Proton-Cotransport of Pravastatin Across Intestinal Brush-Border Membrane

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Purpose. The purpose of the present study is to clarify the intestinal brush-border transport mechanism of a weak organic acid, pravastatin, an HMG-CoA reductase inhibitor.

Methods. The transport of pravastatin was studied by using intestinal brush-border membrane vesicles prepared from rabbit jejunum, and uptake by the membrane vesicles was measured using rapid filtration technique.

Results. The initial uptake of [^{14}C]pravastatin was markedly increased with decreases in extravesicular pH and showed a clear overshoot phenomenon in the presence of a proton gradient ($pH_{in/out}$ = 7.5/5.5). A protonophore, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone, significantly reduced the uptake of [^{14}C]pravastatin. In addition, an ionophore for sodium, potassium and proton, nigericin, stimulated the uptake of [^{14}C]pravastatin in the presence of a potassium gradient ($[K^+]_{in}/[K^+]_{out}$ = 0/145 mM). On the other hand, neither the imposition of an inwardly directed sodium gradient nor an outwardly directed bicarbonate gradient stimulated the uptake of [^{14}C]pravastatin. In the presence of a proton gradient ($pH_{in/out}$ = 7.5/5.5), the initial uptake of pravastatin was saturable with the apparent K_t of 15.2 ± 3.2 mM and J_{max} of 10.6 ± 1.21 nmol/mg protein/10 sec. The uptake of pravastatin was significantly inhibited by monocarboxylic acid compounds such as acetic acid and nicotinic acid in a competitive manner but not by di- or tricarboxylic acids, or acidic amino acid.

Conclusion. It was concluded that a pH-dependent transport of pravastatin across the brush-border membrane occurs by a proton-gradient dependent carrier-mediated mechanism rather than by simple diffusion of its unionized form.

KEY WORDS: pravastatin; intestinal absorption; active transport; pH-dependent transport; HMG-CoA reductase inhibitor.

INTRODUCTION

The apparent pH-dependence observed in the intestinal absorption of weak organic acids has been frequently attributed to a pH-partition theory according to which the protonated molecules of these acids permeate the lipoidal membrane barrier of epithelial cells by passive diffusion (1). However, several organic acids, including acetic acid (2,3), nicotinic acid (4), benzoic acid (5) and salicylic acid (6) have been suggested to be absorbed by carrier-mediated processes with proton-cotransport and/or pH-dependent anion exchange mechanisms. Therefore, some other weak organic acids also may be absorbed by a carrier-mediated mechanism rather than passive diffusion.

Pravastatin is a potent drug for hypercholesterolemia that works by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid in the cholesterol biosynthetic pathway. Pravastatin has tissue selectivity in its pharmacological activity in comparison with other HMG-CoA reductase inhibitors such as simvastatin, lovastatin and compactin (7). It is assumed that the tissue targeting feature of pravastatin comes from its high hydrophilicity compared with others, because although pravastatin is taken up by the major target organ, liver, by the active transport mechanism (8,9), other tissues are likely to take up HMG-CoA reductase inhibitors by simple diffusion rather than specific transport mechanisms. Pravastatin is a monocarboxylic acid with pKa of 4.7 and the extent of its intestinal absorption is as high as the other lipophilic analogues, simvastatin and lovastatin (10). The lipophilic HMG-CoA reductase inhibitors are equally absorbed after oral administration both in the forms of lactone and their hydroxy acid of the parent drugs (11,12), in spite of the hydrophilic nature of the carboxylic acid form. Furthermore, HMG-CoA reductase inhibitors were shown to be transported by a monocarboxylic acid transport system at the blood-brain barrier, although permeability of pravastatin is negligible because of its low affinity to the transporter (13). Taken together, it is thought likely that pravastatin is absorbed by a carrier-mediated mechanism rather than by simple diffusion in the intestine.

The purpose of the present study is to elucidate the intestinal transport mechanism of pravastatin by using small intestinal brush-border membrane vesicles (BBMVs), and to investigate the relationship to the carrier-mediated transport systems postulated previously for monocarboxylic acids (2–6).

MATERIALS AND METHODS

Chemicals

Pravastatin, simvastatin, lovastatin and [^{14}C]pravastatin (9.57 mCi/mmol) were kindly supplied by Sankyo Co., Ltd. (Tokyo, Japan). 4,4′-Diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) and nigericin were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Valinomycin and carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and [^{14}C]nicotinic acid (54.8 mCi/mmol) were purchased from Sigma Chemical Co. (St Louis, MO). [^{3}H]Acetic acid (3.6 Ci/mmol) was purchased from New England Nuclear (Boston, MA) and L-[^{14}C]lactic acid (150 mCi/mmol) and R-[^{3}H]mevalonolactone (20 Ci/mmol) were from ARC Inc. (St Louis, MO). R-[^{3}H]mevalonic acid was prepared by hydrolyzing R-[^{3}H]mevalonolactone. All other chemicals were of reagent grade and used without further purification.

Preparation of the Rabbit Intestinal BBMVs

The present study was performed according to the

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Abbreviations: BBMVs, brush-border membrane vesicles; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; DMSO, dimethyl sulfoxide; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; HEPES, 2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethanesulfonic acid; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; MES, 2-(N-morpholino)ethanesulfonic acid, monohydrate; PCMBS, *p*-chloromercuribenzene sulfonic acid; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

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Guidelines for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University and was approved by the Committee on Animal Experimentation of Kanazawa University, Takara-machi Campus. BBMVs were prepared and purified from the jejunum of male rabbits weighing 1.5–2.0 kg (Sankyo Laboratory Co., Toyama, Japan) according to the calcium precipitation method described previously (14). The final pellets of BBMVs were usually suspended in 25 mM HEPES/Tris buffer, pH 7.5, containing 270 mM mannitol with the protein concentration of 14.2 ± 1.87 mg protein/ml (mean ± S.E.M. n = 14) and were kept on ice until used within the same day. The brush-border enzyme activity of alkaline phosphatase measured as described (14) in the final BBM fraction showed a 20.0 ± 1.35 times enrichment (mean ± S.E.M., n = 14) of the activity compared with a crude homogenate.

