Kinetic Study of the Hepatobiliary Transport of a New Prostaglandin Receptor Agonist

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ABSTRACT
The pharmacokinetics of the hepatobiliary transport of an anionic drug, 7,8-dihydro-5-[(E)-[a-(3-pyridyl)-benzylidene]aminoxy]ethyl]-1-naphthoxy]acetic acid (ONO-1301), a new prostaglandin (PG) I₂ receptor agonist, was investigated in rats. During intravenous infusion of this compound, the drug concentrations in arterial blood, hepatic vein and liver and the biliary excretion rate were measured at steady state. At a low infusion rate, 30% of the ONO-1301 was extracted by the liver during a single pass, and the main clearance organ was demonstrated to be the liver. The total clearance, Cltot, hepatic extraction ratio, E₈, and liver-to-plasma concentration ratio, Kp, values, decreased as the infusion rate increased. Considering the infusion rate-dependent decrease in all three parameters, saturation of hepatic uptake was suggested to be the cause of the nonlinear pharmacokinetics. To confirm this hypothesis, the time profiles of the plasma and liver concentrations of ONO-1301 after intravenous administration of various doses (0.01–25 mg/kg) were analyzed in vivo. The early-phase hepatic uptake clearance at lower doses (0.01–1 mg/kg) was 28 ml/min/kg, which is close to the hepatic plasma flow rate. The uptake clearance also was decreased at the higher doses. The uptake mechanism was investigated with isolated rat hepatocytes. Both Na⁺-dependent and -independent uptake were observed and these were inhibited by hypothermia and ATP depletors, which suggests that the uptake is via carrier-mediated active transport. The initial uptake velocity exhibited concentration dependence, and the kinetic parameters were as follows: Kₘ, 15.6 μM (Na⁺-dependent) and 3.8 μM (Na⁺-independent); Vmax, 5.9 nmol/min/mg (Na⁺-dependent) and 4.8 nmol/min/mg (Na⁺-independent). With these in vitro transport parameters, the plasma unbound fraction and the hepatic plasma flow rate, the hepatic uptake clearance was calculated from a mathematical model. The calculation also indicated that the uptake was so rapid that it was limited by the plasma flow rate. It is concluded that the carrier-mediated active transport systems demonstrated in vitro are responsible for the nonlinear pharmacokinetics of ONO-1301.

During research to develop an orally active and long-lasting PGI₂ analog, a novel compound, ONO-1301, was found. Although this compound possesses a nonprostanoid structure, it exhibits potent PGI₂ activity and inhibits thromboxane A₂ in vitro and in vivo (Kondo et al., 1995).

Besides renal excretion, hepatic metabolism and biliary excretion are the major pathways involved in the removal of xenobiotics. Our quantitative studies have demonstrated that hepatic uptake is the rate-limiting step in the hepatic clearance of several drugs (Miyauchi et al., 1987, 1993). In this case, saturation of membrane transport is one of the factors that causes the nonlinearity of hepatic clearance (Yamazaki et al., 1996). In addition, it has been reported that carrier-mediated transport contributes to hepatic uptake and/or biliary excretion (Petzinger 1994; Elferink et al., 1995). As far as the hepatic uptake is concerned, it is well established that hepatic uptake of the conjugated bile acid, taurocholate, is mediated predominantly by a secondary active transport process driven by an out-to-in Na⁺ gradient (Anwer and Hegen, 1978; Inoue et al., 1982; Yamazaki et al., 1993b). The Na⁺-taurocholate cotransporting different proteins (Ntcp and epoxide hydrolase) have been characterized.

ABBREVIATIONS:
ONO-1301, 7,8-dihydro-5-[(E)-[a-(3-pyridyl)-benzylidene]aminoxy]ethyl]-1-naphthoxy]acetic acid; PG, prostaglandin; TCA, taurocholate; DBSP, dibromosulfophthalein; FCCP, carbonylcyanide-p-(trifluoromethoxy)-phenylhydrazone; Ntcp, Na⁺-taurocholate cotransporting polypeptide; oatp, Na⁺-independent organic anion transporting polypeptide; PGT, prostaglandin transporter; P-gp, P-glycoprotein; SD rats, Sprague-Dawley rats; Kᵦ, Michaelis constant; Vmax, maximum transport velocity; Pdiff, nonspecific diffusion clearance; CLtot, total body clearance; E₈, hepatic extraction ratio; CLₘ₈, hepatic clearance; Qf, hepatic blood flow rate; Qₘ₈, hepatic plasma flow rate; Hₑ, hematocrit; Kp, value, liver-to-plasma concentration ratio; CLₘ₈,plasma and CLₘ₈,liver, biliary excretion clearances based on plasma and liver concentrations; n(ΔH), binding capacity; Kd, dissociation constant; CLuptake,in vivo, hepatic uptake clearance in vivo; AUC(0-t), the area under the plasma concentration-time curve from time 0 to t; PSh, permeability-surface area product obtained in vivo; HPLC, high-performance liquid chromatography; GST, glutathione S-transferase; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N’N’-tetraacetic acid; HEPES, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid.
and cloned by two groups (Hagenbuch et al., 1991, 1994; von Dippe et al., 1996), and it has been reported that the hepatic uptake of various organic anions such as DBSP, pravastatin and leukotriene C4 is mediated by a oatp, which also has been cloned (Jacquemin et al., 1991, 1994; Kullak-Ublick et al., 1995). In addition, the cDNA sequence of a specific PGT has been reported, and the presence of this transporter has been demonstrated in liver as well as other tissues (lung, kidney, etc.); it exhibits similarities to oatp in terms of amino acid sequence and substrate specificity (Kanai et al., 1995).

Regarding biliary excretion, a primary active transport system for several compounds which is coupled directly to ATP-hydrolysis has been reported (Ishikawa et al., 1990; Kobayashi et al., 1990). The existence of transporters for conjugated bile acids, organic anions (canalicular Multiphasic Organic Anion Transporter) and amphipathic organic cations including anticancer drugs (P-gp) was demonstrated (Elferink et al., 1995; Ishikawa et al., 1990; Ito et al., 1996; Meijer et al., 1990; Mayer et al., 1995).

ONO-1301 is an organic anion with a carboxyl group (fig. 1) and is an agonist specifically bound to the PG receptor; therefore, hepatic uptake and biliary excretion may be mediated by the transporters described above. In this study, we carried out a kinetic investigation of the hepatobiliary transport of ONO-1301 and its mechanism of hepatic uptake.

