OATP1B1, OATP1B3, AND MRP2 ARE INVOLVED IN HEPATOBILIARY TRANSPORT OF OLMESARTAN, A NOVEL ANGIOTENSIN II BLOCKER

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ABSTRACT:

Hepatic uptake and biliary excretion of olmesartan, a new angiotensin II blocker, were investigated in vitro using human hepatocytes, cells expressing uptake transporters and canalicular membrane vesicles, and in vivo using Eisai hyperbilirubinemic rats (EHB), inherited multidrug resistance-associated protein (mrp2)-deficient rats. The uptake by human hepatocytes reached saturation with a Michaelis constant (Km) of 29.3 ± 9.9 μM. Both Na+-dependent and Na+-independent uptake of olmesartan by human hepatocytes were observed. The uptake by Na+-independent human liver-specific organic anion transporters OATP1B1 and OATP1B3 expressed in Xenopus laevis oocytes was also saturable, with Km values of 42.6 ± 28.6 and 71.8 ± 21.6 μM, respectively. The Na+-dependent taurocholate-cotransporting polypeptide expressed in HEK 293 cells did not transport olmesartan. The cumulative biliary excretion in EHBR was one-sixth compared with that in Sprague-Dawley rats. ATP-dependent uptake of olmesartan was observed in both human canalicular membrane vesicles (hCMVs) and MR2-expressing vesicles. An MRP inhibitor, MK-571 ([[3-[2-(7-chloro-2-quinolinyl)ethenyl]phenyl][3-(dimethylamino)-3-oxopropyl][thio][methyl][thio]-propanoic acid] completely inhibited the uptake of olmesartan by hCMVs. In conclusion, the hepatic uptake and biliary excretion of olmesartan are mediated by transporters in humans. OATP1B1 and OATP1B3 are involved in hepatic uptake, at least in part, and MR2 plays a dominant role in the biliary excretion.

Olmesartan medoxomil (Benicar in United States; Olmetec in Europe and Japan) is a novel angiotensin II receptor antagonist available for treatment of hypertension. It is a prodrug that is rapidly and completely hydrolyzed in the gastrointestinal tract to a pharmacologically active form, olmesartan. Clinical pharmacokinetic data show that olmesartan is not further metabolized and that 60% of the dose was excreted into the feces via bile as olmesartan, with the remaining approximately 40% of the dose being excreted into the urine also as olmesartan (Laes et al., 2001). This dual excretion route of olmesartan would decrease the risk of increased blood level of olmesartan caused by renal or hepatic impairment. In fact, clinical pharmacokinetic data show that the area under the concentration time curve (AUC0–24h) of olmesartan in the patients with severe renal impairment (creatinine clearance <20 ml/min) increased by just 96% relative to that in healthy subjects. In addition, in the patients with moderate hepatic impairment, the AUC0–24h value of olmesartan increased by just 17% relative to that of healthy subjects (von Bergmann et al., 2001).

The hydrophilic nature of olmesartan as indicated by log D at pH 7.0 (~1.2; in-house data) and a high ratio of the ionized form at pH 7.4 as indicated by the pKa value (4.3; in-house data) were considered to limit greatly permeation of olmesartan through the cell membrane by passive diffusion. Therefore, the high fecal excretion of olmesartan via biliary excretion observed in vivo in humans was thought to be a consequence of the basolateral uptake and biliary excretion of olmesartan mediated by transporters in a concerted manner. In the case of rats, a previous study reported that the biliary excretion of olmesartan is mediated by multidrug resistance-associated protein 2 (mrp2) based on low biliary excretion in Eisai hyperbilirubinemic rats (EHB), which are inherited mrp2-deficient rats, compared with Sprague-Dawley rats (Takayanagi et al., 2005). However, the transporters involved in the hepatobiliary transport of olmesartan in humans have not been fully investigated yet.

