Effect of Pregnane X Receptor Ligand on Pharmacokinetics of Substrates of Organic Cation Transporter Oct1 in Rats

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ABSTRACT:

Because rat organic cation transporter 1 (Oct1, SLC22a1) is expressed mainly in the liver and mediates drug transport, its activity may determine the hepatic handling of cationic drugs. Here, we studied the regulation mechanism of the expression of Oct1, focusing on the nuclear receptors. In vitro studies using cultured hepatocytes indicated that expression of Oct1 was up-regulated by treatment with pregnenolone-16α-carbonitrile (PCN) and by overexpression of rat pregnane X receptor (PXR). In addition, isolated rat hepatocytes exhibited an increase of 1-methyl-4-phenylpyridinium (MPP+) uptake on treatment with PCN. When rats were subcutaneously administered PCN, an increase of biliary excretion clearance and distribution volume was observed for drugs such as MPP+, metformin, and tetaethylammonium, although the effects on pharmacokinetic parameters were variable among the tested drugs. In addition, the expression of Oct2 in kidney was increased by treatment with PCN. Thus, PXR ligands appear to regulate the expression of organic cation transporters in rats and thereby to influence the pharmacokinetic properties of cationic drugs. Because PXR ligands include various clinically used drugs, alterations of hepatic drug handling may arise from interactions between cationic drugs that are substrates of Oct1 and ligands of PXR.

Membrane transport of organic cations, which include many clinically used drugs and endogenous compounds, is mediated by the family of organic cation transporters (OCTs). There are three members of the organic cation transporter family. In humans, OCT1 and OCT2 are exclusively expressed in the liver and kidney, respectively, whereas OCT3 is expressed in multiple tissues, including skeletal muscle, liver, placenta, kidney, and brain (Koepsell et al., 2003). In rodents, Oct1 is expressed in the liver, kidney, and small intestine (Gründemann et al., 1994). Oct2 has a substrate specificity similar to that of Oct1, but is predominantly expressed in the kidney, as is the case in humans (Gründemann et al., 1998, Ishiguro et al., 2005). In addition, it was reported that Oct1 gene-knockout mice showed greatly reduced hepatic uptake and direct intestinal excretion of Oct1 substrates, indicating that this transporter plays an essential role in the disposition of organic cations in the liver and intestine (Jonker et al., 2001; Wang et al., 2002). Despite its expression in the kidney, loss of Oct1 from the kidney is unlikely to affect the renal elimination of organic cations. Furthermore, studies of Oct1 and Oct2 double knockout mice showed that both transporters play key roles in the renal secretion of organic cations (Jonker et al., 2003).

Although OCTs are deeply associated with the pharmacokinetic properties of drugs, information about the involvement of OCTs in drug-induced adverse effects is limited. It has been reported that nephrotoxicity induced by platinum-based drugs is affected by the transport activity of OCTs, since the concentration of platinum agents in kidney is largely determined by the activity of OCTs (Yonezawa et al., 2006). Metformin is a substrate of OCT1, and excessive hepatic levels of such biguanides can lead to lactic acidosis (Wang et al., 2003). In addition, in Oct1 knockout mice, accumulation of metformin in the liver was lower than that in wild-type mice (Wang et al., 2002). Therefore, OCT1 is clearly an important transporter of organic cations in the liver, and alterations of its activity and expression may cause changes in toxicity, as well as in pharmacological effects.

Nuclear receptors are important transcription factors that regulate diverse physiological functions associated with cell differentiation and homeostasis as well as the expression of phase I and phase II

