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Title page

Title:

Involvement of Human Organic Anion Transporting Polypeptide OATP-B (SLC21A9) in pH-Dependent Transport across Intestinal Apical Membrane

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Running title page

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Anion Transport by OATP-B in Human Intestine

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Abbreviations:

BSP, sulfobromophthalein; DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; OATP, organic anion transporting polypeptide.

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Abstract

Some organic anions are absorbed from the gastrointestinal tract through carrier-mediated transport mechanism(s), which may include proton-coupled transport, anion exchange transport, and others. However, the molecular identity of the organic anion transporters localized at the apical membrane of human intestinal epithelial cells has not been clearly demonstrated. In the present study, we focused on human organic anion transporting polypeptide OATP-B and examined its subcellular localization and functionality in the small intestine. Localization of OATP-B was determined by immunohistochemical analysis. Transport properties of estrone-3-sulfate and the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor pravastatin by OATP-B-transfected HEK293 cells were measured. OATP-B was immunohistochemically localized at the apical membrane of intestinal epithelial cells in human. Uptake of [^{3}H]estrone-3-sulfate and [^{14}C]pravastatin by OATP-B at pH 5.5 was higher than that at pH 7.4. [^{3}H]Estrone-3-sulfate transport was decreased by pravastatin, aromatic anion compounds and the anion exchange inhibitor DIDS, but not by small anionic compounds, such as lactic acid and acetic acid. The inhibitory effect of pravastatin on the uptake of [^{3}H]estrone-3-sulfate was concentration-dependent, and the IC_{50} value was 5.5 mM. The results suggested that OATP-B mediates absorption of anionic compounds and its activity may be optimum at the acidic surface microclimate pH of the small intestine. Accordingly, OATP-B plays a role in the absorption of anionic compounds across the apical membrane of human intestinal epithelial cells, although it cannot be decisively concluded that pH-dependent absorption of pravastatin is determined by OATP-B alone.

Members of the organic anion transporting polypeptide (OATP) family are involved in the transport of various endogenous and xenobiotic compounds, such as conjugated metabolites of steroid hormones, thyroid hormones, bile acids, bilirubin, pravastatin, benzylpenicillin and digoxin, and this family is classified as the solute carrier family 21A (SLC21A). So far, 9, 11 and 8 members of the OATP family have been reported in human, rat and mouse, respectively (Hagenbuch and Meier, 2003), though the orthologs of rat or mouse Oatps to human OATPs have not yet been fully clarified (Tamai et al., 2000a). To rationalize the accumulating sequence information on the OATP transporter family, it is essential to clarify the functional roles and pharmacological relevance of each OATP member.

In liver, OATP-B (SLC21A9), OATP-C (SLC21A6, LST1/OATP2) and OATP8 (SLC21A8) are expressed at the basolateral membrane (Kullak-Ublick et al., 2001; König et al., 2000a, 2000b). Among them, OATP-C and OATP8 exhibit specific expression in liver, while OATP-B is expressed in several tissues, including small intestine and liver (Tamai et al., 2000a; Kullak-Ublick et al., 2001). Compared with OATP-C and OATP8, which accept various anionic compounds as substrates, OATP-B has a higher substrate specificity, but as well as transporting the physiological substrates estrone-3-sulfate and dehydroepiandrosterone sulfate, it also mediates the uptake of the xenobiotic sulfobromophthalein (BSP) (Tamai et al., 2000a, 2001a; Kullak-Ublick et al., 2001). Since substrates of OATP-B overlap with those of other OATPs, the role of OATP-B in liver is currently uncertain. Furthermore, it is known that mRNAs of OATP-B, OATP-D (SLC21A11) and OATP-E (SLC21A12) are expressed in human intestine (Tamai et al., 2000a), whereas their roles remain to be clarified. In rat, it was reported that Oatp3 was localized at the apical membrane of intestinal epithelial cells (Walters et al., 2000). Recently, it was reported that fruit juices decreased the absorption of the antihistamic drug fexofenadine in human, and fexofenadine might be absorbed via intestinal OATP transporters (Dresser et al., 2002). These reports suggested that members of

the OATP family might mediate absorption of anionic compounds, including both physiological and xenobiotic compounds, in human small intestine.

The 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitor pravastatin is a water-soluble drug used to treat hypercholesterolemia. It shows a more selective pharmacological effect on liver compared with more lipid-soluble HMG-CoA reductase inhibitors, and this tissue-selectivity may be ascribed to selective tissue distribution (Koga et al., 1990). In spite of the hydrophilicity of pravastatin, it is absorbed to the extent of about 30% after oral administration in healthy volunteers (Singhvi et al., 1990; Pan, 1991), and we suggested involvement of a pH-dependent transporter in the intestinal apical membrane transport of pravastatin (Tamai et al., 1995a). One of the liver-specific OATPs, OATP-C, mediates the uptake of pravastatin by human hepatocytes (Hsiang et al., 1999; Nakai et al., 2001), while it is not clear whether OATP-B transports pravastatin or not. Accordingly, it is possible that OATP family members expressed in human small intestine may mediate the absorption of pravastatin.