Materials and Methods

Materials

[14C]-ONO-1301 (1.10 GBq/mmol) and unlabeled ONO-1301 were donated by ONO Pharmaceutical Co. Ltd. (Osaka, Japan). Rotenone was purchased from Sigma Chemical Co. (St. Louis, MO). FCCP was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were of reagent grade.

Animals

Male Sprague-Dawley rats (250–300 g, Nihon Ikagaku Doubutsu Shizai Kenkyusyo, Tokyo, Japan) were used.

In Vivo Infusion Study

Under ether anesthesia, the femoral vein and artery were cannulated with PE-50 polyethylene tubing for ONO-1301 administration and blood sampling, respectively. The bile duct was cannulated with PE-10 polyethylene tubing and the hepatic vein also was cannulated by the method of Yokota et al. (1976). ONO-1301 dissolved in physiological saline was infused through the femoral vein cannula at a flow rate of 0.8, 8 or 80 mg/kg/hr for 30 min after the beginning of the infusion and then at a flow rate of 0.2, 2 and 20 mg/kg/hr. The concentration of ONO-1301 solutions were 0.2, 2 and 20 mg/ml, respectively. After a certain interval, arterial blood and bile samples were collected in polyethylene tubes. To obtain plasma, blood was centrifuged at 10,000 x g for 2 min in a tabletop microcentrifuge (Microfuge E, Beckman Instruments, Inc., Fullerton, CA). The concentration of ONO-1301 was determined by HPLC. HPLC analysis was performed on a CAPCELLPAK C18 UG-120 column (S-5 μm, 150 mm × 6 mm inside diameter [i.d.]). The mobile phase consisted of CH3CN/0.02 M phosphate buffer (pH 9.0) (1:4) (solvent A) and CH3CN/0.02 M phosphate buffer (pH 9.0) (2:3) (solvent B). A linear gradient was run from 0 to 25 min to increase solvent B from 45% to 100%, followed by a 15 min elution with 100% solvent B; a reverse gradient reduced the solvent B content back to 55% at 40 min. The flow rate was 1.0 ml/min, and the column effluent was monitored at 265 nm. AP-501–01 (0.2 μg; the isomer of ONO-1301) was added to plasma or bile specimens as an internal standard. Plasma and bile specimens (5–100 μl) were mixed with 1 ml ethanol, the mixture was stirred with the vortex mixer and centrifuged at 3,000 rpm for 10 min. The supernatants were evaporated to dryness, and the samples were redissolved in HPLC mobile phase. The liver (0.1 mg) was homogenized with 1 ml ethanol and centrifuged at 3,000 rpm for 10 min. A selected volume (30–100 μl) of supernatant was mixed with internal standard solution and evaporated to dryness, and the sample was redissolved in HPLC mobile phase. Quantitation of ONO-1301 in plasma, bile and liver was accomplished with calibration curves obtained by plotting the ratio of the appropriate peak area to the internal standard (ONO-AP-501–01). Linearity was observed for concentrations of 0.1 to 1 μg/tube. The straight-line equation was y = 1.32x – 0.00050 [y = peak area ratio, ONO-1301/IS; x = amount of ONO-1301 (μg)/tube]. The correlation coefficient, r, was 0.999.

Precision and accuracy of the assay were determined for three injections per concentration, and seven concentrations. The precision was less than 14.3%, and the accuracy was less than 6.7%. Typical HPLC chromatograms of biological samples (plasma, bile and liver) are shown in figure 2.

Total body clearance (CLtot) was calculated from the following equation:

\[ \text{CL}_{\text{tot}} = \frac{I}{C_{a,ss}} \]

where, I represents the infusion rate of ONO-1301, \( C_{a,ss} \) is the arterial plasma concentration of ONO-1301 60 min after the beginning of the infusion.

Hepatic extraction ratio (Eh) was calculated from the following equation:

\[ E_h = \frac{(C_{s,ss} - C_{b,ss})}{C_{a,ss}} \]

where, \( C_{b,ss} \) is the hepatic venous plasma concentration of ONO-1301 65 min after the beginning of the infusion.

The liver-to-plasma concentration ratio (\( K_p \) value) was calculated from the following equation:

\[ K_p = \frac{C_{\text{Liver}}}{C_{b,ss}} \]

where, \( C_{\text{Liver}} \) is the liver concentration of ONO-1301 65 min after the beginning of the infusion.

Biliary excretion clearances based on plasma and liver concentrations of ONO-1301 (\( CL_{\text{bile,plasma}} \) and \( CL_{\text{bile,liver}} \)) were determined as follows:

\[ CL_{\text{bile,plasma}} = V_b/C_{b,ss} \]

\[ CL_{\text{bile,liver}} = V_b/C_{\text{Liver}} \]

where \( V_b \) is the biliary excretion rate of ONO-1301.

Binding of ONO-1301 to Serum or Liver Cytosolic Protein

The equilibrium dialysis method was used to determine the extent of binding of [14C]-ONO-1301. Two-chamber dialysis cells divided by a dialysis membrane (Visking sheet, Sanplate, Osaka, Japan) were used. A volume of 400 μl serum or 33% (w/v) cytosol was put in to one chamber, and the same volume of phosphate buffer (pH 7.4) was put...

Fig. 1. Chemical structure of ONO-1301.
The binding parameters to serum and liver cytosol, respectively, were determined to minimize the denaturation of binding protein(s). ONO-1301 in both serum or cytosol and buffer sides was then measured (cytosol), long enough to reach equilibrium. The concentration of chambers were then incubated at 37°C for 6 hr (serum) or 4°C for 48 hr (cytosol), long enough to reach equilibrium. The concentration of ONO-1301 in both serum or cytosol and buffer sides was then measured. The binding to cytosol was determined at a low temperature to minimize the denaturation of binding protein(s).

The experimental data were fitted to the following equation to determine the binding parameters to serum and liver cytosol, respectively:

\[ C_b = \frac{n(P)_i \cdot C_f}{K_d + C_f} \]  

(6)

\[ C_b = \frac{n(P)_i \cdot C_f}{K_d + C_f} + a \cdot C_f \]  

(7)

where \( C_b \), \( n(P)_i \), \( K_d \) and \( a \) are the ligand concentration bound to protein(s), unbound concentration of ligand, binding capacity, dissociation constant and proportionality constant for nonspecific binding, respectively. Assuming that both the binding affinity and the binding capacity per unit protein are unaffected by the dilution of cytosol, the binding to physiological undiluted cytosol was calculated by use of three times the binding capacity \( n(P)_i \) obtained from binding studies which were carried out with 33% diluted cytosol.

