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RESEARCH PAPER

Intestinal Absorption of HMG-CoA Reductase Inhibitor Pravastatin Mediated by Organic Anion Transporting Polypeptide

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ABSTRACT

Purpose The purpose of this study is to clarify the impact of organic anion transporting polypeptide (Oatp) on intestinal absorption of the HMG-CoA reductase inhibitor, pravastatin.

Methods OATP/Oatp-mediated pravastatin uptake was evaluated with *Xenopus* oocytes systems. Intestinal permeability was measured by an *in situ* closed loop method in rats. *In vivo* pravastatin absorption was kinetically assessed by measuring plasma concentration after oral administration in rats.

Results Uptake of pravastatin by Oatp1a5, Oatp2b1, OATP1A2, and OATP2B1 cRNA-injected *Xenopus* oocytes was significantly increased compared with that by water-injected oocytes. Naringin, a potent inhibitor of Oatps and Mdr1, decreased the Oatp1a5-mediated uptake of pravastatin with IC_{50} value of 30.4 μM. Rat intestinal permeability of pravastatin was significantly reduced in the presence of 1,000 μM naringin. Similar results were obtained in *in vivo* absorption studies in rats. Furthermore, no significant change in the permeability was observed in the presence of elacridar, a potent inhibitor of both Mdr1 and Bcrp, though the permeability was significantly decreased in the presence of both elacridar and naringin, suggesting that Mdr1 and Bcrp are not involved in intestinal absorption of pravastatin.

Conclusion Oatp, but not by Mdr1 or Bcrp, contributes to the intestinal absorption of pravastatin in rats.

KEY WORDS intestinal absorption · Mdr1 · naringin · Oatp · pravastatin

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ABBREVIATIONS

AUC	area under the plasma concentration-time curve
BCRP/Bcrp	breast cancer resistance protein
MDR/Mdr	multidrug resistance
MRP/Mrp	multidrug resistance-associated protein
OAT	organic anion transporter
OATP/Oatp	organic anion transporting polypeptide
P-gp	P-glycoprotein

INTRODUCTION

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) are a class of lipid-lowering compounds used to treat hypercholesterolemia (1,2). Statins are classified into hydrophilic (*e.g.*, pravastatin and rosuvastatin) and lipophilic (*e.g.*, atorvastatin, cerivastatin, fluvastatin, lovastatin, pitavastatin and simvastatin) types according to their log*P* values (2). Since hydrophilic statins cannot cross the plasma membrane by passive diffusion, they require membrane transporters to permeate the plasma membrane. Previous studies have shown that membrane transport of hydrophilic statins, which exist as anions at physiological pH, is mediated by organic anion transporting polypeptides (OATPs) expressed in various tissues (3). In liver, OATP family members, including OATP1B1, OATP1B3, and OATP2B1, play an important role in clearance of many statins and other drugs by transporting them across the sinusoidal membrane into hepatocytes for further metabolism mediated by cytochrome P450 (CYP) isoenzymes (4–7).

Pravastatin (MW=424.25) is a relatively polar hydrophilic statin, and exerts its pharmacological effect more selectively in liver, as compared other lipophilic statins (8)

Hepatic uptake of pravastatin is mainly mediated by liver-specific OATP1B1, and this may account for the apparent liver selectivity (3). Pravastatin undergoes little metabolism by CYP isoenzymes and is excreted mainly in unchanged form to the bile (2,9–11). Since excreted pravastatin is reabsorbed from the intestines, enterohepatic circulation of pravastatin could also contribute to its liver-specific distribution (12). The absorption rate and bioavailability of orally administered pravastatin were reported to be approximately 30% and 20%, respectively (2,13). This relatively high absorption rate of pravastatin, in spite of its hydrophilicity (log*P* value of −0.84), is likely to be due to the involvement of transporter-mediated processes in intestinal absorption. Indeed, we have previously shown that the intestinal absorption of pravastatin is mediated by transporters, including OATP2B1 expressed at the apical membrane of intestinal epithelial cells, in a pH-dependent manner (4,14–16). There is increasing evidence that OATP/Oatp is involved in intestinal absorption of various drugs (17–21). Human OATP1A2, OATP2B1 and rat Oatp1a5 could be determinants of intestinal absorption of fexofenadine, a histamine H_1 receptor antagonist (16,19,22). Oatp1a5 has also been suggested to contribute to intestinal absorption of talinolol, a β_1-adrenergic receptor antagonist, in rats (20,21). However, the contribution of OATP/Oatp to intestinal absorption of a substrate drug can be attenuated if the drug is also a substrate of an efflux transporter such as P-glycoprotein (P-gp, multidrug resistance 1 (MDR1/Mdr1)), and the absorption rate may become difficult to predict (20,21,23,24). Although pravastatin has been shown to be transported by human OATP2B1 in a pH-sensitive manner, little information is available about whether or not influx and efflux transporters are involved in pravastatin absorption *in vivo*. In the present study, we focused on rat Oatp1a5 to evaluate the impact of OATP/Oatp on the intestinal absorption of pravastatin, because Oatp1a5 is the only Oatp transporter that is known to be expressed at the apical membranes in rat small intestine (25–27). Here, we show, by means of *in situ* and *in vivo* experimental techniques, that Oatp1a5, but not Mdr1 or Bcrp, contributes to the intestinal absorption of pravastatin *in vivo*.