We have previously demonstrated that intestinal apical membranes exhibit carrier-mediated transport activity for several organic anions, including acetic acid (Tsuji et al., 1990; Simanjuntak et al., 1991), benzoic acid (Tsuji et al., 1994), lactic acid (Tamai et al., 2000b), nicotinic acid (Simanjuntak et al., 1990; Tanakanaga et al., 1996), pravastatin (Tamai et al., 1995a), and salicylic acid (Tanakanaga et al., 1994) through pH-dependent anion exchange and/or proton-coupled transport mechanisms. We also showed that organic anion transporters expressed in intestinal epithelial cells, including anion exchanger (AE2) (Yabuuchi et al.,1998) and monocarboxylate transporter (MCT1) (Tamai et al., 1995b, 1999; Takanaga et al., 1995) transport some of these organic anions through anion exchange and pH-dependent processes, respectively, while pravastatin was not transported by these transporters. Therefore, it is possible that these transporters play at least a part in the

intestinal absorption of organic anions, and additional transporters may be functional in the intestinal apical membrane (Tamai et al., 2000b).

Rat Oatp3 is expressed in the intestinal apical membrane (Walters et al., 2000), and rat Oatp1 apparently exhibited pH-dependent activity in the transport of BSP and taurocholate (Kanai et al., 1996; Satlin et al., 1997). Based on these previous observations, it was hypothesized that human OATP-B expressed in small intestine might mediate the intestinal absorption of anionic compounds via a pH-dependent mechanism. Accordingly, in the present study we examined the intestinal subcellular localization and functionality of OATP-B by using a typical substrate, estrone-3-sulfate, and a clinically used drug, pravastatin, as model compounds.

Methods

Materials

Pravastatin and [^{14}C]pravastatin sodium salt (529.1 MBq/mmol) were kindly supplied by Sankyo Co., Ltd. (Tokyo, Japan). [^{3}H]Estrone-3-sulfate, ammonium salt (1609.5 GBq/mmol) was purchased from PerkinElmer Life Science Products, Inc. (Boston, MA). pcDNA3 vector was obtained from Invitrogen (Carlsbad, CA). Human adult normal small intestinal tissue slides were purchased from Biochain Institute Inc. (Hayward, CA). HEK293 cells were obtained from Health Science Research Resources Bank (Tokyo, Japan). All other reagents were purchased from Sigma Chemicals (St. Louis, MO) and Wako Pure Chemical Industries (Osaka, Japan).

Immunohistochemical Study of OATP-B in Human Small Intestine

Immunohistochemical staining was performed as described elsewhere (Tamai et al., 2001b) with minor modifications. Paraffin-embedded sections from human small intestine were processed for immunoperoxidase and immunofluorescence staining. Rabbit polyclonal anti-OATP-B antiserum was prepared as described previously by using a synthesized carboxyl-terminal polypeptide of OATP-B with the amino acid sequence CLVSGPGKKPEDSRV as the epitope (Nozawa et al., 2002). Sections were deparaffinized in xylene, rehydrated through a graded series of ethanol, and incubated for 1 h with polyclonal anti-OATP-B antiserum diluted 1:1000 in phosphate buffer containing 1.5% goat IgG (Vector Laboratories, Burlingame, CA) or rabbit normal IgG. Immunoperoxidase staining was performed using a VECTASTAIN Elite ABC-PO kit (Vector Laboratories) and 3,3'-diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries), as the chromogen (brown), in the presence of 0.006% hydrogen peroxide. Methyl green (LABVISION Corporation, Fremont, CA) and VectaMount mounting medium (Vector

Laboratories) were used for nuclear staining and fixation of sections, respectively. For immunofluorescence staining, tissue sections were incubated with Alexa Fluoro™ 594 goat anti-rabbit IgG (Molecular Probes, Inc., Eugene, OR), as the secondary antibody, for 30 min after incubation with primary antibody. Then, they were mounted in VECTASHIELD mounting medium with DAPI (Vector Laboratories) to fix the sample and to stain nuclei. The specimens were examined with a CK40-RFL microscope (Olympus, Tokyo, Japan) and the images were captured with a Penguin 150CL (Pixera Corporation, Los Gotos, CA).

Transport Experiments

For the transport experiments using HEK293 cells, the construct pcDNA3/OATP-B was used to transfect HEK293 according to the calcium phosphate precipitation method. HEK293 cells were routinely grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, penicillin, and streptomycin in a humidified incubator at 37°C under 5% CO_2. After cultivation of HEK293 cells for 24 h in 15 cm dishes, pcDNA/OATP-B or pcDNA vector alone was transfected by adding 20 μg of the plasmid DNA per dish. At 40-48 h after transfection, the cells were harvested and suspended in the transport medium containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 12 mM $MgSO_4$, and 25 mM HEPES, adjusted to pH 7.4. The cell suspension was preincubated at 37°C for 20 min in the transport medium (pH 7.4), then it was centrifuged and the resultant cell pellets were mixed with the uptake medium (pH 5.5 to 7.4) containing a radio-labeled compound to initiate uptake. Uptake medium (pH 5.5 or 6.0) contained 125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 12 mM $MgSO_4$, and 25 mM 2-(N-morpholino)ethanesulfonic acid, adjusted to pH 5.5 or 6.0 with HEPES. Uptake medium (pH 6.5 to 7.4) contained 125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 12 mM $MgSO_4$, and 25 mM HEPES, adjusted to pH 6.5 to 7.4 with

tris(hydroxymethyl)aminomethane. At appropriate times, 160 µL aliquots of the mixture were withdrawn and the cells were separated from the transport medium by centrifugal filtration through a layer of a mixture of silicone oil (SH550, Toray Dow Corning Co., Tokyo) and liquid paraffin (Wako Pure Chemical Industries) with a density of 1.03. Each cell pellet was solubilized in 3 N KOH and then neutralized with HCl. Then, the cell-associated radioactivity was measured by means of a liquid scintillation counter using Cleasol-1 as a liquid scintillation fluid (Nacalai tesque, Kyoto, Japan). HEK293 cells transfected with pcDNA3 vector alone were used to obtain the background activity (termed mock).