Identification of the binding protein in the cytosol

Gel filtration was used to identify the protein responsible for binding ONO-1301. The cytosol specimen was prepared as follows:

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- The experimental data were fitted to the following equation to determine the binding parameters to serum and liver cytosol, respectively:

\[ \frac{dX_t}{dt} = \frac{CL_{uptake, in vivo}}{C_p} \cdot C_p \]  

(8)

where \( X_t \) is the amount of ONO-1301 in the liver at time \( t \), \( CL_{uptake, in vivo} \) is the uptake clearance and \( C_p \) is the plasma concentration of ONO-1301 at time \( t \). The \( C_p \) values were determined by measuring total radioactivity because thin-layer chromatography analysis indicated that >90% of the total radioactivity in the plasma came from unchanged \( ^{14}C \) ONO-1301. Integration of equation 8 gives:

\[ X_t = CL_{uptake, in vivo} \cdot AUC_{0-\infty} \]  

(9)

where \( AUC_{0-\infty} \) represents the area under the plasma concentration-time curve from time 0 to \( t \). Equation 9 divided by \( C_p \) gives:

\[ \frac{X_t}{C_p} = CL_{uptake, in vivo} \cdot AUC_{0-\infty}/C_p \]  

(10)

The \( CL_{uptake, in vivo} \) value can now be obtained from the initial slope of a plot of \( X_t/C_p \) vs. \( AUC_{0-\infty}/C_p \), designated as the “integration plot” (Kim et al., 1988; Yanai et al., 1990).

Measurement of Hepatic Blood Flow

Under ether anesthesia, the femoral vein was cannulated with PE-50 polyethylene tubing for ONO-1301 administration. All doses of ONO-1301 (0.2, 2 and 20 mg/kg/hr) were dissolved in saline and infused through the femoral vein. The abdomen was opened by a downward midline incision extending about 8 cm from the diaphragm. The central lobe of the liver was deflected to the upper left with a gauze soaked in saline. The hepatic artery and portal vein were separated gently, and any fat was removed. The probe was placed around the portal vein and hepatic artery, the central lobe was returned to its normal position and then the abdomen was closed. The hepatic blood flow was measured at steady state before and after the beginning of drug infusion (small animal blood-flow meter, T106, Transonic Systems Inc. Ithaca, NY).
Isolated Rat Hepatocytes

Cell preparation. Hepatocytes were isolated from male SD rats (250–300 g) by a two-step collagenase perfusion method modified from the procedure of Baur et al. (1975). The liver was perfused at 37°C for 20 min with the following medium: 137 mM NaCl, 5.4 mM KCl, 0.5 mM NaH2PO4, 0.42 mM Na2HPO4, 10 mM HEPES, 4.2 mM NaHCO3, 0.5 mM EGTA, 5 mM glucose, equilibrated with 95% O2-5% CO2. Collagenase (from Clostridium histolyticum) hepatocyte isolation grade; Wako Pure Chemical Industries, Ltd., Osaka, Japan), trypsin inhibitor (Type I-S, from Soybean; Sigma Chemical Co., St. Louis, MO) and calcium ion were added to 100 ml EGTA and glucose-free perfusate to give a final concentration of 0.05%, 0.005% and 5 mM, respectively. The liver was then perfused with the collagenase solution for an additional 15 min. After isolation, hepatocytes were suspended (1 mg protein/ml) at 0°C in albumin-free Krebs-Henseleit buffer supplemented with 12.5 mM HEPES (pH 7.3). Cell viability was checked routinely by the trypan blue [0.38% (w/v)] exclusion test. Viability ranged from 85% to 95%.

Uptake study. Uptake of [14C]ONO-1301 (0.5 μM) was initiated by adding ligand to the preincubated (37°C for 3 min) cell suspension (1 mg protein/ml). At designated times, the reaction was terminated by separating the cells from the medium by centrifugal filtration (Schwenk, 1980). Aliquots (200 μl) were placed into 0.4 ml centrifuge tubes containing 50 μl 2 N NaOH, covered by 100 μl of a mixture of silicone and mineral oil (density, 1.015). The samples were then centrifuged for 15 s in a tabletop microfuge (Beckman Instruments, Fullerton, CA). Centrifugation drove the hepatocytes through the oil layer into the 2 N NaOH solution. After the cells dissolved in the alkaline solution, the tube was sliced with a razor blade, and both compartments [medium and bottom compartments (including the cells)] were transferred to scintillation vials. The bottom contents were neutralized with 50 μl 2 N HCl. Then 5 ml of counting solution was added to the vial, and both cell and medium radioactivity was determined in a liquid scintillation spectrophotometer (LS 6000SE, Beckman Instruments, Inc., Fullerton, CA). The time course of ONO-1301 uptake was plotted in terms of the cell-to-medium concentration ratio (CM ratio), so that one could monitor the extent to which the ligand had been concentrated in the cell. Initial uptake velocity was calculated by linear regression of points taken at 20 and 60 s. To estimate Na+-independent ONO-1301 uptake, the uptake study was performed in the absence of external Na+. Under these experimental conditions, choline was used in the incubation buffer. The composition of the choline buffer from the total uptake measured with Krebs-Henseleit buffer containing Na+ (142 mM).

Estimation of Hepatic Uptake CL from the in Vitro Data

Based on the kinetic parameters obtained by the fitting procedure described, under linear condition, the permeability-surface area product, PS\textsubscript{influx,in vitro} (ml/min/kg rat) was calculated from the following equation:

\[
\frac{V_0}{S} - \frac{PS_{\text{influx,in vitro}}}{S} = \left[ \frac{(V_{\text{max}}/K_m) + P_\text{dir}}{a(\beta)}} \cdot (a/\beta) \cdot \gamma \right. \]

where \(a = 1.25 \times 10^6\) (cells/g of liver, Lin et al., 1980), \(\beta = 1.0 \times 10^6\) (cells/mg protein) and \(\gamma = 44\) (g liver/kg rat, Sugita et al., 1982).