Na+-dependent and Na+-independent hepatic basolateral transport systems are known to be responsible for the clearance of various endogenous and exogenous substances from the systemic circulation (Meier, 1988; Tiribelli et al., 1990). As Na+-independent transporters, OATP1B1 (also known as OATP-2, OATP-C, LST-1, and SLC21A6) (Abe et al., 1999) and OATP1B3 (also known as OATP-8, LST-2, and...
SLC21A8 (Abe et al., 2001) are well-known transporters that are specifically expressed on the basolateral membrane of human hepatocytes (Abe et al., 2001) and are involved in the hepatic uptake of various endogenous and xenobiotic substances. On the other hand, Na+-dependent taurocholate-cotransporting polypeptide (NTCP) is the most relevant Na+-dependent transporter identified as a hepatic uptake transporter so far (Trauner and Boyer, 2003). With regard to the transporters involved in biliary excretion, it is known that P-glycoprotein (ABC1), MRPs (ABC2), the bile salt export protein (ABCB2), and the breast cancer resistance protein (ABCG2) are predominantly expressed on canalicular membrane (Chan et al., 2004).

Investigation of the transporters involved in the hepatobiliary transport of drugs will increase our knowledge of potential drug-drug interactions based on competition on transporters and of the effects of single nucleotide polymorphism (SNP) of each transporter on the pharmacokinetics of drugs that are eliminated mostly by hepatic uptake and/or biliary excretion. In fact, our previous study of OATP1B1 as a key molecule for liver-specific distribution of pravastatin, an HMG-CoA reductase inhibitor (Nakai et al., 2001), has led to many investigations being conducted to examine the impact of OATP1B1 SNPs to the pharmacokinetics of pravastatin as a model compound (Nishizato et al., 2003; Niemi et al., 2004, 2005).

In this study, to assess the involvement of transporters in the hepatobiliary transport of olmesartan, we carried out experiments in vitro using human hepatocytes, various transporter expression systems, and human canalicular membrane vesicles (hCMVs), and in vivo using an EHBR animal model.

Materials and Methods

Materials. [14C]Olmesartan (specific activity, 1.3 MBq/mg) and [3H]olmesartan (specific activity, 79 Ci/mmol) were synthesized at Amersham Pharmacia Biotech Ltd. (Tokyo, Japan) (Fig. 1). Olmesartan was synthesized at Santk Organic Chemicals Co., Ltd. (Tokyo, Japan). Cryopreserved human hepatocytes were purchased from In Vitro Technologies Inc. (Baltimore, MD). [3H]Estradiol 17β-β-glucuronide (E2-17G) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). hCMVs were purchased from Tissue Transformation Technologies Inc. (Edison, NJ). Human MRPs (ABC2) were pumps that are predominantly expressed on canalicular membrane (Chan et al., 2004).

Animals. Mature X. laevis females were purchased from Hamamatsu Koyoai Co., Ltd. (Shizuoka, Japan) and maintained in a controlled environment (Goldin, 1992). Male Sprague-Dawley rats and EHBR (7 weeks old) were purchased from SLC Co., Ltd. (Shizuoka, Japan). Animal experiments were carried out according to the guidelines provided by the Institutional Animal Care and Use Committee of Sankyo Co., Ltd.

Uptake by Human Hepatocytes. Cryopreserved human hepatocytes (In Vitro Technologies Inc., Baltimore, MD) were thawed and added into an L-15 medium. After centrifugation (50g, 3 min), nonviable cells were removed by Percoll density centrifugation (100g, 10 min) (Groothuis et al., 1995). The viable cells were suspended in Krebs-Henseleit buffer (118 mM NaCl, 5 mM KCl, 1.1 mM MgSO4, 2.5 mM CaCl2, 1.2 mM KH2PO4, 25 mM NaHCO3, 10 mM glucose, and 10 mM HEPES, pH 7.4), saturated with O2/CO2 (95:5). Viability of the cells was verified by a trypan blue exclusion test, and the cells with viability of not less than 85% were used. The cell suspension was preincubated at 37°C for 5 min. Then, radiolabeled compounds were added to start the uptake experiments. At designated times, the incubation mixture was collected and transferred to a centrifuge tube containing an alkaline solution containing a silicone oil layer overlaid on top of it. Then, the tube was centrifuged in a tube containing the solubilized cell layer was sliced off with a razor blade, and the contents were transferred into a vial for liquid scintillation counting. After the addition of 10 ml of a liquid scintillation fluid (Hionic-Fluor; Packard Bioscience, Groningen, The Netherlands) to the vial, the radioactivity was determined using a Packard TriCarb 2200 CA liquid scintillation analyzer (Packard Instrument Company, Meriden, CT). To examine whether or not the olmesartan uptake by the human hepatocytes is sodium-dependent, the cells were incubated with a choline buffer. The composition of the choline buffer was the same as that of the Krebs-Henseleit buffer, except that the sodium chloride and sodium bicarbonate were replaced with choline chloride and choline bicarbonate, respectively.