Analytical Methods

Cellular protein content was determined according to the method of Bradford (Bradford, 1976) by using a BioRad protein assay kit (Hercules, CA) with bovine serum albumin as the standard. Usually, initial uptake rates were obtained by measuring the uptake at 10 min. The uptake activity of OATP-B by HEK293 cells was evaluated after subtracting the uptake by mock-transfected cells from total uptake by the OATP-B-expressing cells.

All data were expressed as means ± S.E.M., and statistical analysis was performed by using Student's *t* test. Cell-to-medium ratio was obtained by dividing the uptake amount by the cells by the concentration of test compound in the uptake medium. The criterion of significance was taken to be $P < 0.05$.

Results

Immunohistochemical Localization of OATP-B in Human Small Intestine

To clarify the role of OATP-B in human small intestine, immunohistochemical analysis was performed using human intestinal tissue slices. Figures 1a, 1b, 1c and 1d show the results of immunoperoxidase staining, and figures 1e and 1f show the immunofluorescence findings. The signal of OATP-B appears as brown or red in the immunoperoxidase or immunofluorescence method, respectively. Figures 1a and 1e show that OATP-B is localized at the luminal surface of enterocytes in human small intestine. As shown in figure 1c at high magnification, immunoreactivity for OATP-B was localized at the apical membrane of enterocytes. Goblet cells were not stained by anti-OATP-B antiserum. In the control experiment with rabbit normal IgG, the brown or red signal was negligible (figures 1b, 1d, and 1f). These results clearly demonstrate that OATP-B is localized at the apical membrane of enterocytes in human small intestine.

Effect of pH on [^{3}H]Estrone-3-sulfate Uptake by OATP-B

The effect of pH on the initial uptake of [^{3}H]estrone-3-sulfate was examined by changing the extracellular pH over the range of 5.5 to 7.4. As shown in figure 2, the uptake of [^{3}H]estrone-3-sulfate was increased at acidic pH in OATP-B-expressing cells, while the uptake by mock-transfected cells was significantly lower and was not affected by pH. OATP-B-specific uptake of [^{3}H]estrone-3-sulfate, obtained by subtracting the uptake by mock-transfected cells from that by OATP-B-expressing cells, at pH 5.5 was more than 2-fold higher than that at pH 7.4. Accordingly, it was established that OATP-B shows pH-dependent activity, and the following studies were performed mainly at pH 5.5, which is the physiological luminal surface pH, at which OATP-B shows high transport activity.

Inhibitory Effects of Various Compounds on OATP-B-Specific Uptake of [^{3}H]Estrone-3-sulfate

To characterize the pH-dependence of transport by OATP-B, we examined the inhibitory effects of various compounds on OATP-B-specific uptake of [^{3}H]estrone-3-sulfate at pH 5.5. Concentrations of inhibitors were chosen to compare the results in the previous studies on the inhibitory effects on pravastatin uptake examined by rabbit brush-border membrane vesicles (Tamai et al., 1995a). As shown in Table 1, monocarboxylic acids (pravastatin, benzoic acid and nicotinic acid) and a dicarboxylic acid (phthalic acid) significantly decreased the uptake of [^{3}H]estrone-3-sulfate by OATP-B, while other monocarboxylic acids (acetic acid and lactic acid), a dicarboxylic acid (oxalic acid) and a tricarboxylic acid (citric acid) were not significantly inhibitory. Furthermore, 1 mM DIDS, a potent anion exchange inhibitor, markedly reduced the uptake of [^{3}H]estrone-3-sulfate by OATP-B. Accordingly, it was suggested that OATP-B has higher affinity for relatively bulky anions. The affinity of pravastatin for OATP-B was evaluated in terms of the concentration dependence of the inhibitory effect of pravastatin on the uptake of [^{3}H]estrone-3-sulfate by OATP-B (figure 3). The inhibitory effect was indeed concentration-dependent, and the evaluated IC_{50} value was 5.5 ± 1.1 mM.

Time Course of [^{14}C]Pravastatin Uptake by OATP-B

To clarify whether pravastatin is a substrate for OATP-B or not, the uptake of [^{14}C]pravastatin by OATP-B-expressing HEK293 cells was examined. Figure 4 shows the time course of the uptake of [^{14}C]pravastatin by HEK293 cells expressing OATP-B at pH 5.5. Uptake of [^{14}C]pravastatin by OATP-B-expressing cells was higher than that by mock-transfected cells. The OATP-B-mediated steady-state uptake was 13.4 ± 2.1 μL/mg protein, obtained after subtraction of the uptake by mock-transfected cells. Since the

intracellular space of HEK293 cells is 6.3 μL/mg protein (Tamai et al., 1997), [^{14}C]pravastatin is apparently accumulated concentratively within the cells. However, we could not reliably characterize pravastatin transport other than effects of pH (Figure 5) in the present study due to the relatively high background uptake.