ABBREVIATIONS: OCT (Oct), organic cation transporter; AUC, area under the plasma concentration-time curve; CLbio, biliary excretion clearance; Vd, distribution volume; DMEM, Dulbecco’s modified Eagle’s medium; DR, direct repeat; ER, everted repeat; FBS, fetal bovine serum; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; HNF4α, hepatocyte nuclear factor-4α; MPP+, 1-methyl-4-phenylpyridinium; Ntcp, sodium/taurocholate cotransporter; PBS, phosphate-buffered saline; PCN, pregnenolone-16α-carbonitrile; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; PXRE, PXR response element; RT-PCR, reverse transcription polymerase chain reaction; TCA, taurocholic acid; TEA, tetaethylammonium.
drug-metabolizing enzymes and transporters. Although considerable information has been accumulated on the regulatory mechanisms of drug-metabolizing enzymes, less is known about the regulation of drug transporters (Riddick et al., 2004). At present, 48 members of the nuclear receptor superfamily have been identified in human (Nishimura et al., 2004). Pregnane X receptor (PXR), a key regulator of xenobiotic metabolism, heterodimerizes with retinoid X receptor to regulate the expression of xenobiotic-metabolizing enzymes and transporters (Kliewer et al., 1998; Geick et al., 2001). Furthermore, the liver and intestine express similar complement of drug-metabolizing and transporting proteins that are regulated by PXR, including human and rodent orthologs of CYP3A, UGT1A, MR2, MDR1, and Oatp2 (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998). The hepatic uptake of cardiac glycosides was reported to increase after treatment with pregnenolone-16α-carbonitrile (PCN) (Klaassen, 1974), a ligand of PXR.

In the present study, we first examined the effect of various ligands of nuclear receptors on the expression level of Oct1 mRNA in primary-cultured rat hepatocytes. Since the Oct1 mRNA level was increased by exposure to PCN, we then examined the involvement and significance of PXR in the hepatic handling of cationic drugs by means of in vitro and in vivo experiments in rats.

Materials and Methods

Materials. [3H]Taurocholic acid (TCA) (44.0 GBq/mmol) and [3H]digoxin (870.0 GBq/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). [3H]Taurocholic acid (TCA) (44.0 GBq/mmol) and [3H]digoxin (870.0 GBq/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). [3H]Metformin hydrochloride (962.0 GBq/mmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA).

Animal Experiments. Male Wistar rats (200–260 g body weight) were obtained from Satama Experimental Animals Supply Co., Ltd. (Satama, Japan). They were housed three per cage with free access to commercial chow and tap water, and maintained on a 12-h dark/light cycle (8:00 PM to 8:00 AM) in an air-conditioned room (temperature, 24.5 ± 1°C; humidity, 55 ± 5%). They were handled humanely according to the guidelines for animal experimentation of the Tokyo University of Science. PCN was administered subcutaneously at a single dose of 75 mg/kg body weight (15 mg/ml in propylene glycol). The control group received the same volume of vehicle subcutaneously. All studies were performed 24 h after PCN or vehicle administration.

Isolation and Culture of Rat Hepatocytes. Rat hepatocytes were isolated by calcium two-step collagenase perfusion methods according to Tamai and Tsuji (1987). In brief, rats were anesthetized with diethyl ether before portal vein cannulation. The liver was perfused in situ with oxygenated EGTA solution (8.0 g/l NaCl, 0.4 g/l KCl, 0.121 g/l NaHPO4, 0.06 g/l KH2PO4, 0.2 g/l MgSO4, 0.735 g/l CaCl2, 0.35 g/l NaHCO3, 1.0 g/l glucose, and 0.06 g/l phenol red) and collected by filtration through a sterile nylon mesh (150 mesh). The resultant cell pellet was washed and resuspended in Hanks' balanced salt solution. This washing was repeated three times, and finally the hepatocytes were suspended in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS), 70 mg/l streptomycin, 139 mg/l penicillin, 2 g/l bovine serum albumin, 1 µM dexamethasone, and 1 µM insulin. The cell suspension (1.0 × 10^7 viable cells/cm^2) was inoculated into 24-well plates that had been coated with type I collagen, and cultured for 3 h under 5% CO2 at 37°C. The medium was then replaced with fresh DMEM containing 70 mg/l streptomycin, 139 mg/l penicillin, and 2 g/l bovine serum albumin, and culture was continued for 24 h under 5% CO2 at 37°C.