Expression of OATP1B1 and OATP1B3 in X. laevis Oocytes and Uptake by Oocytes. In vitro synthesis of OATP1B1 and OATP1B3 cRNA was performed using cloned cDNAs of OATP1B1 and OATP1B3, as described previously (Abe et al., 1999, 2000). X. laevis oocytes were prepared according to a conventional procedure described previously (Tokui et al., 1999). Briefly, defolliculated oocytes were microinjected with 50 ng of transcribed cRNA or human liver polyadenylated RNA and were cultured for 3 days at 18°C, with daily replacements of modified Barth’s medium [88 mM NaCl, 1 mM CaCl2, 2.4 mM NaHCO3, 0.3 mM Ca(NO3)2, 0.41 mM NaCl, 0.82 mM MgSO4, and 15 mM HEPES, pH 7.6]. Uptake of radiolabeled substrate was examined in a medium containing 100 mM choline chloride, 2 mM MgCl2, 1 mM CaCl2, and 10 mM HEPES, pH 7.5. Oocytes were incubated in 100 µl of the same medium containing a radiolabeled substrate at room temperature. Uptake was terminated by addition of 3 ml of ice-cold uptake buffer, and the oocytes were washed five times. The water-injected oocytes were used as control. Each single oocyte was solubilized in 0.5 mM of 10% (w/w) sodium dodecyl sulfate, and 4 ml of scintillation fluid (Pico-Fluor; Packard Bioscience) was added. The radioactivity was determined using the Packard TriCarb 2200 CA liquid scintillation analyzer.

Uptake Study Using Human NTCP-Transfected HEK-293 Cells. We isolated a human NTCP (hNTCP) clone and introduced it into human embryonic kidney (HEK)-293 cells. Transfected HEK-293 cells were seeded at 1.0 × 105 cells/ml/well on collagen I-coated dishes (BD Biosciences) and cultured for 3 days at 37°C in humidified O2/C02 (95:5). The cells were first washed with DMEM containing 0.2% bovine serum albumin and then incubated with 0.8 ml of DMEM containing 0.2% bovine serum albumin containing 7.5 mM [3H]olmesartan or 75 mM [3H]taurocholate at 37°C. At 5, 15, and 30 min after incubation, the uptake was stopped by three washes with ice-cold phosphate-buffered saline. The cells were then solubilized with 0.5% NaOH, and the
radioactivity taken up by the cells was measured by the Packard TriCarb 2200 CA liquid scintillation analyzer.

**In Vivo Rat Study.** Male Sprague-Dawley rats and EHBR were anesthetized by intraperitoneal administration of pentobarbital (Nembutal; 50 mg/kg), and the common bile duct was cannulated with polyethylene tubing (PE-10) to collect bile samples. [14C]Olmesartan (1 mg/kg) was injected intravenously via the jugular vein, and the plasma was immediately separated by centrifugation. The total radioactivity in the plasma, urine, and bile samples were measured by the Packard TriCarb 2200 CA liquid scintillation analyzer. The radioactivity of [14C]olmesartan in the plasma, urine, and bile samples were measured with an assay by silica gel thin-layer chromatography in combination with a BioImage Analyzer (BAS-2000; Fuji Photo Film Co., Ltd., Tokyo, Japan).

**Uptake by hCMVs and MRP2-Expressing Membrane Vesicles.** hCMVs were purchased from Tissue Transformation Technologies Inc. Human MRP2-expressing vesicles were purchased from SOLVO Technologies Inc. A transport experiment was performed using a rapid filtration technique described in a previous report (Ishikawa et al., 1990). Transport medium (10 mM Tris, 250 mM sucrose, and 10 mM MgCl2, pH 7.4) containing a test compound was preincubated at 37°C for 3 min and was added to a suspension of the membrane vesicles (ca. 10–50 μg of protein) in the presence or absence of 5 mM ATP and an ATP-regenerating system (10 mM creatine phosphate and 100 μg/ml creatine phosphokinase). The mixture was incubated at 37°C for a designated time, and the transport reaction was stopped by the addition of 1 ml of ice-cold stop buffer (250 mM sucrose, 0.1 M NaCl, and 10 mM Tris-HCl, pH 7.4). The reaction mixture was immediately filtered through a 0.45-μm GVWP filter (Millipore Corp., Bedford, MA) and washed twice with 5 ml of the stop buffer. Each filter was placed in a vial with 10 ml of scintillation fluid (Pico-Fluor), and the radioactivity was determined using the Packard TriCarb 2200 CA liquid scintillation analyzer.