MATERIALS AND METHODS

Materials

Pravastatin sodium was kindly provided by Kobayashi Kako Co., Ltd. (Fukui, Japan). Naringin was purchased from Chromadex (Irvine, CA, USA). Elacridar (GF120918) was purchased from Toronto Research Chemicals (North York, Ontario, Canada). All other compounds and reagents were obtained from Sigma-Aldrich Co. (St. Louis, MO), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Nacalai Tesque, Inc. (Kyoto, Japan), or Applied Biosystems (Foster City, CA).

Uptake Experiments in *Xenopus laevis* Oocytes

Preparation of oocytes, *in vitro* synthesis of Oatp1a5, Oatp2b1, OATP1A2, and OATP2B1 cRNA, and uptake experiments were conducted as described previously (16,20). In brief, the construct pGEMHE including Oatp1a5, Oatp2b1, OATP1A2, or OATP2B1 cDNA was used to synthesize cRNA. For standard experiments, defolliculated oocytes were injected with 50 nL of the cRNA solution (1 μg/μL) or water, and then incubated for 3 days at 18°C in modified Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, and 10 mM HEPES, pH 7.4) containing 50 μg/ml gentamicin. To initiate uptake, the oocytes were transferred to a 12-well culture plate and preincubated in uptake buffer (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, and 10 mM 2-(N-morpholino) ethanesulfonic acid, pH 6.5) containing pravastatin at room temperature for the designated time. The uptake was terminated by washing the oocytes three times with ice-cold modified Barth's solution.

Uptake (nL/oocyte) was calculated as the cell-to-medium ratio by dividing the uptake amount by the initial concentration of pravastatin in the uptake medium. Uptake rate (nL/min/oocyte) was expressed as uptake of pravastatin over a specified time. Oatp1a5-, Oatp2b1-, OATP1A2-, and OATP2B1-mediated uptake rates were obtained after subtraction of the uptake by water-injected oocytes from that by Oatp1a5, Oatp2b1, OATP1A2, and OATP2B1 cRNA-injected oocytes, respectively. Kinetic parameters were estimated by means of nonlinear least-squares analysis using Kaleida-Graph (Synergy Software, Reading, PA). The affinity of pravastatin for Oatp1a5 (K_m) and the maximal velocity of Oatp1a5-mediated pravastatin uptake ($V_{\max}$) were obtained by fitting the data to the following equation:

$$V = \frac{V_{\max} \cdot C}{K_m + C} \quad (1)$$

where V is initial uptake rate of pravastatin (fmol/min/oocyte), and C is the concentration of pravastatin in the medium (μM).

The inhibitory effect of naringin on pravastatin uptake was expressed as percent of control, and the naringin

concentration giving half-maximum inhibition (IC_{50}) was obtained from the following equation:

$$\% \text{ of control} = \frac{100 \times IC_{50}}{IC_{50} + [\mathrm{I}]} \quad (2)$$

where [I] is naringin concentration (μM).

In Situ Rat Intestinal Closed Loop Experiment

Male Wistar rats were housed three per cage with free access to commercial chow and tap water and were maintained on a 12 h dark/light cycle (8:00 a.m.–8:00 p.m. light) in an air-controlled room (temperature, 24.0±1°C; humidity, 55±5%). All animal experiments were carried out in accordance with the Declaration of Helsinki and the Guide of Kanazawa University for the Care and Use of Laboratory Animals. Permeability of rat intestinal membrane was evaluated by means of an *in situ* intestinal closed-loop method. Rats (220±20 g body weight) fasted overnight were anesthetized with pentobarbital. The abdominal cavity was opened, and an intestinal loop (length: 10 cm) was made at the lower ileum by cannulation with silicone tubing (i.d., 3 mm). Intestinal contents were removed by slow infusion of saline and air. Phosphate-buffered solution (PBS, pH 6.5) containing pravastatin (10 μM) in the absence or the presence of naringin (1,000 μM) and/or elacridar (1 μM) was applied into the intestinal loop, and then both ends of the loop were ligated. After an appropriate period (60 min), the luminal solution in the loop was collected, and remaining pravastatin in the loop was quantified as described below (LC/MS/MS Analysis). Permeability of pravastatin was evaluated in terms of the percentage of dose absorbed, calculated by subtracting the remaining amount of pravastatin from the administered amount. The following equation was used to calculate the permeability:

