protein expression was quantified by the Gel and Graph Digitizing System.

Statistical analysis: Data were shown as means ± S.E. n represents the number of independent experiments for cell culture studies. In each experiment, a minimum of three wells was used to generate each data point, whereas n of the renal slice experiment is the number of animals. Statistical differences were assessed by using Student’s t-test and one-way analysis of variance (ANOVA) (using GraphPad Prism software, CA, USA) for the renal slice experiment and cell culture studies, respectively. A level of difference was considered to be statistically significant when p < 0.05.

Results

Effect of fenofibrate on [3H]-MPP⁺ uptake in OCT2-CHO-K1 cells: To determine the effect of fibrates on the initial uptake rate of [3H]-MPP⁺, OCT2-CHO-K1 cells were treated with fenofibrate at several concentrations (0–200 µM) for 24 h followed by [3H]-MPP⁺ uptake assay. As shown in Figure 1A, preincubation of cells with fenofibrate at 100 µM resulted in a 50% inhibition of [3H]-MPP⁺ uptake. Fenofibrate at 200 µM did not produce further suppression of the uptake. A time response experiment showed that the inhibitory effect of 100 µM fenofibrate was first observed after 4 h incubation. Increasing the exposure time to 48 h did not further decrease the [3H]-MPP⁺ uptake (Fig. 1B).

cis-Effect of fenofibrate on OCT2-mediated [3H]-MPP⁺ uptake: To be certain that the inhibitory effect of fenofibrate did not result from cis-inhibition of fenofibrate on [3H]-MPP⁺ uptake, we exposed OCT2-CHO-K1 cells to a medium containing [3H]-MPP⁺ and various concentrations of fenofibrate for 1 min. The results showed that in the presence of fenofibrate up to 200 µM concentration, the uptake of [3H]-MPP⁺ was not different from that of the control (Fig. 2A).

Effect of fenofibrate on cell viability: To verify whether the inhibitory effect of fenofibrate was due to its toxicity, OCT2-CHO-K1 cells were incubated with 10–
by incubation with 100 µM of fenofibrate results in a 50% inhibition of protein synthesis; OCT2-CHO-K1 cells were preincubated for 24 h. The data were obtained from 3 independent experiments and expressed as percentage of control (vehicle). *p < 0.05 compared with the vehicle.

Fig. 2. Interaction of fenofibrate with OCT2 and the cytotoxicity of fenofibrate
(A) cis-Effect of fenofibrate on OCT2-mediated [3H]-MPP⁺ uptake; cells were incubated in medium containing [3H]-MPP⁺ alone or in the presence of various concentrations of fenofibrate for 1 min. (B) Cell viability after treatment with vehicle or fenofibrate at several concentrations for 24 h. The data were calculated from 3 independent experiments and expressed as percentage of control (vehicle). *p < 0.05 compared with the vehicle.

Fig. 3. Effect of fenofibrate on [3H]-MPP⁺ uptake during an inhibition of protein synthesis; OCT2-CHO-K1 cells were preincubated with vehicle or 5 µM cycloheximide for 24 h followed by incubation with 100 µM of fenofibrate for a further 24 h. Data presented were obtained from 3 independent experiments performed in triplicate and expressed as percentage control. *p < 0.05 compared with the vehicle control.

200 µM fenofibrate for 24 h prior to test cell viability using the MTT assay. As shown in Figure 2B, OCT2-CHO-K1 cell viability was not changed when compared to the vehicle control.

Effect of fenofibrate on [3H]-MPP⁺ uptake during inhibition of protein synthesis: To determine whether the inhibitory effect of fenofibrate was mediated by inhibition of certain proteins, OCT2-CHO-K1 cells were preincubated under four conditions for 24 h: 1) vehicle, 2) medium containing 100 µM fenofibrate, 3) medium containing 5 µM cycloheximide, and 4) medium containing 5 µM cycloheximide and 100 µM fenofibrate. As expected, preincubating the cells with cycloheximide alone decreased [3H]-MPP⁺ uptake by 50% compared to that of untreated cells. Interestingly, the inhibitory effect of fenofibrate was not abolished by cycloheximide pretreatment (Fig. 3). This data indicated that the inhibitory effect of fenofibrate did not involve protein synthesis.

Effect of fenofibrate on kinetic of OCT2-mediated [3H]-MPP⁺ transport: Next, the mechanism by which fenofibrate reduced the transport function of OCT2 was examined by kinetic analysis to see whether it altered the availability of functional OCT2 in the cell membrane (Jₘₐₓ) or the affinity of the transporter for the substrate (Kₜ). Figure 4 shows that 24 h exposure of OCT2-CHO-K1 cells to fenofibrate significantly decreased Jₘₐₓ from 8.8 ± 0.3 to 1.2 ± 0.1 pmol/min/cm² (p < 0.05), whereas it had no effect on Kₜ (5.6 ± 0.2 µM vs. 7.1 ± 0.7 µM).