**Determination of Kinetic Parameters.** The kinetic parameter for olmesartan uptake by human hepatocytes was calculated according to the following equation:

\[
V_0 = V_{\text{max}} \cdot S/(K_m + S) + P_{\text{oct}} \cdot S
\]

where \(V_0\) is the initial uptake rate (pmol/min/oocyte), \(V_{\text{max}}\) is the maximum uptake rate (pmol/min/oocyte), \(K_m\) is the Michaelis constant (micromolar), \(P_{\text{oct}}\) is the nonspecific uptake clearance (ml/min/10^6 cells), and \(S\) is the radiolabeled olmesartan concentration in the medium (micromolar). The \(K_m\) value and \(P_{\text{oct}}\) value were calculated by an iterative nonlinear least-squares fit of the data to eq. 1.

\[
K_m = \frac{1}{K_{\text{influx}}} = \frac{1}{V_{\text{max}}} \cdot \frac{P_{\text{efflux}}}{P_{\text{influx}}}
\]

where \(K_{\text{influx}}\) is the permeability-surface area product for influx (PS_{influx}) and hepatic intrinsic clearance (CL_{H,in,\text{in}}) were calculated according to the following equation following linear conditions:

\[
PS_{\text{influx}} = (V_{\text{max}}/K_m + P_{\text{oct}}) \times C_n \times W_L
\]

where \(C_n\) is the number of hepatocyte cells (1.0 × 10^6 cells/g liver) (Olinga et al., 1993), and \(W_L\) is human liver weight (1800 g/body) (Davies and Morris, 1993). Hepatic intrinsic clearance was estimated by the following equation:

\[
CL_{H,in,\text{in}} = PS_{\text{influx}} \cdot (CL_{H,in,\text{efflux}}/PS_{\text{influx}} + CL_{H,in})
\]

where \(CL_{H,in,\text{efflux}}\) is intrinsic biliary clearance, and \(PS_{\text{influx}}\) is the permeability-surface area product for influx. In eq. 5, \(CL_{H,in,\text{in}}\) was considered to be equal to \(PS_{\text{influx}}\), assuming that \(PS_{\text{influx}}\) is much lower than \(CL_{H,in,\text{efflux}}\). Finally, the hepatic clearance was estimated according to the equation below, taking into consideration that olmesartan does not enter red blood cells (Yoshida and Kohzuki, 2004).

\[
CL_{H,in,\text{vivo}} = Q_H \cdot (f_H \cdot CL_{H,in,\text{in}} + f_H \cdot CL_{H,in})
\]

where \(Q_H\) and \(f_H\) represent the hepatic plasma flow rate (0.75 l/min) (Sandker et al., 1994) and free fraction of olmesartan in plasma, respectively (fp, 0.01, protein binding of olmesartan over 99%) (Yoshida and Kohzuki, 2004).

**Statistical Analysis.** Data were processed using Microsoft Excel (Microsoft, Redwood, WA) to calculate means and standard deviations. Student’s t test with 95% confidence (\(p < 0.05\)) was performed on the data from the two treatment groups to determine any significant differences between the means.