$$\text{Permeability} = \frac{k_a \cdot V}{2\pi r l} \quad (3)$$

where k_a is the first-order absorption rate constant of pravastatin estimated from the percentage of the dose absorbed during the defined period. V, r and l are the volume of pravastatin solution applied to the loop, the radius and the length of the used segment of intestine, respectively. The length was 10 cm, and the value of the radius (0.18 cm) reported by Fagerholm *et al.* was used (28).

Pharmacokinetic Study in Rats

Prior to experiments, the right jugular vein of male Wistar rats (250±20 g body weight) was cannulated. Groups of rats (n=4) were orally administered pravastatin at a dose of 100 mg/kg (25 mg/mL, pH 6.5) in the absence or presence of naringin (2.32 mg/kg, 25 mg/mL (equivalent to 1,000 μM)), by gavage. Blood samples (250 μL) were collected from the cannula into heparinized tubes before and at the designated times (up to 720 min) after the administration. Each blood sample was replaced with an equal volume of saline, and heparinized saline was used to maintain patency of the catheter. Blood samples were centrifuged at 3,000 *g* for 10 min. The resultant plasma was stored at −30°C until analysis.

Plasma concentrations of pravastatin were analyzed using a non-compartmental method. Area under the plasma concentration-time curve from 0 h to 12 h (AUC_{0-12}) was calculated using the linear trapezoidal rule. The maximum plasma drug concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were obtained directly from the experimental data. The apparent elimination half-life of the log-linear phase ($t_{1/2}$) was calculated based on the terminal elimination rate constant determined by log-linear regression of the final data points (at least 3).

LC/MS/MS Analysis

The concentration of pravastatin in all samples was quantified with a liquid chromatography-tandem mass spectrometry (LC/MS/MS) system consisting of MDS-Sciex API 3200™ triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) coupled with a LC-20AD ultra fast liquid chromatography (UFLC) system (Shimadzu Co., Kyoto, Japan). The UFLC gradient elution was performed using a mobile phase consisting of ammonium acetate (12 mM; pH 4.5) and methanol at a flow rate of 0.3 mL/min. Mercury MS (C_{18}, 10×4.0 mm, Luna 3 μm, Phenomenex, Torrance, CA) was used as the analytical column. In the LC/MS/MS system, the Turbo Ion Spray interface was operated in the positive ion mode at 5,500 V and 200°C. The mass transition (Q1/Q3) of m/z442.2/209.0 was used for pravastatin. Analyst software version 1.4 was used for data manipulation.

Statistical Analysis

Data are shown as the mean with standard error (SEM) of at least three individual experiments. Statistical analyses were performed with the unpaired Student's *t*-test, and a probability of less than 0.05 ($p<0.05$) was considered to be statistically significant.

RESULTS

Uptake of Pravastatin by *Xenopus* Oocytes Expressing Oatp1a5, Oatp2b1, OATP1A2, and OATP2B1

To determine whether pravastatin is transported by Oatp1a5, the time course of pravastatin uptake was

evaluated at pH 6.5 using *Xenopus* oocytes expressing Oatp1a5 (Fig. 1). The uptake of pravastatin (10 μM) by Oatp1a5 cRNA-injected oocytes was significantly increased compared with that by water-injected oocytes, showing that pravastatin is a substrate of Oatp1a5. Because the Oatp1a5-mediated uptake of pravastatin increased linearly up to at least 240 min, uptake at 180 min was routinely measured to evaluate uptake rate in the subsequent studies.

Furthermore, we investigated the uptake of pravastatin by *Xenopus* oocytes expressing rat Oatp1a5 and Oatp2b1 and human OATP1A2 and OATP2B1 at pH 6.5 (Fig. 2). The uptake of pravastatin by all OATP/Oatp cRNA-injected oocytes was significantly increased compared with that by water-injected oocytes, showing that pravastatin is a substrate of Oatp2b1, OATP1A2 and OATP2B1 as well as Oatp1a5.