Cell Culture and Transfection. Rat hepatoma RL-34 cells were cultured in DMEM supplemented with 10% FBS. pShuttle-CMV plasmid vector and pCMV4 plasmid vector contained rPXR cDNA and rRXR cDNA, respectively. RL-34 cells were seeded at the concentration of 2.5 × 10^5 cells/well in six-well plates and transfected with 0.1 µg of total plasmid DNA per well using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). At 48 h after transfection, the cells were used for semiquantitative reverse-transcription polymerase chain reaction (RT-PCR).

Semiquantitative Reverse-Transcription Polymerase Chain Reaction. Total RNA was prepared from the rat hepatocytes using ISOGEN (Wako Pure Chemical Industries, Tokyo, Japan). The total RNA content was determined by measuring the absorbance at 260 nm. The mRNA level of rat hepatocytes was analyzed using semiquantitative RT-PCR. Single-strand cDNAs were constructed using an oligo(dT) primer (Invitrogen) and Improm-II reverse transcriptase (Promega, Madison, WI). These cDNAs provided templates for PCRs with specific primers (Table 1). The PCR conditions were: denaturation at 94°C for 30 s, annealing at 58–62°C for 30 to 60 s, and elongation at 72°C for 30 s in the presence of deoxynucleotides (dNTPs) and Ex Taq polymerase (Takara Shuzo Co., Ltd., Tokyo, Japan). The annealing time and temperature were changed as required, depending on the genes. The PCR cycle numbers were titrated for each primer pair to confirm that amplification was performed within a linear range. PCR products were analyzed by 2% agarose gel (w/v) electrophoresis and the gels were stained with ethidium bromide for visualization. mRNA levels were quantified by using light capture (Atto Co., Tokyo, Japan). PCR amplification data were normalized with respect to glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The quantitation of each gene was repeated at least three times using RNA sources isolated from independently cultured cells, and the results were statistically analyzed by the use of Student’s t test.

Uptake Study with Freshly Isolated Rat Hepatocytes. For uptake measurement, hepatocytes were isolated from the livers of male Wistar rats. Freshly isolated hepatocytes were suspended in uptake medium containing 118 mM NaCl, 23.8 M NaHCO3, 4.8 mM KCl, 5.0 mM d-glucose, 1.5 mM CaCl2, 0.96 mM KH2PO4, 1.2 mM MgSO4, and 23.8 mM HEPES, adjusted to pH 7.3. The cell suspension was preincubated at 37°C for 20 min in the uptake medium, then centrifuged, and the resultant cell pellets were mixed with the uptake medium containing [3H]MPP+, [3H]digoxin, or [3H]TCA. At appropriate times, aliquots of the mixture were withdrawn and the cells were separated from the uptake medium by centrifugation through a layer of a mixture of silicone oil (SH550; Toray Dow Corning Co., Tokyo, Japan) and liquid paraffin (Wako Pure Chemical Industries) at a ratio of 10:3 (density: 1.03) on 3 M KCl solution. Each cell pellet was mixed with 3 N KOH and then neutralized with HCl. The cell-associated radioactivity was measured with a scintillation counter using Celosol-1 as a liquid scintillation fluid (Nacalai Tesque, Kyoto, Japan).

Pharmacokinetic Study. Rats under anesthesia with diethyl ether were cannulated into the femoral vein, femoral artery, and bile duct for drug administration, blood sampling, and bile collection, respectively. After surgical preparation, the rats received a bolus injection of 1 mg/kg [14C]metformin, 32 ng/kg [3H]MPP+, or 8 mg/kg [14C]TEA dissolved in saline solution. Bile

### TABLE 1

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Forward Primers (5'-3')</th>
<th>Reverse Primers (5'-3')</th>
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<tbody>
<tr>
<td>Oct1</td>
<td>CTTGCGTACATGCAGGAACTAGC</td>
<td>CCGGATACAGCACCTTTICC</td>
</tr>
<tr>
<td>Oct2</td>
<td>TGGCATGCTGCAACCCTTTGCC</td>
<td>TGCAAGCGTCGGTGGAGCGATTGCC</td>
</tr>
<tr>
<td>G3PDH</td>
<td>GGTAACGACCATTCGATCCAGA</td>
<td>AGCTGGACACATGCTGCAGCC</td>
</tr>
</tbody>
</table>