Effect of pH on [^{14}C]Pravastatin Uptake by OATP-B

As shown in figure 5, uptake of [^{14}C]pravastatin was significantly increased in OATP-B-expressing cells at acidic pH compared to that in mock-transfected cells, while no significant increase in the uptake of [^{14}C]pravastatin by OATP-B was observed at neutral pH. Uptake of [^{14}C]pravastatin by mock-transfected cells at pH 5.5 was also higher than that at neutral pH. Since the pKa value of pravastatin is 4.7, the apparent increase in [^{14}C]pravastatin uptake by mock-transfected cells at acidic pH seems likely to be due to an increase of the nonionic form of pravastatin, leading to increased diffusion according to the pH partition hypothesis, and/or it may reflect the involvement of an unknown pH-dependent transporter. The absence of pH dependence in estrone-3-sulfate transport by mock-transfected cells shown in figure 2 may be due to the low pKa value (less than 2), which makes the observation of the transport by pH-partition hypothesis difficult, and/or the lack of expression of the pH-dependent transporter for estrone-3-sulfate in mock-transfected HEK293 cells.

Discussion

Although extensive studies on OATP transporters have identified the presence of large numbers of them in mice, rats and humans, the orthologous molecules among species remain to be fully established. Accordingly, it is important to characterize the physiological roles of each member based on the functional characteristics, tissue expression profiles, and regulation mechanism of expression, and to compare them among transporter molecules. Here, we have further characterized human OATP-B by focusing on its role in the small intestine, since no precise information is available on intestinally expressed OATPs in human, though rat Oatp3 is expressed at the intestinal epithelial apical membrane (Walters et al., 2000) and the presence of a transport system for pravastatin and fexofenadine, which are substrates of some OATPs, was suggested in small intestine (Tamai et al., 1995; Dresser et al., 2002).

The present study clarified by immunohistochemical analysis that in human small intestine, OATP-B is localized at the apical membrane of enterocytes (figure 1). To our knowledge, OATP-B is the first OATP molecule shown to be expressed at the apical membrane of human enterocytes, while further studies on the expression level and the regional variation of the expression along the small intestine should be essential to clarify the functional relevance of OATP-B in the absorption of anionic compounds in human. In rat, Oatp3 is localized at the apical membrane of jejunal epithelial cells (Walters et al., 2000) and the localization of OATP-B is the same as that of rat Oatp3. However, amino acid sequence identity between OATP-B and Oatp3 is low (34%) (Hagenbuch and Meier, 2003) and rat Oatp3 may not be an ortholog of OATP-B; indeed, Walters et al. proposed that rat Oatp3 is a homologue of OATP-A (Walters et al., 2000). Nishio et al. cloned moat-1 from rat brain and showed that it has a higher homology (76%) with OATP-B than any other cloned human OATP (Nishio et al., 2000). However, it has not been clarified yet whether OATP-A or moat-1 is localized at the apical membrane of the small intestine or not. In liver, OATP-B is localized at the

basolateral membrane (Kullak-Ublick et al., 2001) in the same manner as OATP-C (König et al., 2000a) and OATP8 (König et al., 2000b), and OATP-B is also localized at the basal membrane of human placental trophoblast cells (St-Pierre et al., 2002). This differential localization of OATP-B among small intestine, liver and placenta is interesting, because the observation suggests the presence of an organ-specific sorting mechanism. Similarly, one of the OATP superfamily, rat Oatp1, is localized at the apical membrane in kidney and at the basolateral membrane in liver (Bergwerk et al., 1996).

We showed that OATP-B mediates pH-dependent transport of estrone-3-sulfate and pravastatin (figures 2 and 5), and this is the first experimental demonstration that human OATP has pH-dependent functionality. It was reported that the substrate specificity of OATP-B is narrower than those of OATP-A, OATP-C and OATP8 at neutral pH (Kullak-Ublick et al., 2001). However, OATP-B might show broader substrate specificity at acidic pH, judging from the results for pravastatin in the present study. We previously showed that intestinal apical membranes are equipped with a proton-coupled transport system for pravastatin, using rabbit intestinal apical membrane vesicles (Tamai et al., 1995a). These observations strongly suggested that OATP-B is involved in the intestinal apical membrane transport of pravastatin. The Km value of pravastatin uptake by the membrane vesicles at pH 5.5 (15.2 mM) (Tamai et al., 1995a) was similar to IC_{50} value of pravastatin for the uptake of estrone-3-sulfate by OATP-B (5.5 mM, figure 3). Uptake of pravastatin by the membrane vesicles was inhibited by monocarboxylic acids and DIDS, but not by di- or tricarboxylic acids (Tamai et al., 1995a). In the present study, uptake of estrone-3-sulfate by OATP-B was similarly affected by several monocarboxylic acids, but not by acetic acid, though it showed a tendency to have an inhibitory effect (table 1). Accordingly, OATP-B has affinity for monocarboxylic compounds, but shows a lower affinity for acetic acid. Phthalic acid, which is a dicarboxylic acid, also showed an inhibitory effect on OATP-B-mediated uptake of