Functional Characterization of Oatp1a5-Mediated Uptake of Pravastatin

To characterize Oatp1a5-mediated transport of pravastatin, we examined the concentration-dependence of pravastatin uptake at pH 6.5 using oocytes expressing Oatp1a5 (Fig. 3). The Oatp1a5-mediated uptake of pravastatin was saturable, with K_m and V_{max} values of 117±11 μM and 161±10 fmol/min/oocyte, respectively. Moreover, when Oatp1a5-mediated uptake of pravastatin was measured at various pH values (pH 5.5–7.4), the uptake decreased as extracellular pH increased (Fig. 4). This pH-dependent pravastatin uptake showed a maximum at pH 5.5, suggesting that Oatp1a5 functions best at the acidic pH in the upper part of the intestine.

Inhibitory Effect of Naringin and Elacridar on Oatp1a5-Mediated Uptake of Pravastatin

We have reported that naringin, the main constituent flavonoid of grapefruit juice, has a significant inhibitory effect on OATP/Oatp- and MDR1/Mdr1-mediated transport of drugs (20,21,29). Therefore, in the present study, naringin was used as a potent inhibitor of Oatp1a5 as well as Mdr1. As shown in Fig. 5, naringin inhibited Oatp1a5-mediated uptake of pravastatin (10 μM) in a concentration-dependent manner with an IC_{50} value of 30.4±3.3 μM (Fig. 5). Since Oatp1a5-mediated uptake of pravastatin was completely abolished in the presence of 1,000 μM naringin, naringin was routinely used at the concentration of 1,000 μM to evaluate Oatp1a5-mediated absorption of pravastatin in the subsequent studies.

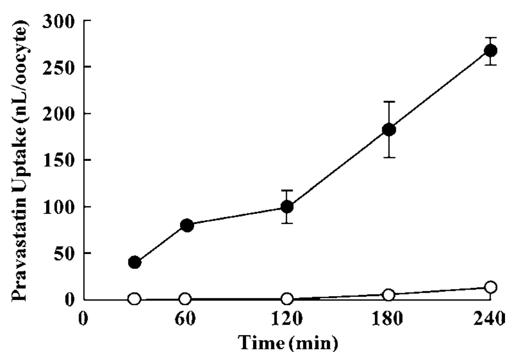

Fig. 1 Time course of pravastatin uptake by *Xenopus* oocytes expressing Oatp1a5. Uptake of pravastatin (10 μM) by water-injected oocytes (*open circles*) or Oatp1a5 cRNA-injected oocytes (*filled circles*) was measured at room temperature and pH 6.5. Data are shown as the mean±SEM (n=8–10).

On the other hand, Oatp1a5-mediated uptake of pravastatin was not affected by elacridar (1 μM), a potent inhibitor of both Mdr1 and Bcrp (Fig. 6). Therefore, elacridar was used at 1 μM to inhibit Mdr1- and Bcrp-mediated secretion, but not Oatp1a5-mediated absorption of pravastatin in the subsequent studies.

Effect of Oatp1a5 and Mdr1a on Intestinal Permeability of Pravastatin in Rats

To evaluate the impact of Oatp1a5 on intestinal absorption of pravastatin, rat intestinal permeability of pravastatin was measured by means of an *in situ* closed loop method (Fig. 7). It was reported that the expression level of Oatp1a5 in ileum is higher than that in jejunum of rats (25). Therefore, Oatp1a5-mediated absorption of pravastatin was studied by measuring the permeability of pravastatin in rat ileum. As shown in Fig. 7, the permeability of pravastatin was $4.20\pm 0.29\times10^{-6}$ cm/sec in the absence of inhibitor. In the presence of naringin (1,000 μM), the permeability was significantly decreased to $1.91\pm0.40\times10^{-6}$ cm/sec, suggesting involvement of Oatp1a5 in the intestinal absorption of pravastatin. Furthermore, no significant change in the permeability was observed in the presence of elacridar (1 μM) alone, though the permeability was significantly decreased to $2.09\pm0.66\times10^{-6}$ cm/sec in the presence of both elacridar and naringin (Fig. 7). These results indicate that intestinal absorption of pravastatin is mediated at least in part by Oatp1a5, but not by Mdr1 or Bcrp.

Effect of Oatp1a5 on Pravastatin Absorption in Rats

When pravastatin was orally administered to rats at a dose of 100 mg/kg (25 mg/mL), the area under the plasma concentration-time curve from 0 h to 12 h (AUC_{0-12}) and the maximum plasma drug concentration (C_{max}) of pravastatin were 574±97 ng·hr/mL and 133±23 ng/mL, respectively (Fig. 8 and Table I). Simultaneous administration of naringin (1,000 μM) significantly decreased the AUC_{0-12} and the C_{max} of pravastatin to 3% and 16%, respectively, suggesting that the Oatp1a5-mediated process was inhibited (Fig. 8 and Table I). On the other hand,