Primer sequences for PCR of the G3PDH and transporter genes.
samples were collected at 15- or 30-min intervals for 120 min. Blood samples were collected at 5, 15, 30, 60, 90, and 120 min after administration. After 120 min, the liver, kidney, spleen, and intestine were removed and homogenized. Tissue samples were solubilized by addition of Soluene 350 (PerkinElmer, Kanagawa, Japan) and heating in a water bath at 50–60°C for the appropriate time, with occasional swirling. Tissue samples were cooled again at 50–60°C for 30 min to complete decolorization. Blood samples were centrifuged at 6500 g for 30 min. The radioactivity was measured with a liquid scintillation counter.

**Determination of Pharmacokinetic Parameters.** Area under the plasma concentration-time curve (AUC), distribution volume (Vd), and total clearance (CL) were determined by moment analyses from the plasma concentration time curve over 120 min. Biliary excretion clearance (CLbile) was determined from the recovery in bile for 120 min.

**Analytical Methods.** Cellular protein content was determined according to the method of Lowry (Lowry et al., 1951) with bovine serum albumin as the standard. Usually, initial uptake rates were obtained by measuring the uptake at each time, as described in the figure legends. Cell-to-medium ratio (CM ratio) was determined from the recovery in bile for 120 min.

**Baseline Studies**

**Results**

**Modified Expression of Oct1 by PCN.** Primary-cultured rat hepatocytes were exposed to various nuclear receptor ligands, and the expression of Oct1 was quantitated. The mRNA level of rOct1 was increased to 2.0 times by PCN, a ligand of PXR in primary-cultured rat hepatocytes (Fig. 1A). In addition, the Oct1 mRNA level was significantly increased by exposure to PCN in the rat hepatoma-derived cell line RL-34 (Fig. 1B). Since PCN is a ligand of rPXR, we further examined the involvement of rPXR in the regulation of Oct1 expression by an overexpression of rPXR in RL-34 cells. The Oct1 mRNA level was significantly increased to 2.5 and 2.0 times by exposure to PCN in both rPXR/rRXRα-overexpressing cells and mock-transfected cells, respectively, in comparison with cells treated with DMSO (Fig. 2). Furthermore, the Oct1 mRNA level in rPXR/ rRXRα-overexpressing cells was slightly increased, about 1.3 times that in mock-transfected cells, by treatment with PCN. These results are consistent with the involvement of rPXR in the regulation of the Oct1 gene.

**Uptake of MPP⁺, Digoxin, and TCA by PCN-Treated Rat Hepatocytes.** We further investigated the effect of PCN on the hepatic uptake of cationic drugs, using freshly isolated hepatocytes from PCN-treated rats. Initial studies indicated that uptake of [3H]MPP⁺ (1 μM), a substrate of Oatp1, and [3H]digoxin (60 nM), a substrate of Oatp2, which is also regulated by rPXR (Guo et al., 2002), increased linearly for 1 min. In addition, uptake activity of [3H]MPP⁺ and [3H]digoxin was increased 1.5- and 2.5-fold, respectively, in PCN-treated rats in comparison with untreated rats (Fig. 3), whereas the uptake of TCA, a substrate of sodium-taurocholate cotransporter (Ntcp) (Zahner et al., 2003), which is not regulated by rPXR, was completely unaffected. Furthermore, the levels of Oct1 and Oatp2 mRNAs were significantly increased 1.5- and 2.5-fold, respectively, in PCN-treated rats, whereas the level of Ntcp mRNA was unchanged (Fig. 4).

**In Vivo Experiments.** The effect of PCN (75 mg/kg body weight) on the pharmacokinetics, such as plasma concentration and cumulative biliary excretion, of cationic drugs was investigated after intra-
metformin (Table 3). In addition, in the small intestine, the cell-to-medium ratio and is shown as the mean ± S.E.M. of three cultures. * indicates a significant difference from the control (Student’s t test; p < 0.05).