**Results**

**Uptake by Human Hepatocytes.** Uptake of [3H]olmesartan by human hepatocytes increased linearly against time up to 2 min (data not shown). Thus, the initial uptake velocity was calculated by linear regression using the data points at 0.5 and 2 min. Substitution of Na⁺ with choline⁺ in the medium decreased the uptake of olmesartan as shown in Table 1. We observed a significant difference in the uptake
between Na\(^+\)-dependent and Na\(^+\)-independent conditions using the donor 2 hepatocytes. However, in the donor 1, we did not observe a significant difference because of interexperiment variation, although a tendency of Na\(^+\)-dependence was observed (\(P = 0.058\)). Taken together, we concluded that there was Na\(^+\)-dependent hepatic uptake of olmesartan. The uptake became saturated with an increasing concentration of [\(^3\)H]olmesartan in the medium (Fig. 2). Kinetic parameters for uptake of olmesartan in the presence of Na\(^+\) were a \(K_m\) value of 29.3 ± 9.9 \(\mu\)M, \(V_{\text{max}}\) of 72.9 ± 65.8 pmol/min/10\(^6\) cells, and \(P_{\text{dif}}\), the nonspecific uptake clearance, of 0.6 ± 0.4 \(\mu\)l/min/10\(^6\) cells, respectively. The permeability-surface area product \(P_{\text{S,flux}}\) was estimated to be approximately 15 (\(\text{l/h/liver}\)) according to eq. 4 under linear conditions. The hepatic clearance \(C_{\text{lin, in vitro}}\), estimated by eq. 6 was approximately 0.36 l/h.

**Uptake by Oocytes.** In the oocytes injected with human liver polyadenylated RNA, the uptake values of olmesartan, taurocholate, and estradiol-17β-d-glucuronide (E2-17G) were higher than that in the oocytes injected only with water (Fig. 3), respectively. The uptake of E2-17G was similar under Na\(^+\)-containing and Na\(^+\)-free conditions (Fig. 3b), indicating that the uptake of E2-17G was independent of sodium ions. Under Na\(^+\)-free conditions, the uptake of taurocholate by oocytes injected with human liver polyadenylated RNA was almost the same as that by oocytes injected with water, indicating that the uptake of taurocholate was totally Na\(^+\)-dependent (Fig. 3C). The uptake of olmesartan partly decreased under Na\(^+\)-free conditions, suggesting that olmesartan was taken up via both Na\(^+\)-independent and Na\(^+\)-dependent mechanisms.

The uptake of olmesartan by OATP1B1- and OATP1B3-expressing oocytes at 10 \(\mu\)M were 2.9 ± 1.0 and 15.6 ± 10.0 times higher than that by the water-injected oocytes, respectively. The OATP1B1- and OATP1B3-mediated uptake of olmesartan was concentration-dependent and reached saturation with \(K_m\) values of 42.6 ± 28.6 and 71.8 ± 21.6 \(\mu\)M, respectively (Fig. 4).

**Uptake by NTCP-Transfected HEK-293 Cells.** No uptake of [\(^3\)H]olmesartan (7.5 nM) by hNTCP-transfected HEK-293 cells was observed, in contrast to the observation that the uptake of tauro-
cholate, a typical substrate for NTCP, was approximately 30-fold higher than that by vector-transfected cells after incubation at 75 nM for 30 min.

**Biliary Excretion in Rats.** [14C]Olmesartan was administered intravenously to bile-fistula Sprague-Dawley rats and EHBR at a dose of 1 mg/kg. EHBR showed a higher plasma level of olmesartan than Sprague-Dawley rats (Fig. 5A), and the AUC(0–2h) in EHBR was approximately 2 times higher than that in Sprague-Dawley rats (Table 2). The cumulative biliary excretion of radioactivity in Sprague-Dawley rats was 68.12 ± 11.81% of the dose by 2 h, being significantly higher than the biliary excretion in EHBR (11.45 ± 3.73%). The CLbile value in EHBR was approximately one-tenth of that in Sprague-Dawley rats (Table 2). Only olmesartan (more than 94% of total radioactivity) was observed as a metabolite in the bile by thin-layer chromatography (data not shown).

**Uptake by hCMVs and Human MRP2-Expressing Vesicles.** The uptake of [3H]olmesartan (0.021 μM) and [3H]E2-17G (0.25 μM) by hCMVs in the presence of ATP was significantly higher than that in the absence of ATP. ATP-dependent uptake of both compounds was almost linear against time up to 30 min (Fig. 6). ATP-dependent uptake of [3H]olmesartan (0.021 μM) and [3H]E2-17G (0.25 μM) was also observed in human MRP2-expressing vesicles (Fig. 7). MK-571 (50 μM), an MRP inhibitor, completely inhibited the uptake of [3H]olmesartan (0.021 μM) and [3H]E2-17G (0.25 μM) by hCMVs (Fig. 8).