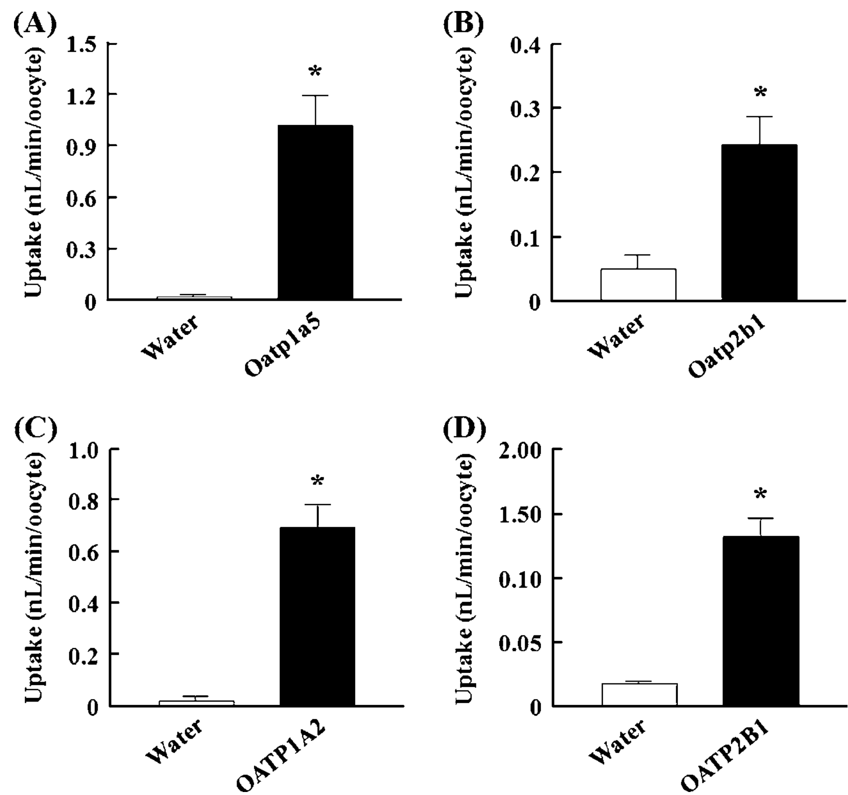

Fig. 2 Uptake of pravastatin by *Xenopus* oocytes expressing Oatp1a5, Oatp2b1, OATP1A2, and OATP2B1. Uptake of pravastatin (10 μM) by water-injected oocytes or Oatp1a5 (**a**), Oatp2b1 (**b**), OATP1A2 (**c**), and OATP2B1 (**d**) cRNA-injected oocytes was measured for 180 min at room temperature and pH 6.5. *$P < 0.05$, significantly different from uptake by water-injected oocytes. Data are shown as the mean±SEM ($n = 8$–10).

naringin did not alter the time to reach maximum plasma concentration (t_{max}) or the apparent elimination half-life ($t_{1/2}$) of pravastatin (Table I).

DISCUSSION

The absorption rate of pravastatin from intestine is relatively high (approximately 30%), despite the hydrophilic nature of the drug, implying an involvement of transporter(s) (13). In the present study, we aimed to investigate the influence of Oatp1a5 on intestinal absorption of pravastatin in rats, using various *in vitro*, *in situ* and *in vivo* methods.

As shown in Figs. 1 and 2, pravastatin was transported by *Xenopus* oocytes expressing Oatp1a5, Oatp2b1, OATP1A2, and OATP2B1, suggesting Oatps are involved in intestinal absorption of pravastatin in rats as well as humans. Although both Oatp1a5 and Oatp2b1 have been reported to be significantly expressed in rodent small intestine, the intracellular localization of Oatp2b1 in the

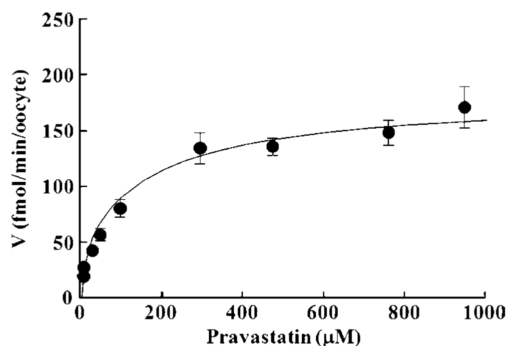

Fig. 3 Concentration dependence of Oatp1a5-mediated pravastatin uptake by *Xenopus* oocytes expressing Oatp1a5. Uptake of pravastatin by water-injected or Oatp1a5 cRNA-injected oocytes was measured at various concentrations for 180 min at room temperature and pH 6.5. Oatp1a5-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that by Oatp1a5 cRNA-injected oocytes. Data are presented as the mean±SEM ($n = 8$–10).

