Human Liver-Specific Organic Anion Transporter, LST-1, Mediates Uptake of Pravastatin by Human Hepatocytes

DAISUKE NAKAI, RIE NAKAGOMI, YOSHITAKE FURUTA, TARO TOKUI, TAKAAKI ABE, TOSHIHIKO IKEDA, and KENJI NISHIMURA

Drug Metabolism and Pharmacokinetics Research Laboratories, Sankyo Co., Ltd., Tokyo, Japan (D.N., R.N., Y.F., T.T., T.I., K.N.); and Department of Neurophysiology, Tohoku University School of Medicine, Sendai, Japan (T.A.)

Received October 19, 2000; accepted January 2, 2001. This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

Involvement of LST-1 (a human liver-specific transporter, also called OATP2) as the major transporter in the uptake of pravastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, by human liver was demonstrated. The hepatic uptake of pravastatin evaluated using human hepatocytes was Na⁺-independent and reached saturation with a Michaelis constant (Kₘ) of 11.5 ± 2.2 μM. The uptake of pravastatin was temperature-dependent and was inhibited by estradiol-17β-D-glucuronide, taurocholic acid, bromosulfophthalein, and simvastatin acid, but not by p-aminohippurate. Estradiol-17β-D-glucuronide competitively inhibited pravastatin uptake with an inhibition constant comparable to the Kₘ value for estradiol-17β-D-glucuronide transport, indicating that a common transporter mediates the transport of pravastatin and estradiol-17β-D-glucuronide in human hepatocytes. The results obtained with human hepatocytes agreed with those obtained with LST-1 expressing Xenopus oocytes. Oocytes microinjected with human liver polyadenylated mRNA showed Na⁺-independent uptake of pravastatin and estradiol-17β-D-glucuronide. A simultaneous injection of LST-1 antisense oligonucleotides completely abolished this uptake. Expression of LST-1 was immunohistochemically demonstrated in the human hepatocytes, but not in Hep G2 cells, which showed very low uptake of pravastatin. Therefore, LST-1 was regarded as a key molecule for pravastatin in liver-specific inhibition of cholesterol synthesis, making pravastatin accessible to the target enzyme, which would otherwise not be inhibited by this hydrophilic drug.

Pravastatin, a water-soluble 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, has been shown to inhibit cholesterol synthesis in vivo specifically in the liver (Koga et al., 1986; Teujin et al., 1990), which is the major site of cholesterol synthesis. Since the adverse effects of HMG-CoA reductase inhibitors during long-term treatment seem to depend in part upon the degree to which they act in extrahepatic tissues (Scott et al., 1989), the inhibitory effect on this enzyme restricted to the liver tissue is pharmacologically and toxicologically of great interest. Pravastatin is reported to be taken up by the liver efficiently through some kind of transport system (Hamelin and Turgeon, 1998), causing liver-specific inhibition of cholesterol synthesis in vivo. Furthermore, due to the hydrophilic nature of pravastatin, it is present only in low levels in other tissues and organs (Hamelin and Turgeon, 1998). This hydrophilicity causes low cellular uptake of pravastatin, as seen by the lack of inhibition of HMG-CoA reductase activity by pravastatin in Hep G2 cells (Cohen et al., 1993), the human hepatoma cell line, in which the hepatic transport activity for pravastatin is absent (Ziegler et al., 1994). These results indicate the importance of the hepatic transport system for pravastatin to exert its liver-specific pharmacological effect.

The hepatic transport system in the basolateral membrane is responsible for the clearance of various endogenous and exogenous substances from the systemic circulation (Meier, 1988; Tiribelli et al., 1990). The uptake of anionic compounds is mediated by Na⁺-dependent and Na⁺-independent systems (Berk et al., 1987; Tiribelli et al., 1990). Na⁺-taurocholic acid cotransporting polypeptides cloned from rat (Hagenbuch et al., 1991) and human (Hagenbuch and Meier, 1994) mediate the uptake of bile acids in a Na⁺-dependent manner. In addition to the Na⁺-dependent transport system, taurocholic acid is also transported via a Na⁺-independent carrier, a so-called multispecific anion transporter. This Na⁺-independent system transports a broad spectrum of substrates, including steroid conjugates, cardiac glycosides, and other xenobiotics (Müller and Jansen, 1997).