The in vivo uptake CL (CL\textsubscript{uptake,in vivo}) ml/min/kg rat was then estimated from the in vitro PS\textsubscript{influx,in vitro} with the dispersion model (equation 13) (Roberts and Rowland, 1986):

\[
\text{CL}_{\text{uptake,in vivo}} = \frac{Q_b}{\gamma^2} 
= \frac{4a}{1 + (a - 1/2D_b)} - \left(1 - \frac{1}{(1 + a)^2} \cdot \exp((a - 1/2D_b) - (1 - a)^2)} \cdot \exp((a - 1)2D_b) \right. \]

\[
= \frac{R_N \cdot PS_{\text{influx,in vitro}}}{Q_b \cdot \gamma^2} \]

where,

\[
a = \left(1 + 4 R_N \cdot D_b \right)^{1/2} \]

\[
R_N = \frac{f_u \cdot PS_{\text{influx,in vitro}}}{Q_b \cdot \gamma^2} \]

\(D_b\) is the dispersion number, \(Q_b\) is the hepatic blood flow rate in rats, \(f_u\) is the unbound fraction of ONO-1301 and \(R_N\) is the blood-to-plasma concentration ratio. In the analysis with the dispersion model, a \(D_b\) of 0.17 was used (Iwatsubo et al., 1996).

Results

In vivo infusion study. After intravenous infusion (0.2, 2, and 2 mg/kg/hr), the plasma concentrations in both arterial and hepatic venous blood, the liver concentration and the biliary excretion rate of ONO-1301 were measured at steady state. CL\textsubscript{rat}, E\textsubscript{H} and K\textsubscript{p} were calculated from equations 1, 2 and 3, respectively. These results are shown in table 1. All parameters remained constant at 50 and 60 min after the beginning of the intravenous infusion, 0.2 to 20 mg/kg/hr, having reached steady state. The E\textsubscript{H} value was estimated to be 0.31 for the 0.2 mg/min/kg infusion, which indicates that 30% of ONO-1301 molecules were extracted by the liver during a single pass. CL\textsubscript{H} was estimated to be 12.2 to 6.7 ml/min/kg at 0.2 to 2 mg/min/kg and was similar to CL\textsubscript{tot} (6.5–5.7 ml/min/kg), so the main clearance organ was suggested to be the liver. The CL\textsubscript{rat}, E\textsubscript{H} and K\textsubscript{p} values in the liver decreased as the infusion rate increased (table 1). The biliary excretion rates of ONO-1301 normalized for the infusion rate (V\textsubscript{bile}/I) at steady state at 0.2, 2 and 20 mg/kg/hr were 9.15 ± 0.71%, 10.6 ± 1.1% and 4.96 ± 0.89%, respectively. The biliary excretion clearance based on the plasma concentration of ONO-1301 (CL\textsubscript{bile,plasma}) decreased as the infusion rate increased; on the other hand, the clearance rates based on the liver concentration (CL\textsubscript{bile,liver}) increased (table 1).

Binding to serum protein. The binding of ONO-1301 to serum protein was studied by equilibrium dialysis; distribution of ONO-1301 to blood cells also was studied. ONO-1301 distribution in blood cells was negligible. The Scatchard plot for the binding of ONO-1301 to rat serum protein (fig. 3) revealed the presence of a single kind of binding site. The binding capacity [n(P)] and dissociation constant (K\textsubscript{d}) were...
concentrations of four binding sites on an albumin molecule. The free fraction concentration (0.4–0.6 mM), which suggests the existence of a value was approximately four times the serum albumin concentration estimated to be 1860 \( \times 10^{-6} \) M. The dialysis cells were then incubated at 37°C for 6 hr. \( C_a \) and \( C_t \) represent the concentration bound to serum protein and the unbound concentration of ONO-1301, respectively. Each symbol and vertical bar represents the mean ± S.E. of three data points. The solid line represents the fitted line (equation 6).

### Table 1

<table>
<thead>
<tr>
<th>Dose dependence of the kinetic parameters for ONO-1301 at steady state (60 min after the beginning of the intravenous infusion)(^a)</th>
<th>0.2 mg/kg/hr</th>
<th>2 mg/kg/hr</th>
<th>20 mg/kg/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{ext} (\mu g/ml) )</td>
<td>0.518 ± 0.027</td>
<td>5.90 ± 0.11</td>
<td>175 ± 2</td>
</tr>
<tr>
<td>( C_{ext,av} (\mu g/ml) )</td>
<td>0.358 ± 0.018</td>
<td>4.96 ± 0.15</td>
<td>171 ± 2</td>
</tr>
<tr>
<td>( E_t )</td>
<td>6.48 ± 0.35</td>
<td>5.65 ± 0.10(^{ab})</td>
<td>1.90 ± 0.023(^{abc})</td>
</tr>
<tr>
<td>( V_{sol} (\mu g/min/kg) )</td>
<td>0.306 ± 0.034</td>
<td>0.158 ± 0.026(^{abc})</td>
<td>0.0240 ± 0.013(^{abc})</td>
</tr>
<tr>
<td>( V_{sol}/F (%) )</td>
<td>9.15 ± 0.71</td>
<td>3.53 ± 0.37</td>
<td>16.5 ± 3.0</td>
</tr>
<tr>
<td>( V_{solv} (\mu g/g) )</td>
<td>9.34 ± 0.95</td>
<td>74.8 ± 6.6</td>
<td>4.96 ± 0.89(^{ab})</td>
</tr>
<tr>
<td>( K_{b,av}(g/ml/min/kg) )</td>
<td>26.4 ± 3.7</td>
<td>15.2 ± 1.4(^{ab})</td>
<td>286 ± 17</td>
</tr>
<tr>
<td>( V_{b,lp}(g/ml/min/kg) )</td>
<td>0.86 ± 0.10</td>
<td>0.714 ± 0.089</td>
<td>0.0967 ± 0.017(^{abc})</td>
</tr>
<tr>
<td>( V_{b,lp}(g/ml/min/kg) )</td>
<td>0.0331 ± 0.0032</td>
<td>0.0472 ± 0.0042</td>
<td>0.0573 ± 0.0085(^{ab})</td>
</tr>
</tbody>
</table>

\(^{a}\) Each value represents the mean ± S.E. of three rats.