**Discussion**

In the present study, we demonstrated that the hepatic uptake and biliary excretion of olmesartan, a pharmacologically active form of olmesartan medoxomil, in humans is mediated by transporters. The uptake of olmesartan by human hepatocytes reached saturation with a $K_m$ value of 29.4 μM (Fig. 2). The maximum plasma concentration of olmesartan ranged from 0.22 to 2.1 mg/l (0.49 to 4.7 μM) after a single oral dosage of 10 to 160 mg of olmesartan medoxomil to healthy volunteers (Warner and Jarvis, 2002). The $C_{max}$ of olmesartan was much lower than the $K_m$ value of the hepatic uptake of olmesartan obtained in this study. Therefore, we concluded that transporters play an important role in the hepatic uptake of olmesartan over the therapeutic dosing range for olmesartan medoxomil.

We believe that cryopreserved hepatocytes are useful for extrapolation of in vivo hepatic clearance based on the in vitro data as described below. In vivo $CL_{H}$ was calculated to be 0.86 l/h based on the total clearance and the renal clearance after intravenous administration of [14C]olmesartan to Sprague-Dawley rats (○, bile; ●, urine) and EHBR (○, bile; △, urine) at a dose of 1 mg Eq of [14C]olmesartan/kg. Each point represents the mean ± S.D. ($n$ = 4).
the pharmacokinetic parameters in vivo based on in vitro data is considered as an acceptable allowance.

Uptake of olmesartan by human hepatocytes had both Na\(^+\)/H\(^+\)-dependent and Na\(^+\)/H\(^+\)-independent fractions as indicated by the facts that the substitution of sodium ion with choline in the uptake medium led to a decrease in the uptake of olmesartan (Table 1), and the uptake under Na\(^+\)-free conditions was higher than the uptake via passive diffusion. Supporting this view, the oocytes injected with human liver polyadenylated RNA also transported olmesartan in both Na\(^+\)/H\(^+\)-dependent and Na\(^+\)/H\(^+\)-independent manners (Fig. 3).

As for the Na\(^+\)/H\(^+\)-independent uptake of olmesartan, both OATP1B1- and OATP1B3-expressed oocytes transported olmesartan (Fig. 4). OATP1B1- and OATP1B3-mediated uptake of olmesartan reached saturation with \(K_m\) values of 42.5 and 70.1 \(\mu M\), respectively (Fig. 4). These values were comparable with the \(K_m\) values obtained from human hepatocytes, indicating that OATP1B1 and OATP1B3, at least in part, contribute to the uptake of olmesartan into human liver.

As for the Na\(^+\)-dependent uptake of olmesartan, NTCP-transfected HEK-293 cells did not transport olmesartan. Only NTCP has been identified as an Na\(^+\)-dependent basolateral hepatic transporter (Lecuire et al., 2000), although it has not been reported to transport xenobiotic substrates so far. Thus, there is a possibility that olmesartan is taken up by unknown Na\(^+\)-dependent hepatic basolateral transporter(s) other than NTCP. Akhteruzzaman et al. (1999) have also reported that Na\(^+\)-dependent fraction is involved in hepatic uptake of an anionic cyclopentapeptide, BQ-123, but NTCP did not transport it.

According to the results obtained in the present study, MRP2 quite likely plays a dominant role in the biliary excretion of olmesartan as indicated by the fact that the biliary excretion of olmesartan was low in the experiment using EHBR, which have inherited mrp2 deficiency, compared with Sprague-Dawley rats (Fig. 5). The AUC\(_{0–2h}\) in

<table>
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<tr>
<th>Strain</th>
<th>AUC(_{0-2h})</th>
<th>Cumulative Biliary Excretion (0–2 h)</th>
<th>(\text{CL}_{\text{bile}}^{a})</th>
</tr>
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<tbody>
<tr>
<td>SDR</td>
<td>5.58 ± 1.48</td>
<td>68.12 ± 11.81</td>
<td>6.22 ± 0.89</td>
</tr>
<tr>
<td>EHBR</td>
<td>8.34 ± 0.86</td>
<td>11.45 ± 3.73</td>
<td>0.24 ± 0.09</td>
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SDR, Sprague-Dawley rats.  
\(^{a}\)\(\text{CL}_{\text{bile}}\) was calculated according to eq. 3.
contrast to pravastatin, however, olmesartan is taken up in both administration of radiolabeled olmesartan (Laeis et al., 2001). In vivo treatment of olmesartan was approximately 40% of the dose in humans after exposure of olmesartan caused by OATP1B1 SNPs is considered to be responsible for the impaired pathway. Thus, the extent of the increase in bodily clearance of olmesartan might be a substrate for P-glycoprotein in rats. However, the results obtained in the present study indicate that P-glycoprotein is not a dominant transporter involved in the biliary excretion of olmesartan.