Previously, we demonstrated that pravastatin is taken up actively by rat hepatocytes through a Na⁺-independent multispecific anion transporter (Komai et al., 1992; Yamazaki et al., 1993) and that cloned organic anion transporting

ABBREVIATIONS: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; OATP2, organic anion transporting polypeptide 2; LST-1, human liver-specific organic anion transporter; KHL, keyhole limpet hemocyanin; Kₘ, Michaelis constant; Vₘₜₐₓ, maximum uptake rate; Pₛₜₜ, nonspecific uptake clearance; Kᵢ, inhibition constant.
polypeptide 2 (oatp2) is the transporter responsible for the active hepatocellular uptake of pravastatin in rats (Abe et al,. 1998; Tokui et al,. 1999). A previous study of inhibition of sterol synthesis using various human cells suggested that pravastatin is also taken up by human hepatocytes via liver-specific transporter(s) (van Vliet et al,. 1995). However, the uptake characteristics of pravastatin by human hepatocytes have not been investigated yet.

Recently, we also isolated a novel human liver-specific organic anion transporter, LST-1, which transports a wide variety of endogenous and exogenous anionic compounds into hepatocytes and is expressed exclusively in the liver (Abe et al,. 1999). LST-1 has a moderate sequence homology to both the organic anion transporter polypeptide family and the prostaglandin transporter. The overall amino acid sequence homology is 42.2% with human OATP (Kullak-Ublick et al,. 1999) and 34.9% with human prostaglandin transporter (Lu et al,. 1996). LST-1 transports taurocholic acid, conjugated steroids (dehydroepiandrosterone sulfate, estradiol-17β-D-glucuronide, and estrone-sulfate), eicosanoids (prostaglandin E2, thromboxane B2, leukotriene C4, and leukotriene E4), and thyroid hormones in a Na+-independent manner, which demonstrates its multispecificity (Abe et al,. 1999). As the hepatic expression level of human OATP is negligible (Abe et al,. 1999), transport by LST-1 is believed to be the principal mechanism for Na+-independent clearance of bile acids and organic anions in the human liver. Hsiang et al,. (1999) also reported the identical transporter to be OATP2 and the pravastatin transport by OATP2 expressed in 293c18 cells. König et al,. (2000) also demonstrated by immunohistochemistry that LST-1 (OATP2) was localized to the basolateral membrane of human hepatocytes. However, whether LST-1 was the major transporter in the hepatocellular uptake of pravastatin in humans was still unclear.

To estimate the involvement of LST-1 in pravastatin transport in human liver, we investigated the uptake characteristics of pravastatin using human hepatocytes, Xenopus oocytes injected with human liver polyadenylated mRNA or cRNA derived from LST-1, and Hep G2 cells.

**Experimental Procedures**

**Materials.** [14C]Pravastatin (specific activity: 14.3 mCi/mmole) and [3H]pravastatin (specific activity: 44.6 Ci/mmole) were synthesized at Amersham Japan (Tokyo, Japan). The radiochemical purity of [14C]pravastatin and [3H]pravastatin, determined by high performance liquid chromatography, was 99 and 96%, respectively. [3H]Estradiol-17β-D-glucuronide and [3H]taurocholic acid were purchased from NEN Life Science Products (Boston, MA). Simvastatin acid was synthesized at Sankyo (Tokyo, Japan). Cryopreserved human hepatocytes were purchased from In Vitro Technology (Baltimore, MD) and Tissue Transformation Technologies (Edison, NJ). Hep G2 cells were purchased from American Type Culture Collection (Rockville, MD). Percoll was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Polyadenylated mRNA from human liver was purchased from Clontech Laboratories (Palo Alto, CA). Antisense oligonucleotides against LST-1 were synthesized at Amersham Japan (Tokyo, Japan). All other chemicals used were of reagent grade.

**Animals.** Mature Xenopus laevis females were purchased from Hamamatsu Kyozai (Hamamatsu, Japan) and maintained in a controlled environment (Goldin, 1992). All experiments using Xenopus laevis were approved by the Ethical Committee of Sankyo for Animal Experiments.