\(^{b}\) Statistically significant (analysis of variance): * \( P < 0.05 \), ** \( P < 0.1 \), *** \( P < 0.001 \) vs. 0.2 mg/kg/hr; † \( P < 0.2 \), †† \( P < 0.1 \), ††† \( P < 0.001 \), vs. 2 mg/kg/hr.

\(^{c}\) Measured from 55 to 65 min.

\(^{d}\) Calculated by dividing \( V_{b,lp} \) by the infusion rate, \( I \).

\(^{e}\) Calculated by dividing \( V_{b,lp} \) by \( C_{ext,av} \).

\(^{f}\) Calculated by dividing \( V_{b,lp} \) by \( C_{ext} \).

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### Fig. 3

Scatchard plot for serum protein binding of ONO-1301. ONO-1301 was added to the serum side of the dialysis cell to produce final concentrations of 3 to 1000 \( \mu \)M. The dialysis cells were then incubated at 37°C for 6 hr. \( C_b \) and \( C_t \) represent the concentration bound to serum protein and the unbound concentration of ONO-1301, respectively. The ratio of the amount in the cytosol to the total amount in homogenate was 23%. The binding of ONO-1301 to cytosolic protein was studied by gel filtration. The Scatchard plot for the binding of ONO-1301 to cytosolic protein is shown in figure 4. The binding of ONO-1301 to cytosolic protein was studied by gel filtration. The Scatchard plot for the binding of ONO-1301 to cytosolic protein is shown in figure 4.

### Fig. 4

Scatchard plot for cytosol protein binding of ONO-1301. ONO-1301 was added to the cytosol side of the dialysis cell to produce final concentrations of 3 to 1000 \( \mu \)M. The dialysis cells were then incubated at 37°C for 6 hr. \( C_b \) and \( C_t \) represent the concentration bound to cytosol protein and the unbound concentration of ONO-1301, respectively. Each symbol and vertical bar represents the mean ± S.E. of three data points. The solid line represents the fitted line (equation 7).

### Binding to cytosolic protein

The distribution of ONO-1301 between cytosol and other organelles was measured. The ratio of the amount in the cytosol to the total amount in homogenate was 23%. The binding of ONO-1301 to 33% cytosol was quantitated by equilibrium dialysis. Figure 4 shows the Scatchard plot for the binding of ONO-1301 to cytosolic protein. The binding data were fitted to equation 7. For the binding of ONO-1301, saturable and nonsaturable components were observed. The \( K_d \) value was 5.4 \( \mu \)M. The \( n(P) \) value of ONO-1301 in 100% cytosol was estimated to be 117 \( \mu \)M by extrapolating the binding data obtained in diluted cytosol specimens (33% cytosol). The free fraction remained constant (2.50–2.86%) from 3 to 20 \( \mu \)M, but was increased at concentrations >50 \( \mu \)M. The free fraction was estimated to be 4.95 ± 0.60% at a concentration of 600 \( \mu \)M, corresponding to the liver concentration of ONO-1301 in the in vivo study, with the highest infusion rate (20 mg/kg/hr).

### Identification of binding protein in the cytosol

The binding of ONO-1301 to cytosolic protein was studied by gel filtration. The elution pattern for the protein and associated radioactivity is shown in figure 5. The determination of the GST activity coincided with that of the radioactivity, and the protein responsible for binding ONO-1301 in liver cytosol may be ligandin (Sugiyama et al., 1983; Takenaka et al., 1995).

### Measurement of hepatic blood flow

The effect of a constant infusion of ONO-1301 (0.2, 2 and 20 mg/kg/hr) on the hepatic blood flow rate was measured. Before beginning the infusion, the hepatic blood flow rate was 59.1 ml/min/kg. The hepatic blood flow rate was not affected by the infusion of saline but was increased by ONO-1301 in a dose-dependent manner. The hepatic blood flow rate increase reached almost constant (by 30%) at the infusion rate of 2 mg/kg/hr (table 2).

### Initial uptake clearance in vivo (integration plot analysis)

To investigate directly the saturation of hepatic uptake, the time profiles of plasma and liver concentrations of ONO-1301 after intravenous administration of various doses (0.01–25 mg/kg) were analyzed in vivo (fig. 6). The early-phase hepatic uptake clearance (\( C_{I,uptake,in vivo} \)) over the linear range (0.01–1 mg/kg) was 27 to 30 ml/min/kg.
which is close to the hepatic plasma flow rate. CL<sub>uptake,in vivo</sub> decreased as the dose increased and fell to 14.3 ml/min/kg at a dose of 25 mg/kg (fig. 7).

**Isolated rat hepatocytes.** [14C]ONO-1301 uptake by isolated rat hepatocytes increased in a time-dependent manner and a highly concentrative uptake was observed, e.g., the cell-to-medium (C/M) concentration ratio at 5 min was 8000. ONO-1301 exhibited both Na<sup>+</sup>-dependent and -independent uptake (fig. 8). The initial uptake velocity exhibited a concentration dependence, and the Eadie-Hofstee plot of the uptake data is shown in figure 9. The kinetic parameters were as follows: Na<sup>+</sup>-dependent parameters; K<sub>m</sub> = 15.6 ± 5.4 μM, V<sub>max</sub> = 5.9 ± 1.6 nmol/min/mg, P<sub>dir</sub> = 12.9 ± 5.4 μl/min/mg (mean ± computer-calculated S.D.), Na<sup>+</sup>-independent parameters; K<sub>m</sub> = 3.8 ± 0.8 μM, V<sub>max</sub> = 4.8 ± 0.7 nmol/min/mg, I<sub>dir</sub> = 29.3 ± 4.8 μl/min/mg (mean ± computer-calculated S.D.).

Furthermore, the uptake of ONO-1301 was inhibited in the presence of an ATP depletor, such as rotenone (30 μM) and FCCP (2 μM), and was markedly inhibited also by hypothermia (table 3). The effect of the mutual inhibition of ONO-1301 uptake by TCA or pravastatin (Yamazaki et al., 1993a), which are typical substrates of Na<sup>+</sup>/TCA cotransporter and the oatp, respectively, was examined. TCA and pravastatin inhibited the uptake of ONO-1301 both in the presence and absence of external Na<sup>+</sup> in a concentration-dependent manner; however, their inhibitions were only partial (table 3). That is, the highest concentration (500 μM) of TCA and pravastatin, which is more than 10 times the K<sub>m</sub> values for their own uptake, inhibited the ONO-1301 uptake only by 50%. The half-inhibition concentrations of TCA and pravastatin for the inhibitable ONO-1301 uptake were approximately 20 μM and 50 μM, respectively (table 3). On the other hand,

**Fig. 5.** Elution pattern for the binding of ONO-1301 to the liver cytosol of rats. [14C]ONO-1301 was added to 0.5 ml of liver cytosol to produce final concentrations of 10 μM. Specimens were incubated at 37°C for 15 min and then applied to HPLC with a gel filtration column (Asahipak GS-510, 50 cm × 7.6 mm i.d.). The solvent system used was 50 mM potassium phosphate buffer (pH 7.4) at a flow rate of 0.5 ml/min. See details in the text. Solid line, protein absorbance (UV 280 nm); ○: radioactivity of [14C]ONO-1301, 152 ; GST activity.