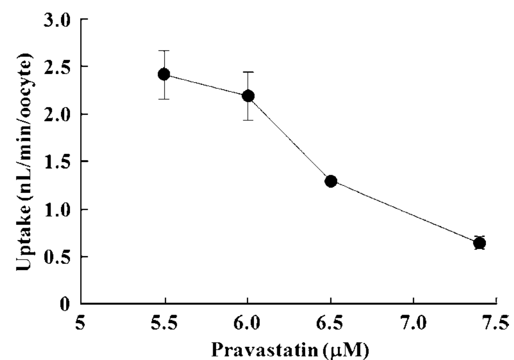

Fig. 4 pH dependence of Oatp1a5-mediated pravastatin uptake by *Xenopus* oocytes expressing Oatp1a5. Uptake of pravastatin (10 μM) by water-injected or Oatp1a5-cRNA-injected oocytes was measured at various concentrations for 180 min at room temperature and various pH values (pH 5.5–7.4). Oatp1a5-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that by Oatp1a5 cRNA-injected oocytes. Data are presented as the mean±SEM ($n = 8$–10).

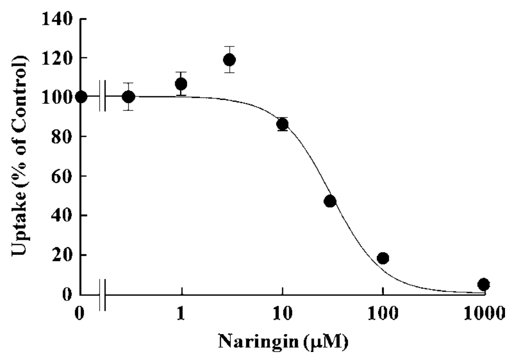

Fig. 5 Inhibitory effect of naringin on pravastatin uptake by *Xenopus* oocytes expressing Oatp1a5. Uptake of pravastatin (10 μM) by water-injected or Oatp1a5 cRNA-injected oocytes was measured in the absence or presence of various concentrations of naringin for 180 min at room temperature and pH 6.5. Oatp1a5-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that by Oatp1a5 cRNA-injected oocytes. Data are shown as the mean±SEM ($n=8$–10).

intestinal epithelial cells remains obscure (25–27). Therefore, in the present study, we focused on rat Oatp1a5, which is expressed at the apical membrane, as an influx transporter for pravastatin involved in its intestinal absorption in rats (25). The Oatp1a5-mediated uptake of pravastatin was saturable and was increased at acidic pH (Figs. 3 and 4). This pH dependence of pravastatin transport is consistent with the pH-sensitive OATP2B1-mediated transport of organic anions, including fexofenadine and pravastatin (15). Therefore, Oatp1a5 may play a role in intestinal absorption of anionic drugs, especially through the upper part of the small intestine, in view of the acidic microclimate pH around the intestinal epithelial cells (30).

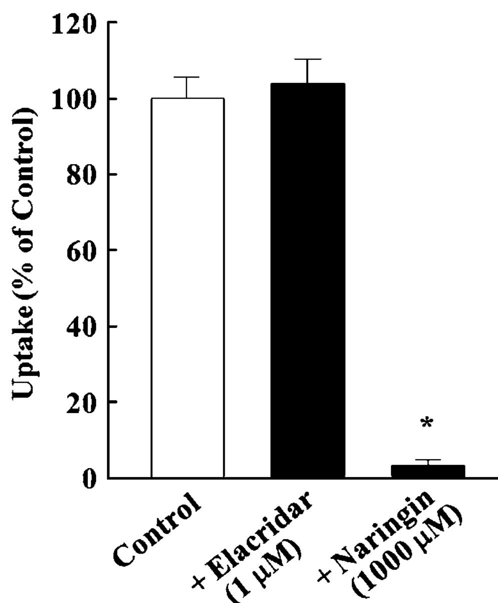

Fig. 6 Inhibitory effect of elacridar on pravastatin uptake by *Xenopus* oocytes expressing Oatp1a5. Uptake of pravastatin (10 μM) by water-injected or Oatp1a5 cRNA-injected oocytes was measured in the absence or presence of elacridar (1 μM) and naringin (1,000 μM) for 180 min at room temperature and pH 6.5. Oatp1a5-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that by Oatp1a5 cRNA-injected oocytes. *$P<0.05$, significantly different from uptake without elacridar and naringin (control). Data are shown as the mean±SEM ($n=8$–10).