**Uptake Experiments.** Cryopreserved human hepatocytes 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 (pH 7.4) containing 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, saturated with O2/CO2 (95/5). Viability of the cells was verified by the Trypan Blue exclusion test, and the cells with >90% viability were used. The cell suspension was preincubated at 37°C for 5 min. Then, the radiolabeled compounds were added to start the uptake experiment. At designated times, the incubation mixture was centrifuged, and the cells were transferred through a silicone oil layer to the alkaline solution layer, which thus terminated the uptake reaction (Yamazaki et al,. 1993). After the cells were solubilized in the alkaline layer, the bottom of the tube containing the solubilized cell layer was sliced off with a razor blade and transferred into a vial for liquid scintillation counting. After addition of 10 ml of 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. To examine whether pravastatin uptake by the human hepatocytes was sodium-dependent, 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 NaCl and NaHCO3 were replaced with choline chloride and choline bicarbonate, respectively. Hep G2 cells were cultured in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum, 1 mM pyruvate, and penicillin/streptomycin (100 U/100 μg/ml). Approximately 106 Hep G2 cells were seeded in a 24-well plate 24 h before the uptake study. The cells were then washed with prewarmed, serum-free medium, and uptake studies were performed. The cells were incubated with a radiolabeled compound in a CO2 incubator for 1 h, washed with ice-cold phosphate-buffered saline, and lysed with 0.1 N NaOH. After solubilization, the radioactivity was determined using a Packard TriCarb 2200 CA liquid scintillation analyzer (Packard Instrument, Meriden, CT). Protein concentrations were determined using a commercially available protein assay kit (Bio-Rad, Richmond, CA).

**Expression of LST-1 in Xenopus laevis Oocytes.** In vitro synthesis of LST-1-cRNA was performed using the cloned cDNA of LST-1, as described previously (Abe et al,. 1999). Xenopus laevis oocytes were prepared according to a procedure described previously (Tokui et al,. 1999). The oocytes were injected with 50 ng of polyadenylated human liver mRNA, with 50 ng of transcribed cRNA of LST-1, or with the same volume of water for the control. After injection, the oocytes were cultured for 3 days at 18°C, with daily replacements of the modified Barth’s solution (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO3, 0.3 mM Ca(NO3)2, 0.41 mM CaCl2, 0.82 mM MgSO4, and 15 mM HEPES, pH 7.6). Hybrid-depletion experiments were performed using antisense oligonucleotides of LST-1 (5’-TTGATTTTGCTTCA-3’, position 1–15). Fifty nanograms of polyadenylated human liver mRNA was incubated for 15 min at 42°C with 5.0 μg antisense oligonucleotides of LST-1. Samples were then cooled on ice and injected into oocytes.

**Uptake by Oocytes.** Uptake experiments were started by incubating the oocytes at room temperature in 100 μl of either the sodium-free or sodium-containing uptake buffer (100 mM choline chloride or NaCl, respectively, with 10 mM HEPES/5 mM Tris, pH 7.5, 1 mM KCl, 1 mM CaCl2, and 2 mM MgCl2). At the indicated intervals, uptake was terminated by the addition of 3 ml of ice-cold incubation buffer, and the oocytes were washed three times with the same ice-cold buffer. The water-injected oocytes were used as the control. A single oocyte was solubilized in 0.5 ml of 10% (w/v) sodium dodecyl sulfate, and 4 ml of scintillation fluid (Pico Fluor, Packard Bioscience) was added. The radioactivity was determined using a Packard TriCarb 2200 CA liquid scintillation analyzer.
Preparation of Rabbit Antibodies against Human LST-1. A peptide containing 12 amino acids (CNLDMQDNAAAN, position 691–702) at the carboxy-terminus of human LST-1 was synthesized and linked to maleimide-activated keyhole limpet hemocyanin (KHL; Pierce, Rockford, IL). The KHL-linked peptide (1 mg/injection) was emulsified by mixing with an equal volume of Freund’s adjuvant and injected into the foot pads of female rabbits. Booster injections were performed at 2, 6, and 8 weeks, and the animals were sacrificed at 10 weeks. The antibodies were affinity-purified using CNBr-activated Sepharose CL-4B (Amersham Pharmacia Biotech, Uppsala, Sweden) coupled with synthetic peptides according to a standard procedure (Shigemoto et al., 1994).