**Fig. 6.** Dose-dependent change in the “integration plot” for the hepatic uptake of ONO-1301 after intravenous administration to rats. After intravenous administration of [14C]ONO-1301 (•, 0.01 mg/kg; △, 5 mg/kg; □, 25 mg/kg), both plasma concentration (C<sub>p</sub>) time profiles and uptake by the liver were measured and the data expressed as an integration plot (equation 11). The slope represents CL<sub>uptake,in vivo</sub>.

**Fig. 7.** Dose dependence of ONO-1301 uptake clearances (CL<sub>uptake,in vivo</sub>). ONO-1301 was administered at 0.01, 0.2, 1, 5, 10 and 25 mg/kg, and CL<sub>uptake,in vivo</sub> was estimated by integration plot analysis as shown in figure 6.

**Fig. 8.** Time course of ONO-1301 uptake by isolated rat hepatocytes. Uptake of [14C]ONO-1301 was measured by incubating the isolated rat hepatocytes in Krebs-Henseleit buffer (pH 7.3) containing 0.5 μM [14C]ONO-1301 in the presence (□) and absence (●) of Na<sup>+</sup> at 37°C after preincubation for 3 min. Na<sup>+</sup>-dependent ONO-1301 uptake (○) was calculated by subtracting the uptake with choline buffer from the total uptake measured. The uptake value (C/M ratio) means the cellular uptake amount divided by the extracellular concentration. Each symbol and vertical bar represents the mean ± S.E. of nine determinations in three different preparations.

**TABLE 2**

<table>
<thead>
<tr>
<th>Steady State&lt;sup&gt;a&lt;/sup&gt; (%) increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
</tr>
<tr>
<td>0.2 mg/kg/hr</td>
</tr>
<tr>
<td>2 mg/kg/hr</td>
</tr>
<tr>
<td>20 mg/kg/hr</td>
</tr>
</tbody>
</table>

<sup>a</sup> The average (± S.E.) hepatic blood flow rate before infusion was 59.1 ± 2.7 ml/min/kg (n = 20).

<sup>b</sup> Percent increase compared with that before infusion; mean ± S.E. of five rats.

<sup>c</sup> Statistically significant (analysis of variance): * P < .05; ** P < .01 vs. saline.
Fig. 9. Eadie-Hofstee plot of ONO-1301 uptake by isolated rat hepatocytes. Uptake of ONO-1301 was measured at concentrations of 0.2, 0.5, 1, 2, 5, 10, 20, 100 and 500 μM in the absence (Na⁺-independent uptake, ○) and presence of Na⁺. Na⁺-dependent ONO-1301 uptake (●) was calculated by subtracting the uptake with choline buffer from the total uptake measured. Solid (Na⁺-independent uptake) and dotted (Na⁺-dependent uptake) lines represent the fitted line.

TABLE 3
Effects of various inhibitors and hyperthermia on uptake of ONO-1301, and effects of ONO-1301 on the uptake of TCA and pravastatin by isolated rat hepatocytes

<table>
<thead>
<tr>
<th>Uptake</th>
<th>Na⁺-dependent (%)</th>
<th>Na⁺-independent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONO-1301 uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>O°C</td>
<td>1.2 ± 1.2***</td>
<td>5.0 ± 1.6***</td>
</tr>
<tr>
<td>Rotenone, 30 μM</td>
<td>51.5 ± 17.2</td>
<td>47.4 ± 1.8***</td>
</tr>
<tr>
<td>FCCP, 2 μM</td>
<td>82.2 ± 27.9</td>
<td>34.3 ± 2.2***</td>
</tr>
<tr>
<td>ONO-1301 500 μM</td>
<td>13.4 ± 7.2***</td>
<td>4.0 ± 1.4***</td>
</tr>
<tr>
<td>TCA, 20 μM</td>
<td>64.2 ± 17.7#</td>
<td>78.4 ± 3.2</td>
</tr>
<tr>
<td>TCA, 50 μM</td>
<td>109.4 ± 65.7</td>
<td>53.1 ± 5.8***</td>
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<tr>
<td>TCA, 100 μM</td>
<td>71.4 ± 18.9</td>
<td>50.0 ± 2.7***</td>
</tr>
<tr>
<td>TCA, 500 μM</td>
<td>23.4 ± 11.6***</td>
<td>44.3 ± 3.9***</td>
</tr>
<tr>
<td>Pravastatin, 20 μM</td>
<td>33.9 ± 14.2***</td>
<td>95.1 ± 4.9</td>
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<td>Pravastatin, 50 μM</td>
<td>51.3 ± 15.7#</td>
<td>74.1 ± 3.1#</td>
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<tr>
<td>Pravastatin, 100 μM</td>
<td>44.4 ± 20.6***</td>
<td>67.6 ± 2.8***</td>
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<td>Pravastatin, 500 μM</td>
<td>49.4 ± 19.4***</td>
<td>45.5 ± 2.8***</td>
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<td>Pravastatin uptake</td>
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<td>Control</td>
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<td>100</td>
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<td>Pravastatin, 500 μM</td>
<td>16.8 ± 1.1***</td>
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<td>ONO-1301, 1 μM</td>
<td>87.1 ± 4.1</td>
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<td>ONO-1301, 10 μM</td>
<td>40.3 ± 3.1***</td>
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<td>ONO-1301, 100 μM</td>
<td>6.5 ± 1.1***</td>
<td></td>
</tr>
<tr>
<td>ONO-1301, 500 μM</td>
<td>4.6 ± 2.0***</td>
<td></td>
</tr>
<tr>
<td>TCA uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>TCA, 500 μM</td>
<td>4.3 ± 0.6***</td>
<td>6.9 ± 3.3***</td>
</tr>
<tr>
<td>ONO-1301, 1 μM</td>
<td>90.2 ± 4.0</td>
<td>95.6 ± 13.5</td>
</tr>
<tr>
<td>ONO-1301, 10 μM</td>
<td>44.7 ± 3.5**</td>
<td>60.4 ± 6.1**</td>
</tr>
<tr>
<td>ONO-1301, 100 μM</td>
<td>9.2 ± 1.8***</td>
<td>28.8 ± 5.0***</td>
</tr>
<tr>
<td>ONO-1301, 500 μM</td>
<td>2.5 ± 0.7***</td>
<td>19.2 ± 3.3***</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± S.E. of six determinations in two different preparations.
** Statistically significant (analysis of variance), * P < .05, ** P < .01, *** P < 0.001 vs. control.