FIG. 3. Effect of PCN on uptake of MPP⁺, digoxin, and TCA by freshly isolated rat hepatocytes. Seven-week-old male Wistar rats were given subcutaneous injections of PCN (75 mg/kg body weight) or propylene glycol (vehicle) once a day for 4 days. At 24 h after the final administration of PCN, rat hepatocytes were prepared. Freshly isolated rat hepatocytes were preincubated at 37°C for 20 min. Uptake of [3H]MPP⁺ (1 μM) (A), [3H]digoxin (60 nM) (B), and [3H]TCA (1 μM) (C) by the isolated hepatocytes derived from rats treated with PCN (closed symbols) or propylene glycol (open symbols) was measured at pH 7.4 and 37°C. Uptake was expressed as intravenous administration of radiolabeled metformin, MPP⁺, and TEA. PCN treatment caused a small but significant decrease in the plasma concentration of [14C]metformin but did not affect the plasma concentration of [3H]MPP⁺ or [14C]TEA (Fig. 5). However, PCN treatment significantly increased the bile excretion of [14C]TEA and slightly increased that of [14C]metformin and [3H]MPP⁺ (Fig. 6, A–C), whereas the bile flow itself remained constant (Fig. 6, D–F). Pharmacokinetic parameters, such as AUC, Vₚ, and CLbic over 120 min, are summarized in Table 2. The AUC of [14C]metformin was decreased by PCN treatment, whereas the AUC of [3H]MPP⁺ and [14C]TEA was unchanged (Table 2). PCN significantly increased the values of Vₚ and CLbic of [14C]metformin, [3H]MPP⁺, and [14C]TEA (Table 2).

The values of tissue-to-plasma concentration ratio (Kᵢₑ) in the liver, kidney, intestine, and spleen are shown in Table 3. In the liver, PCN significantly increased the Kᵢₑ of [14C]metformin, but not those of [3H]MPP⁺ and [14C]TEA. In the kidney, PCN treatment significantly increased the Kᵢₑ of [3H]MPP⁺ and [14C]TEA, but not that of [14C]metformin (Table 3). In addition, in the small intestine, the Kᵢₑ values of [14C]metformin and [3H]MPP⁺ were significantly increased by treatment with PCN (Table 3). No change in Kᵢₑ in the spleen was observed following PCN treatment. Furthermore, in vivo treatment with PCN resulted in an increase of Oct1 mRNA level in the liver, but not in the kidney. Interestingly, Oct2 in the kidney was increased by PCN treatment (Fig. 7). Accordingly, it is possible that Oct2 is also regulated by PXR in the kidney.

**Discussion**

Cellular uptake and metabolism of endobiotic and xenobiotic substances are essential functions of the liver (van Montfoort et al., 2003). Many pharmacologically active agents are cationic and are taken up by the liver before biotransformation and/or excretion. Oct1 (Slc22a1) is a major organic cation transporter at the basolateral membrane of the liver, and the gene product is responsible for the hepatocellular uptake of numerous organic cations. In fact, in Oct1 gene-knockout mice, accumulation of TEA in the liver was 4- to 6-fold lower than in wild-type mice (Jonker et al., 2001).