Immunohistochemistry. Air-dried smears of human hepatocytes and Hep G2 cells were fixed for 10 min in 10% formaldehyde in phosphate-buffered saline and permeabilized by one cycle of freeze-thawing. Treated cells were incubated with the antibody raised against LST-1 (diluted in DAKO Antibody Diluent, DAKO, Carpinteria, CA) for 60 min at room temperature. Immunostaining was performed using a commercially available immunostaining kit (Nichirei, Tokyo, Japan). Nuclei were stained with hematoxylin after the immunostaining.

Determination of Kinetic Parameters. The kinetic parameters for pravastatin uptake were calculated according to the following equation:

$$V_0 = V_{\text{max}} \cdot S/(K_m + S) + P_{\text{dif}} \cdot S$$

where $V_0$ is the initial uptake rate (pmol/min/10^6 cells), $V_{\text{max}}$ is the maximum uptake rate (pmol/min/10^6 cells), $K_m$ is the Michaelis constant ($\mu$M), $P_{\text{dif}}$ is the nonspecific uptake clearance ($\mu$l/min/10^6 cells), and $S$ is the pravastatin concentration in the medium ($\mu$M). The data collected in the uptake experiments were fitted to the above equation by an iterative nonlinear least-squares method using WinNonlin (version 1.1, Scientific Consulting, Inc., Cary, NC). The apparent kinetic parameters ($K_m^{\text{app}}$, $V_{\text{max}}^{\text{app}}$, and $P_{\text{dif}}^{\text{app}}$) for pravastatin uptake in the presence of estradiol-17$\beta$-D-glucuronide were also estimated by fitting the data to eq. 1. The $V_{\text{max}}^{\text{app}}$ and $P_{\text{dif}}^{\text{app}}$ values for pravastatin thus obtained were practically the same as those in the absence of estradiol-17$\beta$-D-glucuronide, whereas the $K_m^{\text{app}}$ value was increased with the addition of estradiol-17$\beta$-D-glucuronide. Thus, the inhibition constant ($K_i$) was calculated according to the following equation, assuming competitive inhibition:

$$K_i = K_m \cdot i/(K_m^{\text{app}} - K_m)$$

where $K_m$ is the Michaelis constant for pravastatin uptake in the absence of estradiol-17$\beta$-D-glucuronide ($\mu$M), $K_m^{\text{app}}$ is the Michaelis constant for pravastatin uptake in the presence of estradiol-17$\beta$-D-glucuronide ($\mu$M) and $i$ is the estradiol-17$\beta$-D-glucuronide concentration in the medium ($\mu$M). The LST-1-mediated uptake rate of pravastatin was calculated by subtracting the uptake in water-injected oocytes from that in LST-1 cRNA-injected oocytes. The kinetic parameters were calculated according to the following equation:

$$V_0 = V_{\text{max}} \cdot S/(K_m + S)$$

where $V_0$ is the initial uptake rate (pmol/min/10^6 cells), $V_{\text{max}}$ is the maximum uptake rate (pmol/min/oocyte), $K_m$ is the Michaelis constant ($\mu$M), and $S$ is the pravastatin concentration in the medium ($\mu$M). The uptake data were fitted to the above equation by an iterative nonlinear least-squares method using WinNonlin (version 1.1, Scientific Consulting, Inc.). All obtained kinetic parameters are shown as mean ± S.E.