hand, ONO-1301 inhibited the uptake of TCA and pravastatin almost completely, and the half-inhibition concentration of ONO-1301 was approximately 10 μM (table 3).

Estimation of hepatic uptake clearance from the in vitro uptake data. Based on the kinetic parameters obtained from in vitro experiments, the PS_{influx,in vitro} was calculated from equation 12 as 9400 ml/min/kg. Considering that the free fraction of ONO-1301 was 0.018, f_a · PS_{influx,in vitro} was calculated as 171 ml/min/kg. This value was five times that of the hepatic plasma flow rate (32.5 ml/min/kg), which is the product of the hepatic blood flow rate (59.1 ml/min/kg) and the hematocrit (0.45), which suggests that the hepatic uptake is blood-flow rate-limited. Actually, the CL_{uptake,in vivo} estimated from equations 13 to 15 was 31.5 ml/min/kg, which is close to the hepatic plasma flow rate and agrees with the value (29 ml/min/kg) obtained from the in vivo study at a dose of 0.01 mg/kg (fig. 7).

Discussion

The hepatobiliary transport of ONO-1301 was analyzed kinetically in rats in vivo. During intravenous infusion of this compound, CL_{tot} at steady state decreased as the infusion rate increased and exhibited nonlinearity (table 1). After a single intravenous administration of [14C]ONO-1301, most of the total radioactivity from the injected dose was recovered in the bile (H. Imawaka, unpublished data). These results suggest that hepatic uptake, hepatic metabolism and biliary excretion may be important factors governing the disposition of this drug.

Both CL_{tot} and E_{H} decreased as the infusion rate increased. At 2 mg/kg/hr infusion, CL_{tot} decreased to only approximately 90% of the CL_{tot} at 0.2 mg/kg/hr, whereas the decrease in E_{H} was approximately 50%. The decrease was more marked for E_{H} than CL_{tot} at 20 mg/kg/hr (8% and 30% of that at 0.2 mg/kg/hr, respectively) (table 1). This phenomenon may be caused by an increase in hepatic blood flow rate with ONO-1301 administration. Therefore, to investigate the effect of ONO-1301 on the hepatic blood flow rate, Q_{B} was measured directly with a blood flow meter under ether anesthesia. Ether anesthesia affects both blood flow as well as drug metabolism (Watkins and Klaassen, 1983). It is doubtful whether the blood flow rate observed in this experiment is the physiological hepatic blood flow rate. However, all in vivo experiments (in vivo infusion study and initial uptake clearance in vivo) were done under the same conditions of anesthesia. As for hepatic blood flow rate, the dose-dependent effect of ONO-1301 can be discussed. Before beginning the ONO-1301 infusion, the hepatic blood flow rate was 59.1 ml/min/kg, similar to reported values (Nagata et al., 1990; Yokota et al., 1976). After intravenous infusion of ONO-1301, the hepatic blood flow rates increased. The increase in hepatic blood flow rate at infusion rates of 0.2, 2 and 20 mg/kg/hr was 21%, 31% and 34%, respectively (table 2). Such an increase in hepatic blood flow rate may be one reason why CL_{tot} did not decrease as much as E_{H}. However, there may be another reason, because the decrease in CL_{tot} at 2 mg/kg/hr compared with 0.2 mg/kg/hr was smaller than the corresponding decrease in hepatic clearance (CL_{H} = Q_{B} · E_{H}).

In general, CL_{H} is expressed as a function of Q_{B}, f_{u} and CL_{int,all}. Where f_{u} is the blood unbound fraction, and CL_{int,all} represents the overall intrinsic clearance which includes not only metabolism and biliary excretion but also membrane permeability, as described by the following equation (Miyachi et al., 1987; Pang and Gillette 1978):

\[ CL_{int,all} = \frac{PS_{int} \cdot CL_{int,met} + CL_{int,bile}}{PS_{eff} + CL_{int,met} + CL_{int,bile}} \]  

where, PS_{int} and PS_{eff} represent the membrane permeability clearance of unbound ligand for the influx and efflux pro-
cesses, respectively, and $\text{CL}_{\text{int,met}}$ and $\text{CL}_{\text{int,bile}}$ represent the "exact" intrinsic clearances for metabolism and biliary excretion of the unbound ligand, respectively. From equation 16, possible explanations for the decrease in $C_L_{\text{Q}}$ as the infusion rate increases are: 1) saturation of hepatic uptake ($\text{PS}_{\text{eff}}$), 2) saturation of biliary excretion ($\text{CL}_{\text{int,bile}}$), and 3) saturation of metabolism ($\text{CL}_{\text{int,met}}$). After intravenous administration of $[^{14\text{C}}]$ONO-1301 at various doses, the fraction of the metabolites to the total radioactivity in plasma and bile did not change with dose (H. Imawaka, unpublished data). Therefore, saturation of metabolism may not have occurred.