Effect of fenofibrate on OCT2-mediated [3H]-MPP⁺ uptake during PPARα inhibition: Since fenofibrate is a PPARα agonist, we examined whether inhibition of fenofibrate was via PPARα activation by using GW6471, a specific inhibitor of PPARα. OCT2-CHO-K1 cells were preincubated under four conditions: 1) vehicle, 2) medium containing 100 µM fenofibrate, 3) medium containing 25 µM GW6471, and 4) medium containing 25 µM GW6471 for 4 h followed by 100 µM fenofibrate plus 25 µM GW6471 for another 24 h. At the end of the preincubation periods, the [3H]-MPP⁺ uptake was determined. The results showed that fenofibrate significantly reduced OCT2-mediated [3H]-MPP⁺ uptake by about 49.8 ± 18.9%. Similarly, preexposure of cells to GW6471 decreased [3H]-MPP⁺ uptake by 50.1 ± 9.6% (Fig. 5A). Moreover, the inhibitory effect of fenofibrate was not attenuated by GW6471, suggesting that fenofibrate action did not involve an activation of PPARα. This was confirmed by the finding that preincubation with both fenofibrate and GW6471 produced additive suppressive effect on OCT2-mediated [3H]-MPP⁺ uptake.

Effect of clofibrate and WY14643 on [3H]-MPP⁺ uptake in OCT2-CHO-K1 cells: After showing that the inhibitory effect of fenofibrate was unlikely to be mediated by PPARα activation, we tested whether activation of PPARα by other agonists could modulate the OCT2 transport function. OCT2-CHO-K1 cells were preincubated in a medium containing PPARα activator (500 µM clofi-
Fibrates, the PPARα agonists, are widely prescribed for the treatment of hyperlipidemia-related diseases such as hypertension and diabetes, therefore, they ran a high risk of drug-drug interaction. Renal excretions of several of these cationic drugs involve transporters such as p-glycoprotein, OATP, and OCT1, which have been shown to be regulated by PPARα. It has been suggested that fibrates might regulate the transport function of OCT2. To explore this possibility, we investigated the effect of fibrates on OCT2-mediated organic cation transport in both a heterologous system and intact renal tissue.

The data obtained from our study showed that fenofibrate inhibited OCT2-mediated organic cation basolateral uptake. The active concentration of fenofibrate used in the in vitro study was higher than that of the pharmacological concen-

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**Fig. 5. Role of PPARα in fenofibrate-induced inhibition of OCT2 function**

(A) Effect of fenofibrate on OCT2-mediated [3H]-MPP⁺ transport during PPARα inhibition; OCT2-CHO-K1 cells were incubated with vehicle, 100 µM fenofibrate, 25 µM GW6471 (a PPARα antagonist), and fenofibrate plus GW6471 for 24 h prior to 1 min [3H]-MPP⁺ uptake. (B) Effect of PPARα activators (clobifibrate and WY14643) on OCT2-mediated [3H]-MPP⁺ uptake; OCT2-CHO-K1 cells were preincubated with vehicle, 500 µM clobifibrate, 50 µM WY14643 for 24 h prior to the measurement of [3H]-MPP⁺ uptake. The data were calculated from 3 independent experiments and expressed as percentage of control (vehicle). *p < 0.05 compared with vehicle whereas #p < 0.05 compared with fenofibrate-treated group.

**Fig. 6. Effect of fenofibrate [3H]-MPP⁺ uptake in renal proximal tubule cells (A and B) and protein expression of mOCT2 (C)**