**Results**

Uptake of $[^{14}\text{C}]$Pravastatin, $[^{3}\text{H}]$Estradiol-17$\beta$-D-glucuronide, and $[^{3}\text{H}]$Taurocholic Acid by Human Hepatocytes. The time course of uptake of $[^{14}\text{C}]$pravastatin (10 $\mu$M), $[^{3}\text{H}]$estra
diol-17$\beta$-D-glucuronide (10 $\mu$M), and $[^{3}\text{H}]$taurocholic acid (10 $\mu$M) by human hepatocytes is shown in Fig. 1. Pravastatin uptake increased linearly up to at least 5 min. The uptake of estradiol-17$\beta$-D-glucuronide and taurocholic acid increased linearly up to 120 s. Thus, the initial uptake rate was calculated by linear regression using the six data points collected between 0.5 and 5 min for pravastatin
The uptake of $[^{14}C]$pravastatin, $[^{3}H]$estradiol-17β-D-glucuronide, and $[^{3}H]$taurocholic acid became saturated with increasing concentrations of the substrate in the medium (Fig. 2). The kinetic parameters of pravastatin, estradiol-17β-D-glucuronide, and taurocholic acid were 11.5 ± 2.2 µM (n = 3), 14.0 ± 6.5 µM (n = 3), and 25.5 ± 5.0 µM (n = 3), respectively, for $K_m$, 10.2 ± 2.6 pmol/min/10⁶ cells (n = 3), 59.3 ± 38.9 pmol/min/10⁶ cells (n = 3), and 77.0 ± 25.3 pmol/min/10⁶ cells (n = 3), respectively, for $V_{max}$; and 0.30 ± 0.14 µl/min/10⁶ cells (n = 3), 0.23 ± 0.06 µl/min/10⁶ cells (n = 3), and 0.16 ± 0.08 µl/min/10⁶ cells (n = 3), respectively, for $P_{diff}$.

The uptake of $[^{14}C]$pravastatin and $[^{3}H]$estradiol-17β-D-glucuronide was Na⁺-independent as shown in Table 1. On the other hand, taurocholic acid uptake showed Na⁺ dependence (Table 1). The fraction of Na⁺-independent taurocholic acid uptake was 20% of the total uptake (n = 3). The pravastatin uptake exhibited a remarkable temperature dependence, decreasing to 20.0 ± 4.8% at 0°C, compared with that at 37°C (n = 3).

To characterize the pravastatin uptake by human hepatocytes, the effect of organic anions on the uptake of $[^{14}C]$pravastatin was examined. The uptake of pravastatin was inhibited by taurocholic acid, simvastatin acid, and bromosulfophthalein, but not by $\gamma$-aminohippurate, a substrate for renal organic anion exchanger OAT1 (Sekine et al., 1997) as shown in Table 2. In the presence of estradiol-17β-D-glucuronide (20 µM), the apparent $K_m$ of pravastatin increased by 2.6 times, compared with the $K_m$ in the absence of estradiol-17β-D-glucuronide. However, the apparent $V_{max}$ and $P_{diff}$ values did not change significantly. This result demonstrates that estradiol-17β-D-glucuronide competitively inhibits the uptake of pravastatin. According to eq. 2, the $K'_o$ of estradiol-17β-D-glucuronide in inhibiting pravastatin uptake was calculated to be 13.9 ± 4.0 µM (n = 3).

### Table 1

<table>
<thead>
<tr>
<th>Uptake Rate</th>
<th>Na⁺-Independent Ratio</th>
<th>Choline⁺/Na⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmol/min/10⁶ cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pravastatin</td>
<td>10.5 ± 4.3</td>
<td>13.5 ± 6.1</td>
</tr>
<tr>
<td>E₂-17G</td>
<td>31.3 ± 8.4</td>
<td>39.5 ± 14.4</td>
</tr>
<tr>
<td>TCA</td>
<td>23.7 ± 3.1</td>
<td>47.0 ± 6.6</td>
</tr>
</tbody>
</table>

E₂-17G, estradiol-17β-D-glucuronide; TCA, taurocholic acid.