Because transporters were found to be involved in the hepatobiliary transport of olmesartan, we need to take into consideration the effects of SNPs of certain transporters, such as MRP2, OATP1B1, and OATP1B2, on the pharmacokinetics of olmesartan in humans. Recently, many genetic variants of OATP1B1 (Tirona et al., 2001; Nozawa et al., 2002; Nishizato et al., 2003; Niemi et al., 2004) and MRP2 (Ito et al., 2001; Itoda et al., 2002; Suzuki and Sugiyama, 2002; Hirouchi et al., 2004) have been reported. The SNPs on these transporters have been demonstrated to alter the transport activity in vitro studies using transfected cells (Hirouchi et al., 2004; Iwai et al., 2004). Several mutations on the coding sequence of MRP2 have been reported in patients with Dubin-Johnson syndrome and some of which led to abolishing the function of MRP2 (Keitel et al., 2003; Ito et al., 2005). Recently, a reduction in the methotrexate elimination half-life was observed in patients with Dubin-Johnson syndrome (Hulot et al., 2005). Thus, there is a possibility that a defect of MRP2 gene may affect the pharmacokinetics of olmesartan. Monitoring of such MRP2-deficient patients may be necessary in clinical situations.

As for OATP1B1 SNPs, Niemi et al. (2004, 2005) reported that SNPs on OATP1B1 affect the pharmacokinetics of pravastatin and repaglinide. In the case of pravastatin, the mean maximum concentration of pravastatin in heterozygous carriers of OATP1B1*15B (containing the 388A allele) was approximately 2 times higher than that in noncarriers of OATP1B1*15B (Niemi et al., 2004). AEHBR was ca. 2 times higher than that in Sprague-Dawley rats, although the CL_bile value in EHBBR was ca. one-tenth of that in Sprague-Dawley rats (Table 2). It has not been clarified why the AUC(0–2h) in EHBBR was not elevated very much when the CL_bile clearance pathways. Regarding the hepatic uptake of pravastatin, this compound is a dual clearance pathways, that is, hepatobiliary and urinary excretion of pravastatin in heterozygous carriers of OATP1B1*15B (containing the 388A allele) was approximately 2 times higher than that in noncarriers of OATP1B1*15B (Niemi et al., 2004).

Although there is a possibility of OATP1B1 SNPs somewhat affecting the pharmacokinetics of olmesartan, we believe that OATP1B1 SNPs will not have a great impact on olmesartan pharmacokinetics for the following reason. Olmesartan, as pravastatin, has dual clearance pathways, that is, hepatobiliary and urinary excretion pathways. Regarding the hepatic uptake of pravastatin, this compound is taken up by the liver only in an Na+-independent manner. In contrast to pravastatin, however, olmesartan is taken up in both Na+-dependent and Na+-independent manners. Therefore, even if one of the clearance pathways is impaired, the other could compensate for the impaired pathway. Thus, the extent of the increase in bodily exposure of olmesartan caused by OATP1B1 SNPs is considered to be small.

In addition to the hepatobiliary excretion, there may be a transport-mediated urinary excretion system for olmesartan. Urinary excretion of olmesartan was approximately 40% of the dose in humans after administration of radiolabeled olmesartan (Lacis et al., 2001). In vivo renal clearance (0.53 l/h) (Davies and Morris, 1993) was higher than the glomerular filtration rate corrected by plasma-free fraction, suggesting the involvement of secretion even though no experimental data are currently available. Further investigation is necessary to clarify renal handling of olmesartan.

In conclusion, OATP1B1 and OATP1B3 contribute to the hepatic uptake of olmesartan, at least in part. MRP2 plays a dominant role in the biliary excretion of olmesartan.

References


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