We initially treated the primary cultured rat hepatocytes and rat liver-derived cell line RL-34 with a PXR ligand, PCN, and found that the cells exhibited a significant increase of Oct1 gene expression (Fig. 2). In addition, we examined whether the effect of PCN on the expression of Oct1 is mediated by PXR or not (Fig. 3). The involvement of PXR was suggested by the observation that the Oct1 mRNA level was increased by overexpression of PXR/RXR. Therefore, it appears that Oct1 expression in the liver is regulated by PXR. Although the fractional increase of Oct1 expression by PCN in PXR-transfected cells was not different for mock-transfected and PXR-transfected cells, the expression level in pRXR/rRXRα-overexpressing cells was higher than that in mock-transfected cells by treatment with PCN. Since RL-34 cells natively express some amount of PXR (data not shown), mock-transfected cells exhibited an increase in expression of Oct1 by PCN. In addition, it is possible that there are ligands of PXR in used cultured medium containing FBS. Therefore, if the cell line that does not express the PXR is cultured in medium without PXR ligands, the clearer effect of PXR overexpression could be observed.
To examine whether the expression of Oct1 by PXR was regulated directly or indirectly, the presence of conserved regions of the PXR binding site [PXR response element (PXRE)], such as direct repeat (DR) 3 or DR4 and everted repeat (ER) 6 or ER8, was investigated in the promoter region of Oct1 (Kliewer et al., 2002). We found no sequence corresponding to DR3, DR4, ER6, or ER8 in the rat Oct1 promoter region up to 10 kilobases. Oatp2, expression of which is mainly taken up by Ntcp, so that the absence of any change in the apparent uptake of TCA upon PCN treatment is not unreasonable (Kouzuki et al., 1998). Although TCA could be transported by Oatp2, it is thought that TCA is such an increase was not observed in the case of [3H]TCA uptake. Thus, the increased uptake activity can be explained in terms of increased expression level of Oct1 and Oatp2 in vitro (Fig. 4). Although TCA could be transported by Oatp2, it is thought that TCA is mainly taken up by Ntcp, so that the absence of any change in apparent uptake of TCA upon PCN treatment is not unreasonable (Kouzuki et al., 1998).

Since the plasma concentrations of metformin, MPP⁺, and TEA were hardly changed (Fig. 6), it was suggested that the pharmacokinetics of metformin, MPP⁺, and TEA were not affected by an increase in Oct1 expression level. However, the Vd of metformin, MPP⁺, and TEA increased upon PCN treatment. The Kp values of metformin in the liver and small intestine were significantly increased by treatment with PCN (Table 3). It was reported that, although no significant reduction was observed in the urinary excretion of metformin, the

**TABLE 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control</th>
<th>PCN</th>
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<tbody>
<tr>
<td>Metformin</td>
<td>AUC (µg · min/ml/kg)</td>
<td>812 ± 3.50</td>
</tr>
<tr>
<td>TEA</td>
<td>AUC (µg · min/ml/kg)</td>
<td>1.41 ± 0.07</td>
</tr>
<tr>
<td>MPP⁺</td>
<td>AUC (µg · min/ml/kg)</td>
<td>2.09 ± 0.09</td>
</tr>
<tr>
<td>MPP⁺</td>
<td>Vd (l/kg)</td>
<td>3.09 ± 0.25</td>
</tr>
<tr>
<td>MPP⁺</td>
<td>CLint (l/min/kg)</td>
<td>125 ± 7.44</td>
</tr>
<tr>
<td>TEA</td>
<td>Vd (l/kg)</td>
<td>0.896 ± 0.03</td>
</tr>
<tr>
<td>TEA</td>
<td>CLint (l/min/kg)</td>
<td>47.1 ± 3.49</td>
</tr>
</tbody>
</table>

* Significant difference from the control (Student’s t test; p < 0.05).

**TABLE 3**

<table>
<thead>
<tr>
<th>Compound and Tissue</th>
<th>Tissue to Plasma Ratio (Kp)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Metformin</td>
<td>Liver</td>
</tr>
<tr>
<td>Metformin</td>
<td>Kidney</td>
</tr>
<tr>
<td>Metformin</td>
<td>Spleen</td>
</tr>
<tr>
<td>MPP⁺</td>
<td>Intestine</td>
</tr>
<tr>
<td>MPP⁺</td>
<td>Liver</td>
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<tr>
<td>MPP⁺</td>
<td>Kidney</td>
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<tr>
<td>MPP⁺</td>
<td>Spleen</td>
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<tr>
<td>TEA</td>
<td>Intestine</td>
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<tr>
<td>TEA</td>
<td>Liver</td>
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<tr>
<td>TEA</td>
<td>Kidney</td>
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<tr>
<td>TEA</td>
<td>Spleen</td>
</tr>
<tr>
<td>TEA</td>
<td>Intestine</td>
</tr>
</tbody>
</table>

N.T., not tested.