To clarify the possibility that saturation of hepatic uptake and biliary excretion might occur, various pharmacokinetic parameters at steady state were analyzed. The biliary excretion clearance based on the plasma concentration of ONO-1301 ($\text{CL}_{\text{bile,plasma}}$, 0.86 ml/min/kg) was much smaller than $\text{CL}_{\text{H}}$ (12.2 ml/min/kg) at 0.2 mg/kg/hr, which suggests that the biliary excretion did not contribute so much to the decrease in hepatic clearance of ONO-1301. Although $\text{CL}_{\text{bile,plasma}}$ decreased as the infusion rate increased, the clearances based on the liver concentration ($\text{CL}_{\text{bile,liver}}$) increased. We, therefore, suggest that the decrease in $\text{CL}_{\text{H}}$ was not caused by saturation of biliary excretion. The increase in $\text{CL}_{\text{bile,liver}}$ with increasing infusion rate might be explained by saturation of the tissue binding of ONO-1301, because the hepatic concentration of ONO-1301 (150 $\mu$M) at 2 mg/kg/hr, was higher than the concentration of ligandin (50–100 $\mu$M) (Sathirakul et al., 1993; Arias et al., 1976), which was identified as the cytosolic binding protein of ONO-1301 by gel filtration (fig. 5). The finding that the binding capacity for the high-affinity binding is approximately 100 $\mu$M (fig. 4) also supports this idea.

In addition to these considerations, the decrease in $K_p$ as well as in $E_{\text{H}}$ with increasing infusion rate suggests that the nonlinear pharmacokinetics are caused by saturation of hepatic uptake. The $K_p$ of this compound at the lowest infusion rate is large, 26, despite extensive plasma protein binding (98% binding, fig. 3). Such a high $K_p$ may be explained by two possible mechanisms; one is carrier-mediated active hepatic uptake as mentioned above, and/or more extensive binding to intracellular proteins than to plasma proteins. The binding to cytosolic proteins (ligandin as the major binding protein, fig. 5) was quantitated with 33% cytosol (fig. 4). From the binding parameters ($K_d$, $n(P)$), obtained, the binding of ONO-1301 to undiluted cytosol (100% cytosol) over the linear binding range was estimated from equation 7. The extrapolated percentage binding (97%) was close to the binding to plasma proteins (98%). Consequently, a high $K_p$ value of 26 cannot be accounted for only by tissue binding, but carrier-mediated active uptake must also be considered.

To investigate directly the saturation of hepatic uptake, the time profiles of plasma and liver concentrations of ONO-1301 after intravenous administration at various doses (0.01–25 mg/kg) were analyzed in vivo. The early-phase hepatic uptake clearance ($\text{CL}_{\text{uptake,in vivo}}$) over the linear range (0.01–1 $\mu$g/kg) was approximately 29 ml/min/kg (fig. 7), which is close to the hepatic plasma flow rate (32.5 ml/min/kg, table 2). $\text{CL}_{\text{uptake,in vivo}}$ decreased as the dose increased and saturation of the uptake was observed (fig. 7). Such a nonlinearity in $\text{CL}_{\text{H}}$ and the $K_p$ value at steady state (table 1) could thus be attributed to saturation of hepatic uptake.

Furthermore, by use of isolated rat hepatocytes, the analysis of the kinetics in the Na$^+$- dependent and -independent uptake provided one saturable component with a $K_m$ value of 15.6 $\mu$M (Na$^+$-dependent) and 3.8 $\mu$M (Na$^+$-independent), a $V_{\text{max}}$ value of 5.9 nmol/min/mg (Na$^+$-dependent) and 4.8 nmol/min/mg (Na$^+$-independent) and nonspecific diffusion. From the kinetic parameters obtained, the uptake clearance ($\text{PS}_{\text{influx,in vitro}}$) in the presence of an external Na$^+$ (corresponding to physiological conditions) was calculated to be 9400 ml/min/kg from equation 12. Furthermore, based on the in vitro parameters ($f$, $\text{PS}_{\text{influx,in vitro}}$), $\text{CL}_{\text{uptake,in vivo}}$ was estimated from equations 13 to 15 to be 31.5 ml/min/kg, which is close to the hepatic plasma flow rate and agrees with the result of the in vivo study at a dose of 0.01 mg/kg (29 ml/min/kg, fig. 6). Although the results from both in vitro and in vivo studies indicate that the hepatic uptake clearance is close to the hepatic plasma flow rate, the $E_{\text{H}}$ value at steady-state was 0.31 (much lower than 1). As understood easily from equation 16, this can be explained by the hypothesis that the $\text{PS}_{\text{eff}}$ value is much larger than the sum of the $\text{CL}_{\text{int,met}}$ and $\text{CL}_{\text{int,bile}}$ values.

We investigated the uptake mechanism of ONO-1301 with isolated rat hepatocytes. Both Na$^+$-dependent and -independent uptake was observed. The uptake characteristics of ONO-1301, i.e., highly concentrative (equilibrium C/M ratio about 8000) (fig. 8), temperature dependent and sensitive to ATP depletors (table 3), demonstrate that the hepatic uptake of ONO-1301 is mediated by both a Na$^+$-dependent and -independent carrier mediated active transport system. ONO-1301 is an organic anion with a carboxyl group and is an agonist specifically bound to PG receptors, hence, hepatic uptake processes may be mediated by the transporters (Ntcp, epoxide hydrolase, oatp or PGT) described in the introduction. To investigate which transporter is responsible for the hepatic uptake of ONO-1301, the mutual inhibition of hepatic uptake was examined.

The uptake of ONO-1301 both in the presence and absence of external Na$^+$ was inhibited by TCA and pravastatin (a typical non-bile acid organic anion) only partly. However, the half-inhibition concentrations of TCA and pravastatin for the inhibitable ONO-1301 uptake were 29 and 50 $\mu$M, and were comparable with the $K_m$ values for the uptake of TCA ($K_m = 12$ (Na$^+$-dependent), 57 (Na$^+$-independent) $\mu$M, Anwer and Hegner, 1978) and pravastatin ($K_m = 29$ $\mu$M, Yamazaki et al., 1993a) themselves, respectively (table 3). ONO-1301 also inhibited the uptake of TCA and pravastatin almost completely, and the half-inhibition concentration of ONO-1301 was approximately 10 $\mu$M (table 3), which agreed well with the $K_m$ values (4–7 $\mu$M) of ONO-1301 uptake (fig. 9). These mutual inhibition studies suggest that the hepatic uptake of ONO-1301 may be mediated at least partly by Na$^+$-dependent TCA transporters and Na$^+$-independent oatp. In addition, we cannot exclude the possibility of a contribution from PGT.

To clarify the quantitative contribution of Ntcp, epoxide hydrolase, oatp and PGT to ONO-1301 uptake by hepatocytes, detailed kinetic studies involving the analysis of the mutual inhibition pattern in isolated rat hepatocytes and the mammalian cells to which these transporters are transfected are necessary and are currently underway in our laboratory.

Acknowledgments

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References