estrone-3-sulfate, although it did not inhibit the uptake of pravastatin by rabbit intestinal apical membrane vesicles (Tamai et al., 1995a). Therefore, the affinity of OATP-B may depend on the size of compounds rather than the number of carboxylic acid moieties. The inhibitory effect of 1 mM DIDS on OATP-B (8.4 % of control, table 1) is stronger than that observed in rabbit apical membrane vesicles (74.9 % of control) (Tamai et al., 1995a). Since DIDS is a potent inhibitor of anion exchange rather than proton-gradient-stimulated uptake (Tamai et al., 2000b), OATP-B might be an anion exchange transporter that shows apparent pH dependence, like rat Oatp1, which shows bicarbonate/anion exchange transport (Satlin et al., 1997), while further studies would be needed as to whether OATP-B is an anion exchanger or not. Accordingly, OATP-B seems to have similar, though not identical, characteristics to those observed in rabbit intestinal apical membrane vesicles. The difference in the selectivity of inhibitors between OATP-B and rabbit intestinal apical membrane vesicles may be due to the difference of test compounds (estrone-3-sulfate in the present study and pravastatin in the previous rabbit membrane vesicle study), species difference or the presence of other transporter(s) for pravastatin in rabbit small intestine.

Figures 4 and 5 showed that pravastatin was transported at acidic pH by OATP-B. This is the first report that pravastatin is a substrate of a transporter localized at the apical membrane of enterocytes, though OATP-B did not significantly transport pravastatin at neutral pH (figure 5). Accordingly, it is suggested that transport activity of pravastatin may be low at the basolateral membrane of liver or lower part of the intestine, where OATP-B is expressed, because the pH in these regions is not so acidic. In a study of healthy subjects, pravastatin was mainly absorbed from the duodenum (Triscari et al., 1995), and this report supports the idea that OATP-B plays a role in the intestinal transport of pravastatin.

A recent report suggested that intestinal OATP might be an important determinant of the absorption of the antihistamic drug, fexofenadine (Dresser et al., 2002). The report showed

that fruit juices decreased the AUC and Cmax of fexofenadine in man after an oral administration (Dresser et al., 2002). Since the AUC and Cmax of fexofenadine were decreased after administration of antacids, aluminium/magnesium hydroxide (Product information, Aventis Pharma Ltd., Tokyo, Japan), the absorption of fexofenadine also may be regulated by intestinal pH, like that of pravastatin (Tamai et al., 1995), though there is no information as to whether OATP-B has affinity for fexofenadine or is sensitive to fruit juices. One report showed that grapefruit juice did not significantly affect the AUC value of pravastatin, despite a tendency to decrease the AUC and Cmax (Lilja et al., 1999). Further study is needed to clarify the absorption mechanism of pravastatin and fexofenadine.

In conclusion, the present study showed that OATP-B might play a role in the pH-dependent intestinal absorption of anionic drugs across the apical membrane of human intestinal epithelial cells. It will be important to elucidate the in vivo contribution of OATP-B to the intestinal absorption of drugs by further clarifying the driving force and substrate selectivity of OATP-B at acidic pH, where its activity is optimum.

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Footnotes

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Figure legends

Figure 1

Immunohistochemical localization of OATP-B in human small intestine.

Sections of human small intestine were incubated with the anti-OATP-B antiserum (1,000-fold dilution, a,c,e) or rabbit normal IgG (b,d,f). Expression of OATP-B is indicated by brown or red coloration with the immunoperoxidase method (a-d) or the immunofluorescence method (e,f), respectively. OATP-B was localized at the luminal (L) surface (a,e) and the apical membrane (c) of epithelial cells of small intestine, but not in goblet cells (arrowhead). Each bar represents 50 μm.

Figure 2

Effect of extracellular pH on uptake of [^{3}H]estrone-3-sulfate by OATP-B.

Uptake of [^{3}H]estrone-3-sulfate (4.82 nM) by OATP-B cDNA- (closed circles) or mock-transfected HEK293 cells (squares) was measured for 10 min. The cells were preincubated for 20 min at 37°C in the incubation buffer (pH 7.4). Open circles represent OATP-B specific uptake of [^{3}H]estrone-3-sulfate obtained by subtracting the uptake by mock-transfected cells from that by OATP-B cDNA-transfected cells. Uptake was expressed as cell-to-medium ratio. Each result represents the mean and S.E.M. (n=3 or 4) and (*) indicates a significant difference from the uptake by mock-transfected cells. (p<0.05)

Figure 3

Inhibitory effect of pravastatin on the uptake of [^{3}H]estrone-3-sulfate by OATP-B.

OATP-B cDNA- or mock-transfected cells were preincubated for 20 min at 37°C in the uptake buffer (pH 7.4). Uptake of [^{3}H]estrone-3-sulfate (4.76 nM) was measured for 10 min at 37°C by incubating the cells in the uptake buffer (pH 5.5), and the results are shown as

percentage of control uptake measured in the absence of inhibitor after correcting for the uptake by mock-transfected cells. Each column represents the mean and S.E.M. (n=3 or 4) and (*) indicates a significant difference from the control (p<0.05)

Figure 4

Time courses for the uptake of [^{14}C]pravastatin by OATP-B.