(A) LLC-PK1 cells were 24 h preincubated with fenofibrate, WY14643, or clobifibrate at 200 µM, 50 µM, or 500 µM, respectively. Data were collected from triplicate 3 independent experiments. (B) The renal cortical slices were prepared from mice treated with vehicle or fenofibrate for 14 days prior to the 30 min uptake of [3H]-MPP⁺. The data were calculated as tissue per medium ratio (T/M) and represented as percentage uptake of control (vehicle-treated mice). The numbers in the bars indicate number of animals. *p < 0.05 compared with the vehicle. (C) Renal protein expression of mOCT2 following fenofibrate treatment for 14 days. Equal amounts of both membrane fraction and whole cell lysate (50 µg total protein) were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane. Subsequently, membranes were blotted with anti-mOCT2 antibody. The data are expressed as being relative to vehicle value. *p < 0.05 compared with the vehicle (n = 6).
Although there is no data available for the transport mechanism of fenofibrate into renal proximal tubules, based on the chemical structure of fenofibrate, it is possible that organic anion transporters (OATs) may contribute to fenofibrate uptake. This hypothesis was supported by evidence showing that an active metabolite of fenofibrate, fenofibrinic acid, might be transported by OAT3. We speculated that the transport of fenofibrate into the CHO-K1 cells that show no expression of OATs would be less than that of intact renal proximal tubules. Therefore, a high concentration of fenofibrate was required for the in vitro study. There were 3 possibilities that may explain the underlying mechanism of fenofibrate inhibition including: 1) inhibiting the transport function by binding and immobilizing the transporters; 2) inhibiting synthesis of new protein; and 3) interfering with the trafficking process of transporters. The present results showing an absence of fenofibrate effect on cis-inhibition indicated that its inhibitory action did not involve binding and immobilization of the transporter. In addition, since 24 h exposure of the cells to fenofibrate had no effect on cell viability, the drug was not cytotoxic. The second possibility was tested by exposing the cell to cycloheximide for 24 h followed by a test of the fenofibrate effect. Although cycloheximide itself through suppressing synthesis of new protein led to a 50% reduction in OCT2 transport function, however, this notion was ruled out. Since fenofibrate still caused a further reduction in OCT2 transport as compared to the cycloheximide control. Furthermore, the kinetic analysis revealed that fenofibrate decreased OCT2 transport function by reducing the number of functional transporters available on the membrane as shown in a decrease in Jmax. Since the total protein expression of OCT2 was not altered by fenofibrate, therefore, we further determined the effect of fenofibrate on plasma membrane expression of OCT2 using crude membrane extract. Although the crude membrane extract was used to study the plasma membrane protein, the impurity with intracellular protein should be of concern. Our data showed that fenofibrate reduced membrane expression of OCT2 supporting the possibility that fenofibrate may increase an internalization or decrease an insertion of the transporters between membrane and intracellular compartment. This notion was supported by the previous study showing that fenofibrate interfered with trafficking of proteins involved in the phosphorylation process in hepatocellular carcinoma cells by reduction of ERK1/2 phosphorylation. Moreover, our previous data demonstrated that the basal activity of OCT2 required ERK1/2 phosphorylation.

Since fenofibrate is a member of the PPARα agonists, we determined whether the inhibitory effect of fenofibrate on [3H]-MPP+ transport was influenced by a PPARα-dependent mechanism. Surprisingly, by using PPARα antagonist GW6471, it was shown that the effect of fenofibrate did not involve PPARα activation. The PPARα-independent effects of fenofibrate had been revealed as evidence showing that fenofibrate suppressed growth in human endothelial cells and hepatocellular carcinoma and inhibited cysteinyl leukotriene production in mast cells via a PPARα-independent mechanism. We also determined whether PPARα activation by other ligands could affect OCT2 activity. Exposure of the cells to PPARα agonists, clofibrate and WY14643, for 24 h did not affect organic cation transport in CHO-K1 cells. It seemed unlikely that the absence of PPARα agonist (WY14643 and clofibrate) action on OCT2 resulted from disfunction of PPARα in the CHO-K1 transfected with cDNA of OCT2. This notion was supported by the evidence showing no effect of PPARα agonists in LLC-PK1 cells that endogenously expressed OCT2. Taken together, these results supported the idea that the inhibitory effect of fenofibrate did not require PPARα activation. However, our data demonstrated that inhibition of PPARα by using GW6471 reduced the transport function of OCT2 by 50%, which indicated that basal activity of OCT2 was under-regulated by PPARα (Fig. 5). The additive effect of fenofibrate and GW6471 supported the possibility that the inhibitory effect of fenofibrate and PPARα inactivation on OCT2 may use different pathways.

Although the heterologous expression cell system (OCT2-CHO-K1 cells) is widely used to investigate transporter activity, the environment of non-renal cells may make the cells significantly different from the renal cells. We further investigated the effect of fenofibrate in LLC-PK1 cells, which are the renal proximal tubular cell line endogenously expressing OCT2. The data revealed that similar to its effect in OCT2-CHO-K1 cells, fenofibrate inhibited OCT2-mediated organic cation transport in this cell model. Since the local environment of the cell line may affect the regulation of OCT2 transport function, the effect of fenofibrate was evaluated in intact renal proximal tubules. The inhibitory effect of fenofibrate on OCT2-mediated organic cation transport was confirmed in renal cortical slices (mainly proximal tubules) prepared from mice treated with fenofibrate for 14 days. The magnitude of fenofibrate effect in intact renal proximal tubules was however less than that in OCT2-CHO-K1 cells, implying that other transport pathways may contribute to organic cation transport in intact tissue. Although, OCT2 is the major transporter in proximal tubules, OCT1, which is also present, may contribute to the organic cation transport in the renal proximal tubule. This hypothesis was supported by the evidence showing that OCT1 was up-regulated by PPARα activation.

In summary, OCT2-mediated organic cation transport was reduced by fenofibrate in both cell culture systems and intact renal proximal tubules. The mechanism by which fenofibrate inhibited OCT2-mediated [3H]-MPP+ transport involved a reduction in the number of functional transporters on the membrane, but not the affinity of the transporters and this mechanism was likely to be PPARα-independent. Modulation of OCT2 activity influences the pharmacokinetics of cationic drugs; therefore, drug-drug...
interaction in hyperlipidemic patients who receive polypharmacy containing fenofibrate needs to be monitored. Indeed, understanding of the OCT2-mediated drug-drug interaction will lead to better prediction and management of the therapeutic outcomes.

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References