### Table 2

<table>
<thead>
<tr>
<th>Pravastatin Uptake</th>
<th>Human Hepatocytes</th>
<th>LST-1-Expressing Oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>+TCA</td>
<td>24.5 ± 8.8</td>
<td>19.7 ± 3.9</td>
</tr>
<tr>
<td>+SVA</td>
<td>6.5 ± 5.8</td>
<td>16.0 ± 1.3</td>
</tr>
<tr>
<td>+BSP</td>
<td>4.7 ± 2.6</td>
<td>13.6 ± 2.0</td>
</tr>
<tr>
<td>+PAH</td>
<td>12.0 ± 8.6</td>
<td>118.0 ± 4.9</td>
</tr>
</tbody>
</table>

TCA, taurocholic acid; SVA, simvastatin acid; BSP, bromosulfophthalein; PAH, $\gamma$-aminohippurate.
Effect of ion substitution and LST-1 antisense inhibition of the uptake of [3H]pravastatin (2.21 μM) and [3H]estradiol-17β-d-glucuronide (0.76 μM) in oocytes injected with human liver polyadenylated mRNA

Uptake was measured in the medium containing sodium chloride [Na⁺ (+)], or choline chloride [Na⁺ (−)] (see Experimental Procedures). Antisense (+) indicates the uptake into the oocytes after the coinjection of LST-1 antisense oligonucleotides and polyadenylated human liver mRNA. They were preincubated for 15 min at 42°C before the injection. Data are expressed as the mean ± S.E. of 4 to 15 uptake measurements.

<table>
<thead>
<tr>
<th>Uptake Conditions</th>
<th>Substrates</th>
<th>Na⁺</th>
<th>Antisense</th>
<th>Pravastatin</th>
<th>E2-17G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>+</td>
<td>−</td>
<td>0.011 ± 0.001</td>
<td>0.29 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Poly(A) mRNA</td>
<td>+</td>
<td>−</td>
<td>0.022 ± 0.001</td>
<td>0.40 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Poly(A) mRNA</td>
<td>−</td>
<td>−</td>
<td>0.028 ± 0.003</td>
<td>0.38 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Poly(A) mRNA</td>
<td>+</td>
<td>−</td>
<td>0.011 ± 0.000</td>
<td>0.31 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Concentration dependence of the uptake of [14C]pravastatin (A) and [3H]estradiol-17β-d-glucuronide (B) in oocytes injected with LST-1 cRNA. ○, LST-1 cRNA-injected oocytes; ●, water-injected oocytes. Uptake was measured in the medium containing sodium chloride (see Experimental Procedures). Data are expressed as the mean ± S.E. of three to seven uptake measurements in one representative preparation of three separate oocyte preparations. The solid line represents the least-squares fit of the data to eq. 3.

Fig. 5. Concentration dependence of the uptake of pravastatin (●) and estradiol-17β-d-glucuronide (○) by Hep G2 cells. Data are expressed as the mean ± S.E. of three uptake measurements.

Discussion

The active transport of pravastatin by human hepatocytes has been demonstrated in the present study. In the human hepatocytes, the uptake of pravastatin became saturated with increasing pravastatin concentrations (Fig. 2), and was Na⁺-independent (Table 1) and temperature-dependent. This indicates that pravastatin uptake by human hepatocytes is a transporter-mediated and energy-requiring process. The uptake of pravastatin was inhibited by estradiol-17β-d-glucuronide, taurocholic acid, simvastatin acid, and bromosulfophthalein, but not by p-aminohippurate (Table 2).
All these characteristics of pravastatin uptake by human hepatocytes were in good agreement with those by rat hepatocytes (Yamazaki et al., 1993). However, the $V_{\text{max}}$ value for pravastatin was about 30 times lower in the human hepatocytes than in the rat hepatocytes (Yamazaki et al., 1993), suggesting lower transporter activity in humans than in rats and/or a decrease in the transporter activity of human hepatocytes after cryopreservation (De Loecker et al., 1990).

In the same manner as above, the uptake of estradiol-17β-D-glucuronide and taurocholic acid by human hepatocytes became saturated with increasing substrate concentrations (Fig. 2). The uptake of estradiol-17β-D-glucuronide was Na$^+$-independent, whereas that of taurocholic acid was Na$^+$-dependent (Table 1). The $K_m$ values were 25.5 ± 5.0 μM for taurocholic acid (Fig. 2), which was lower than the values reported previously (Azer and Stacy, 1993: 45.7 μM; Sandker et al., 1994: 62 μM) and was 14.6 ± 6.5 μM for estradiol-17β-D-glucuronide (Fig. 2). This is the first observation and report for Na$^+$-independent uptake of estradiol-17β-D-glucuronide by human hepatocytes. The kinetic analysis demonstrates that estradiol-17β-D-glucuronide competitively inhibits pravastatin uptake by human hepatocytes (Fig. 3). The $K_i$ value of estradiol-17β-D-glucuronide in the inhibition of the pravastatin uptake was comparable to the $K_m$ value for the hepatocellular uptake of estradiol-17β-D-glucuronide, suggesting that a common transporter is responsible for the uptake of both pravastatin and estradiol-17β-D-glucuronide.