Uptake of [^{14}C]pravastatin (14.7 μM) was measured over 15 min using OATP-B cDNA- (closed circles) or mock-transfected cells (squares) at 37°C in the incubation buffer (pH 5.5). Open circles represent OATP-B-specific [^{14}C]pravastatin uptake obtained by subtraction of the uptake by mock-transfected cells from that by OATP-B cDNA-transfected cells. Uptake was expressed as cell-to-medium ratio. Each result represents the mean and S.E.M. (n=3 or 4) and (*) indicates a significant difference from uptake by mock-transfected cells. (p<0.05)

Figure 5

Effect of extracellular pH on the uptake of [^{14}C]pravastatin by OATP-B

Uptake of [^{14}C]pravastatin (14.7 μM) was measured for 10 min using OATP-B cDNA- (closed circles) or mock-transfected cells (squares). The cells were preincubated for 20 min at 37°C in the incubation buffer (pH 7.4). Open circles represent OATP-B-specific [^{3}H]pravastatin uptake obtained by subtraction of the uptake by mock-transfected cells from that by OATP-B cDNA-transfected cells. Uptake was expressed as cell-to-medium ratio. Each result represents the mean and S.E.M. (n=3 or 4) and (*) indicates a significant difference from the uptake by mock-transfected cells. (p<0.05)