* Significant difference from the control (Student’s t test; p < 0.05).
hepatic uptake of metformin was markedly reduced in Oct1 gene-deficient mice (Wang et al., 2002). Furthermore, the distribution of cationic compounds into hepatic and intestinal tissues was decreased in Oct1 gene-deficient mice (Jonker et al., 2001). Therefore, hepatic and intestinal accumulation of metformin could be determined by Oct1, and an increase of the expression of hepatic Oct1 via PXR should lead to an increased tissue concentration without significant change in plasma level. Since metformin causes dose-dependent lactic acidosis in the liver (Pearlman et al., 1996; Wang et al., 2003), interaction of PXR with metformin may result in metformin-induced toxicity.

Treatment with PCN increased the \( K_a \) values of TEA and MPP\(^+\) in the kidney, but not in the liver. The Oct2 expression level in the kidney was also increased by treatment with PCN (Fig. 7). Therefore, the Oct2 expression might also be regulated by PXR in the kidney. Moreover, alteration of Oct2 expression in the kidney may affect the pharmacokinetics of Oct2 substrates, as well as alteration of Oct1 expression in the liver. Those results suggest that TEA and MPP\(^+\) are transported mainly by Oct2 rather than Oct1 in vivo.

It was reported that the biliary excretion of digoxin was increased by treatment with PCN (Klaassen, 1974). Digoxin is a substrate of both P-glycoprotein and Oatp2, which are regulated by PXR (Tangawara et al., 1992; Geick et al., 2001). Thus, the increase of biliary excretion of digoxin by PCN treatment should be accounted for by the increases of the hepatic influx and canalicular efflux transporters, Oatps and P-glycoprotein, respectively. Accordingly, it is possible that cationic compounds that are recognized by P-glycoprotein or other cationic efflux transporters would show increased hepatic distribution and biliary excretion.

At present, organic carrier transporters consist of OCTs, multidrug and toxin extrusion (MATE) (Otsuka et al., 2005) and organic cation/ carnitine transporters (OCTNs). Although, it is possible that MATE and OCTNs affect the PK parameters of those compounds, it is unknown whether MATE and OCTNs are regulated by PXR. Therefore, further studies will be required to confirm whether MATE and OCTNs are regulated by PXR. On the other hand, since metformin, MPP\(^+\), and TEA used in the present study were not metabolized, the PK parameters of those compounds should not be affected by drug metabolizing enzymes that are regulated by PXR.

Recently, it was reported that mouse Oct1 was regulated by peroxisome proliferator-activated receptors \( \alpha \) and \( \gamma \) (PPAR\( \alpha \) and PPAR\( \gamma \)) (Nie et al., 2005). PPAR\( \alpha \) is associated with the proteins involved in lipid metabolism and \( \beta \)-oxidation of fatty acids. In contrast, the expression level of rat Oct1 was down-regulated by accumulation of bile acids (Denk et al., 2004), which suppress the expression of hepatocyte nuclear factor-4\( \alpha \) (HNF4\( \alpha \)). Moreover, human OCT1 is regulated by HNF4\( \alpha \), which is a liver-enriched nuclear receptor and plays a crucial role in hepatocyte differentiation and maintenance (Saborowski et al., 2006). Therefore, it is possible that ligands of PPAR\( \alpha \), PPAR\( \gamma \), and HNF4\( \alpha \), as well as PXR, may influence the pharmacokinetics of organic cations.

In conclusion, our results indicate that PXR ligands affect the pharmacokinetics of organic cations in vivo and in vitro by up-regulating the expression of Oct1 and probably also Oct2. Accordingly, the regulatory mechanisms of drug transporters, as well as drug-metabolizing enzymes, by nuclear receptors may have an important influence on the pharmacokinetic properties, and consequently, on the efficacy and/or toxicity, of various drugs.

References


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