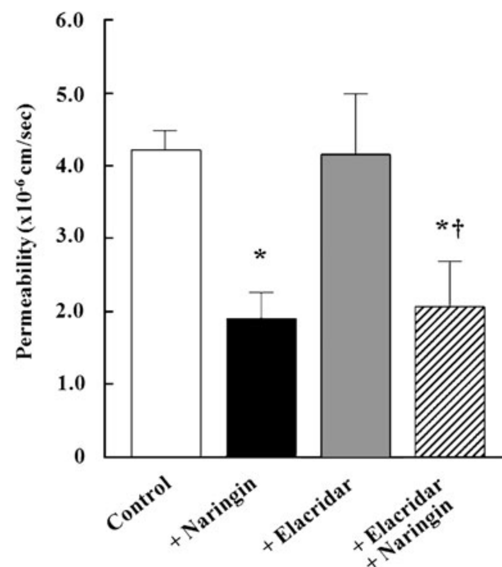

Fig. 7 Effects of naringin and elacridar on pravastatin absorption in rat small intestine. Permeability of pravastatin (10 μM, pH 6.5) in rat small intestine (ileum) was determined by means of an *in situ* closed loop method in the absence or presence of naringin (1,000 μM) and/or elacridar (1 μM) for 60 min at 37°C. *$P<0.05$, significantly different from permeability without naringin and elacridar (control). †$P<0.05$, significantly different from permeability with elacridar. Data are shown as the mean±SEM ($n=4$).

On the other hand, naringin inhibited the Oatp1a5-mediated transport of pravastatin with an IC_{50} value of 30.4 μM, and 1,000 μM naringin completely inhibited Oatp1a5-mediated transport of pravastatin (Fig. 5). Recent investigations have indicated that pravastatin does not interact with MDR1 in *in vitro* transport assay (31,32). Hence, the effect of naringin on only Oatp1a5-mediated influx would be reflected in the apparent intestinal absorption of pravastatin measured in *in situ* and *in vivo* rat experiments, even though naringin is a potent inhibitor of both Oatp and Mdr1. The rat intestinal permeability of pravastatin measured by means of the *in situ* closed loop method was indeed significantly decreased in the presence of 1,000 μM naringin, indicating an involvement of Oatp1a5 in the intestinal absorption of pravastatin (Fig. 7). In addition, elacridar, a potent inhibitor of both Mdr1 and Bcrp, alone had no significant effect on the permeability of pravastatin, but the permeability was significantly decreased in the presence of both elacridar and naringin (Fig. 7). These results suggest that neither Mdr1- nor Bcrp-mediated transport is involved in the intestinal absorption of pravastatin. Our present data do not rule out the possibility that some other influx and/or

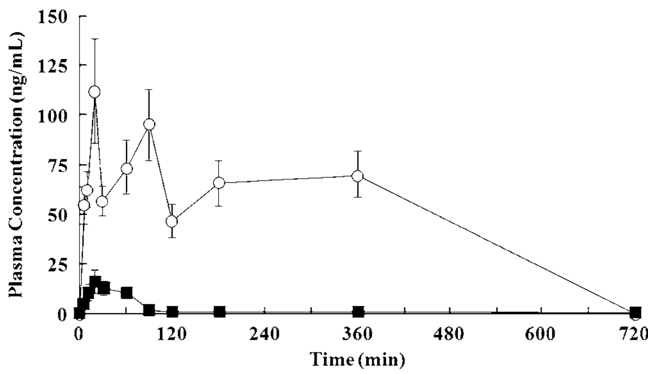

Fig. 8 Mean plasma concentration-time profiles of pravastatin in rats after oral administration. Pravastatin solution (100 mg/kg, 25 mg/mL, pH 6.5) was orally administered in the absence (*open circles*) or the presence of 1,000 μM naringin (2.32 mg/kg, 0.58 mg/mL) (*filled squares*). Data are shown as the mean±SEM ($n=4$).

efflux transporter(s) susceptible to naringin plays a role in the absorption of pravastatin. Previously, pravastatin was reported to be a substrate of Oat3 (33,34). However, Oat3-mediated transport does not account for the naringin-sensitive pravastatin absorption observed in the present study because Oat3 is not expressed in rat intestine (35). Pravastatin is also reported to be a substrate of multidrug resistance-associated protein 2 (Mrp2) expressed in the bile canalicular membrane, based on comparative studies of biliary excretion of pravastatin in normal and Mrp2-dysfunctional Eisai hyperbilirubinemic rats (EHBR) (10,36). Therefore, Mrp2 is considered to be one of the transporters involved in the intestinal absorption process of pravastatin. However, there are few reports indicating an impact of Mrp2 on the intestinal absorption of drugs, although Dahan and Amidon recently demonstrated that Mrp2 may affect the intestinal absorption of sulfasalazine, by using an *in situ* single-pass perfusion method (37). In addition, it is unclear whether naringin inhibits Mrp2-mediated transport of drugs. Further studies would need to investigate the inhibitory effect of naringin on Mrp2-mediated absorption of pravastatin.