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Figure 1

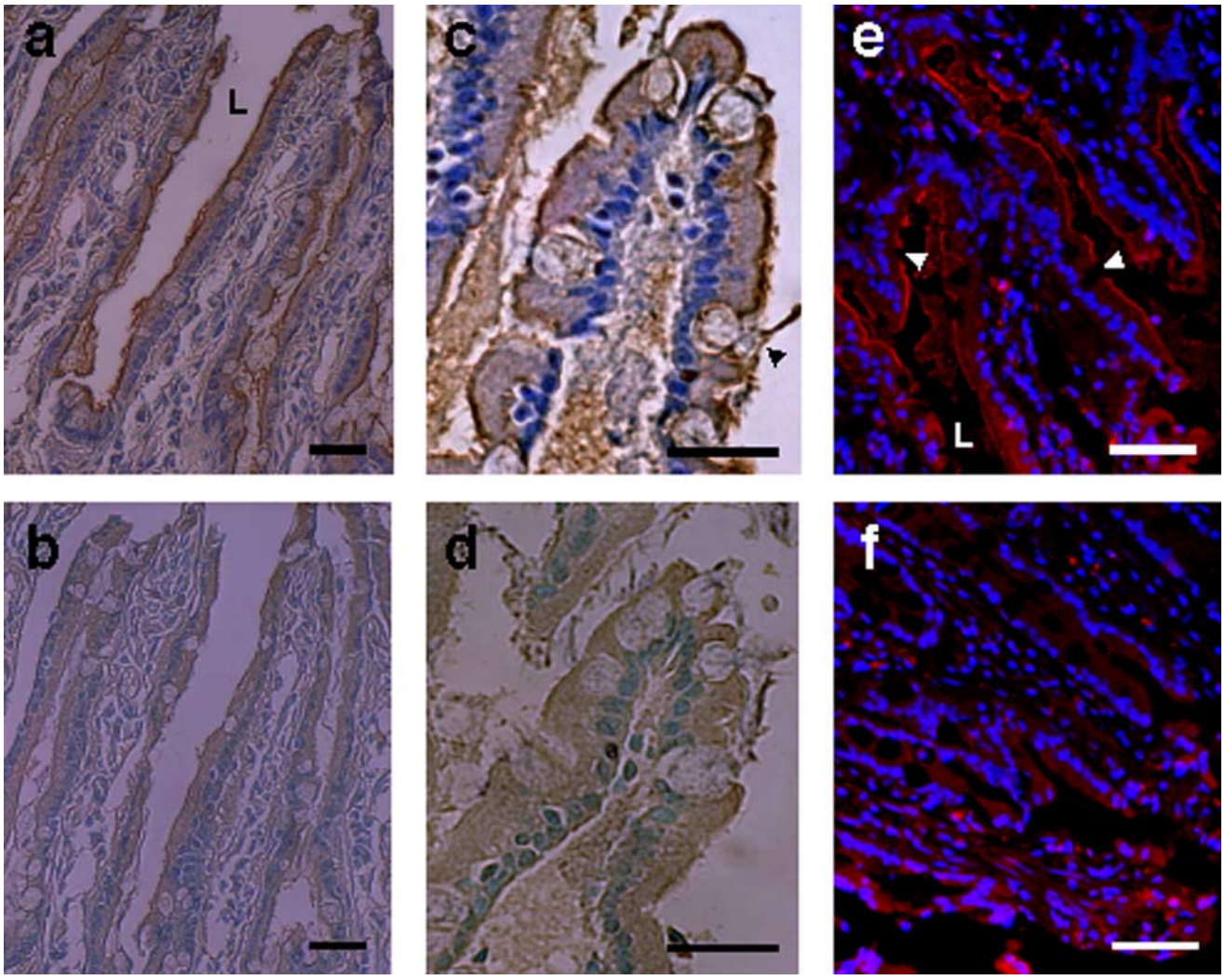

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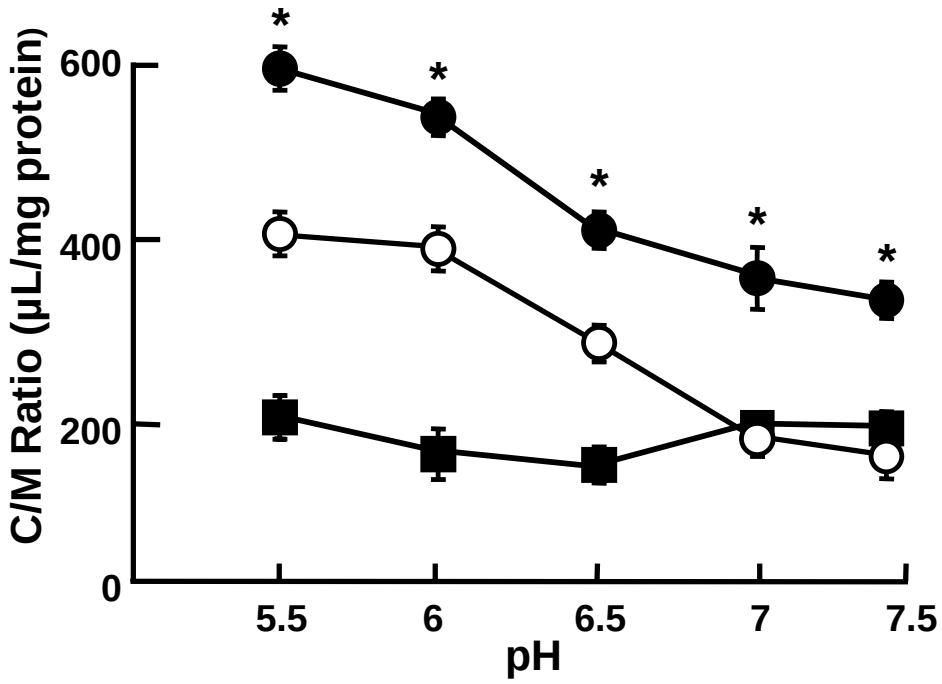
Figure 2



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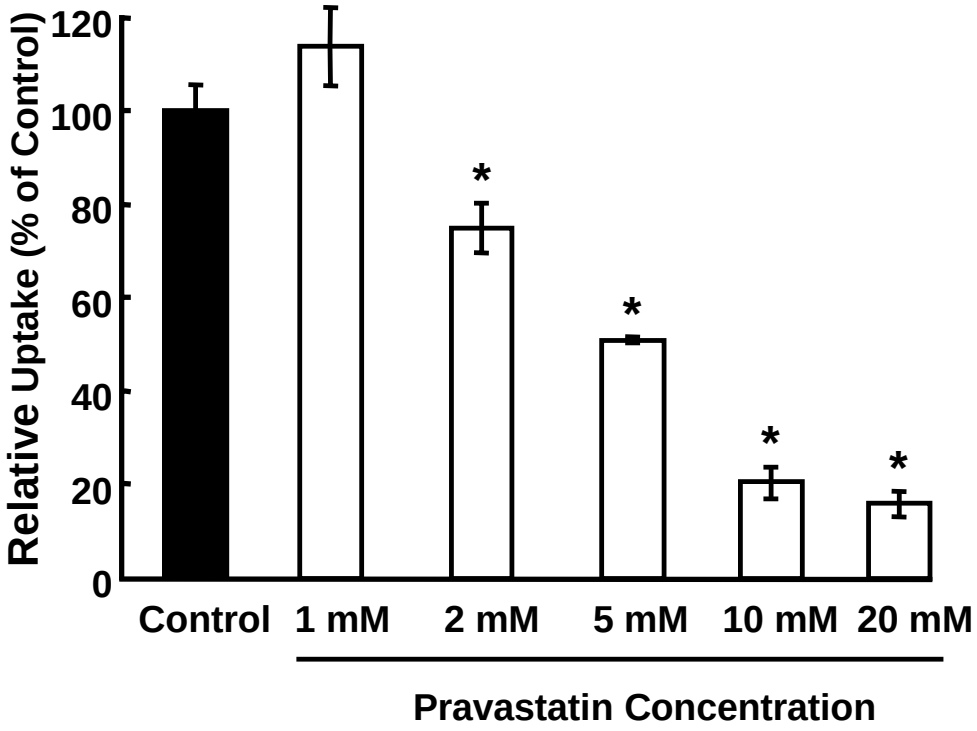