Oocytes expressing LST-1 showed essentially the same characteristics of pravastatin uptake as those observed in human hepatocytes. The uptake of both $[^{14}\text{C}]$pravastatin and $[^3\text{H}]$estradiol-17β-D-glucuronide in LST-1 cRNA-injected oocytes was Na$^+$-independent and became saturated with increasing concentrations (Fig. 4). The $K_m$ values were 13.7 ± 4.0 μM for pravastatin and 9.7 ± 2.0 μM for estradiol-17β-D-glucuronide, which were comparable to those obtained from the uptake study using human hepatocytes. The inhibitory
effects of organic anions on pravastatin uptake in the LST-1-expressing oocytes were also similar to those in the human hepatocyte (Table 2).

Our hybrid-depletion study demonstrates that the uptake of pravastatin and estradiol-17β-D-glucuronide by the human liver is via LST-1. The Na+-independent uptake of [3H]pravastatin and [3H]-estradiol-17β-estradiol by oocytes microinjected with human liver polyadenylated RNA, as well as in human hepatocytes (Tables 1 and 3). The LST-1 antisense oligonucleotide completely abolished the Na+-independent uptake of [3H]pravastatin and [3H]-estradiol-17β-estradiol into the oocytes (Table 3). Thus, LST-1 plays a predominant role in the uptake of pravastatin and estradiol-17β-estradiol in this experimental system. Because the contribution of transporters, which are not expressed in oocytes microinjected with human liver polyadenylated RNA, cannot be ruled out, further studies, for example, an inhibition study using an LST-1 transporter activity neutralizing antibody, may need to be performed.

A previous in vitro study on HMG-CoA reductase inhibition (Cohen et al., 1993) suggests the importance of LST-1 for the pharmacological action of pravastatin in the target cells. In Hep G2 cells, a frequently used cell model of human hepatocytes, the inhibitory effect of pravastatin on the cholesterol synthesis was much less potent than those of other lipophilic HMG-CoA reductase inhibitors, simvastatin and lovastatin, although, in the cell homogenates, the inhibitory effects of the three inhibitors were very similar (Cohen et al., 1993). In contrast, in primary cultured human hepatocytes, the inhibitory effect of pravastatin was comparable to that of simvastatin (Cohen et al., 1993). Hep G2 cells were immuno-histochemically demonstrated to express no LST-1, and failed to show saturable uptake at increasing concentrations of pravastatin or estradiol-17β-D-glucuronide (Figs. 5 and 6). Their uptake by Hep G2 cells was considered to proceed by simple, passive diffusion. These clearly indicated that the transporter activity regulated by LST-1 made pravastatin accessible to the HMG-CoA reductase, which would otherwise not be inhibited by this hydrophilic molecule.

In conclusion, LST-1 has been demonstrated to be involved as the major transporter in active, Na+-independent uptake of pravastatin by human hepatocytes. Furthermore, since cells that do not express LST-1 showed no inhibitory effect of HMG-CoA reductase by pravastatin, we conclude that LST-1 is the key molecule for the liver-specific inhibition of cholesterol synthesis by pravastatin in humans.

Acknowledgments

We thank Dr. K. Fukuda for helpful suggestions in the immunohistochemical study.

References


Send reprint requests to: Dr. Taro Tokui, Drug Metabolism and Pharmacokinetics Research Laboratories, Sankyo Co., Ltd., 2-58 Hiromachi, Shima-gawa-kou, Tokyo, 140-8710, Japan. E-mail: tokui@shina.sankyo.co.jp