In order to confirm the validity of these *in situ* findings, we performed an *in vivo* pharmacokinetic study of pravastatin in rats (Fig. 8 and Table I). The plasma concentration of pravastatin after oral administration was significantly decreased by coadministration of 1,000 μM naringin. This is in good agreement with the result obtained by means of the *in situ* closed loop method (Fig. 7). Although direct evidence of the role of Oatp1a5 in the absorption of pravastatin was not obtained from these *in situ* and *in vivo* studies, these results are consistent with a role of Oatp1a5 in intestinal uptake of pravastatin. In the present study, we did not examine the influence of naringin on pravastatin disposition (*e.g.*, by administration of intravenous pravastatin with naringin), but we consider that the systemic clearance of pravastatin would not be affected by naringin, since there was no significant difference between the $t_{1/2}$ values of pravastatin after oral administration with and without naringin (Table I).

To date, it has been thought that pravastatin is rapidly absorbed from the upper part of the small intestine through passive diffusion according to the pH-partition hypothesis. Our present results, showing rapid absorption after oral administration with a T_{max} value of 0.571 h, support this hypothesis (Fig. 8 and Table I). However, contribution of passive diffusion to intestinal absorption of pravastatin is considered to be very low, since plasma concentration of pravastatin was drastically decreased by coadministration of naringin, indicating that Oatp is a major determinant of the intestinal absorption of pravastatin even at the upper part of the small intestine (Fig. 8). Therefore, regional difference of pravastatin absorption may be observed depending on site-specific expression and/or function of Oatp along the gastrointestinal tract. Absorption from the lower part of the intestine is important for enterohepatic circulation of pravastatin, so Oatp expressed in the lower part as well as

Table I Pharmacokinetic Parameters of Pravastatin After Oral Administration in the Absence and Presence of Naringin in Rats

Pravastatin[a]	Pharmacokinetic parameters of pravastatin			
	AUC_{0-12}	C_{max}	t_{max}	$t_{1/2}$
Control	574±97	133±23	0.571±0.243	1.54±0.06
+ Naringin[b]	17.4±1.0*	21.4±2.3*	0.417±0.036	1.57±0.11

Data are presented as the mean±SEM ($n=4$)

AUC_{0-12} area under plasma concentration-time curve from 0 h to 12 h, C_{max} peak plasma drug concentration, t_{max} time to reach maximum plasma concentration, $t_{1/2}$ elimination half-life

[a] Pravastatin solution was orally administered at 100 mg/kg,25 mg/mL, pH 6.5

[b] Applied concentration of naringin was 1,000 μM (2.32 mg/kg, 0.58 mg/mL)

*$P<0.05$, significantly different from values without naringin

the upper part of the small intestine must play a key role in the intestinal absorption of pravastatin. In Figs. 7 and 8, the effect of naringin on plasma concentration of pravastatin after oral administration is apparently inconsistent with that on permeability of pravastatin measured by *in situ* closed loop method. Previous studies have shown that regional difference of drug permeability along the gastrointestinal tract makes it difficult to predict *in vivo* drug absorption (28, 38–40). Accordingly, our apparently inconsistent findings may also be explained by regional difference of Oatp-mediated absorption of pravastatin along the gastrointestinal tract.

The present study indicates that Oatp1a5 is the major determinant of the intestinal absorption of pravastatin in rats, although contributions of other intestinal Oatp members, including Oatp2b1 shown in Fig. 2, to intestinal absorption of pravastatin cannot be ruled out. In Fig. 2, pravastatin was shown to be a substrate of OATP1A2 and OATP2B1 expressed at the apical membranes in human small intestine (15, 22). Since human OATP1A2 and OATP2B1 share high sequence homology with rat Oatp1a5 and Oatp2b1, respectively, the present study indicates strongly that OATPs contribute to intestinal absorption of pravastatin in humans. Recently, several investigations have indicated that OATP/Oatp-mediated absorption of drugs is a putative site of grapefruit juice and drug interactions (21, 22, 41). However, the effect of grapefruit juice on the pharmacokinetics of pravastatin in humans has not yet been reported (42). Further studies seem warranted to clarify the molecular mechanisms and species differences of drug-drug and/or juice-drug interaction of statins, such as pravastatin, in order to fully establish the impact of OATP/Oatp on intestinal absorption of pravastatin *in vivo*.

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