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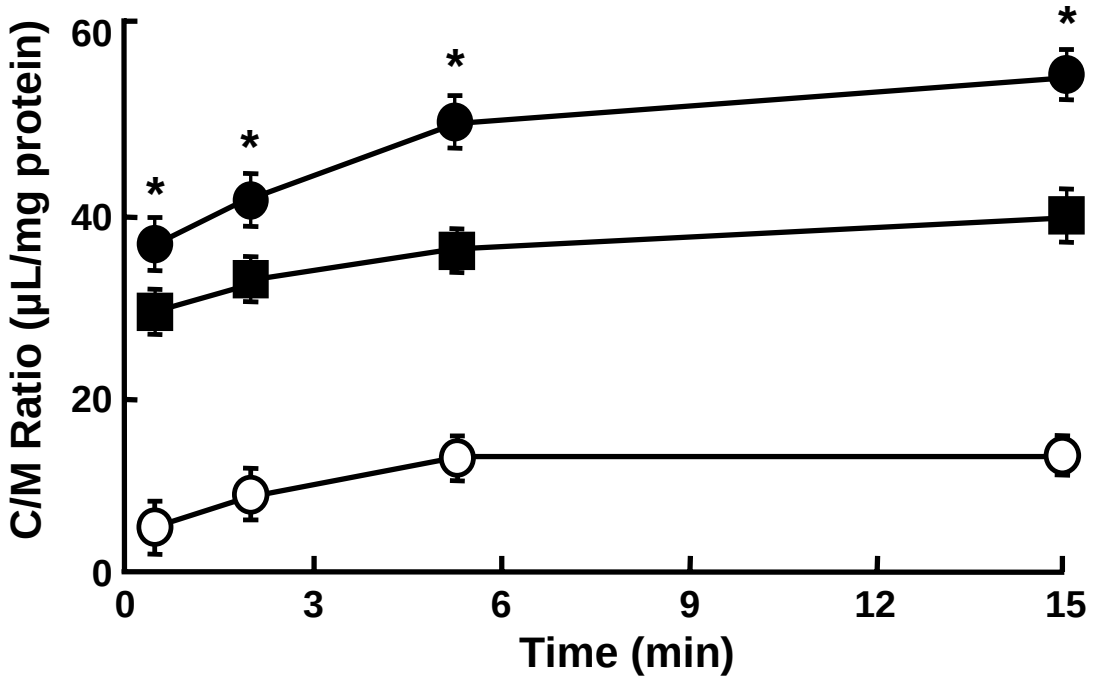
Figure 4



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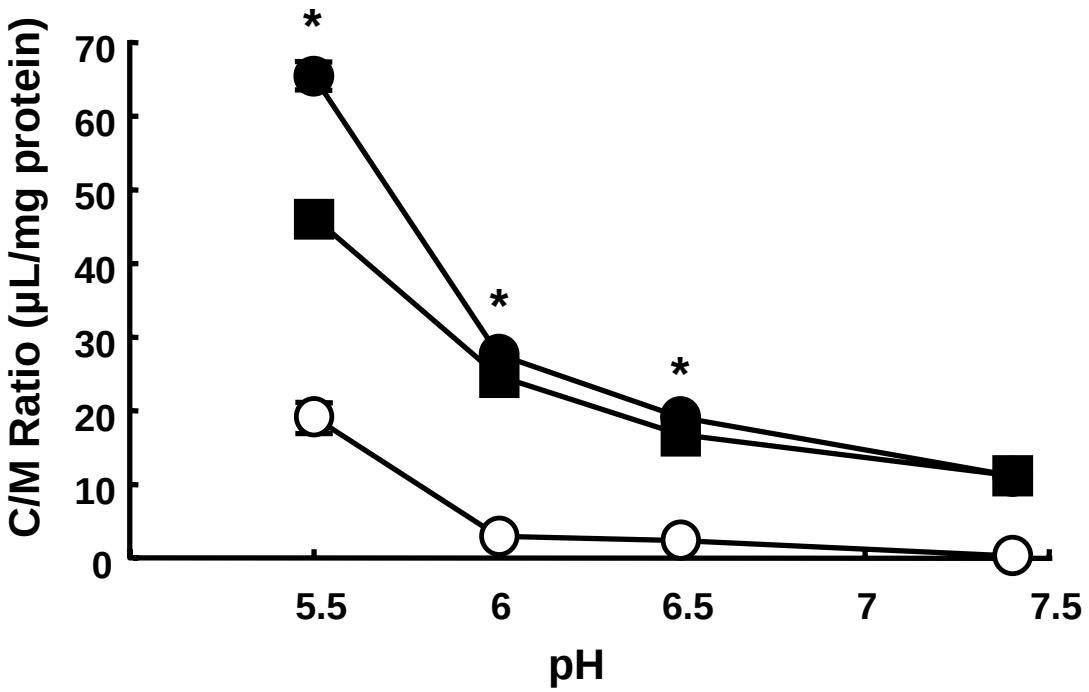

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Table 1

Inhibitor	Concentration (mM)	Relative Uptake (% of Control)
Pravastatin	10	32.5±3.0 *
Acetic Acid	10	86.1±3.1
Lactic Acid	10	85.6±2.9
Benzoic Acid	10	56.1±3.5 *
Nicotinic Acid	10	59.7±2.9 *
Oxalic Acid	10	83.9±1.9
Phthalic Acid	10	42.7±3.5 *
Citric Acid	10	97.7±2.2
DIDS	1	8.4±1.3 *

The Inhibitory Effects of Various Compounds on the Uptake of [^{3}H]Estrone-3-sulfate by OATP-B Expressed in HEK293 Cells.

OATP-B cDNA- or mock-transfected cells were preincubated for 20 min at 37°C in the uptake buffer (pH 7.4). Uptake of [^{3}H]estrone-3-sulfate (4.76 nM) was measured for 10 min at 37°C by incubating the cells in the uptake buffer (pH 5.5) and the results are shown as percentage of control uptake measured in the absence of inhibitor after correcting for the uptake by mock-transfected cells. Each value represents the mean and S.E.M. (n=3 or 4), and (*) indicates a significant difference from the control (p<0.05)