Transport Experiment

The uptakes of radio-labeled compounds by the membrane vesicles were carried out by a rapid filtration method in the same manner described previously (14). Briefly, the uptake was initiated by adding 90 μl of incubation medium containing radio-labeled compound to 10 μl of membrane vesicle suspension. The pH of an incubation medium appropriate for the intended study was adjusted by Tris, MES or HEPES. The transport reaction was carried out at 37°C, except for acetic acid measured at 27°C and was terminated at the desired time by adding 1 ml of ice-cold stop solution containing 330 mM mannitol and 25 mM HEPES/Tris (pH 7.5). The initial uptake rate was determined from the uptake at 10 sec, since 10 sec was the shortest time for technically reliable measurements of the uptake, although a little back flux during the initial 10 sec was expected considering the time course of the uptake shown in Fig. 2. The diluted samples were immediately applied on a Millipore filter (HAWP, 0.45 μm pore size, Nihon Millipore Ltd., Tokyo, Japan) and washed rapidly twice with 4 ml of ice-cold stop solution. Nonspecific binding to the BBMVs and filters was determined by diluting the suspension of membranes with ice-cold stop solution before adding the test compound. Although the nonspecific binding was so small as to be negligible, all uptakes were corrected by subtraction of the bound amount. The specific conditions for each experiment are given in the figure legends and table footnotes.

Analytical Method

The radio-labeled compound taken up was determined by its total radioactivity, since no metabolism of [^{14}C]pravastatin was observed during the uptake measurement when analyzed by HPLC after the incubation of [^{14}C]pravastatin with BBMVs for 3 hrs (data not shown). The Millipore filters were transferred to counting vials and dissolved in 4 ml of scintillation fluid, Clear-sol I (Nacalai Tesque Inc., Kyoto, Japan), and counted by means of a liquid scintillation counter, LSC-1,000 (Aloka Co Ltd., Tokyo, Japan). Protein was measured by the method of Bradford (15) using a Bio-Rad protein assay kit (Bio-Rad Richmond, CA) with bovine serum albumin as a standard.

Data Analysis

The kinetic parameters for the uptake of pravastatin by BBMVs were estimated by solving the following equation, consisting of both a saturable term and an apparently non-saturable linear term, using the nonlinear least-squares regression analysis as described previously (14):

$$J = J_{max}[C]/(K_t + [C]) + k_d[C]$$

where J and [C] represent the apparent uptake rate and the concentration of pravastatin, respectively. J_{max} and K_t are the maximum uptake rate and the apparent Michaelis constant for a carrier-mediated process, respectively, and k_d is the first-order rate constant for the apparently nonsaturable component which was regarded as the uptake rate determined at 4°C.

RESULTS

Effect of pH and Temperature on [^{14}C]Pravastatin Uptake

The effect of pH on the initial uptake of [^{14}C]pravastatin was examined by changing the extravesicular pH (pH_{out}) over a range of 5.0 to 7.5 with a constant intravesicular pH (pH_{in}) at 7.5. As shown in Fig. 1, the uptake of [^{14}C]pravastatin was increased at acidic pH. Since the pKa value of pravastatin is 4.7, about 90% of pravastatin exists as an ionized form at pH 5.5, and the uptake at pH 5.5 was about 37-fold compared with that at neutral pH. Furthermore, the initial uptake rate at pH 5.5 was significantly lowered at 4°C (Fig. 1), suggesting that a specific transporter mediates the uptake. Therefore, pH 5.5 was selected as the standard extravesicular pH in the following study to characterize the transport of pravastatin.

Effect of a Proton Gradient on [^{14}C]Pravastatin Uptake

In order to clarify the nature of the stimulative effect of low pH_{out} on the uptake of [^{14}C]pravastatin, three different

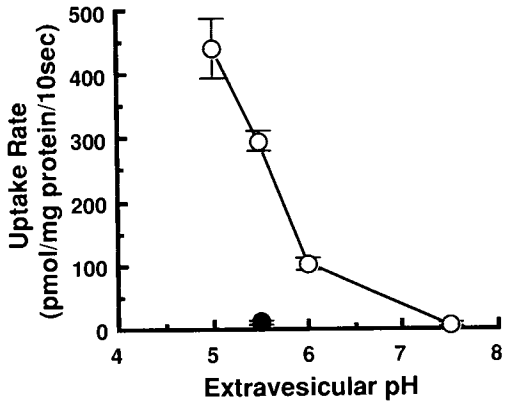

Fig. 1. Effect of extravesicular pH on the initial uptake of [^{14}C]-pravastatin. Membrane vesicles were preloaded with 25 mM HEPES/Tris buffer (pH 7.5) containing 270 mM mannitol. The uptake of [^{14}C]pravastatin (0.20 mM) was measured for 10 sec at 37°C (open circle) or 4°C (closed circle) by incubating membrane vesicles in 25 mM MES/Tris buffer (pH 5.0–6.0) or 25 mM HEPES/Tris buffer (pH 7.5) containing 270 mM mannitol. Each point represents the mean ± S.E.M. of three to four experiments.

types of experiments were carried out. The first was to compare the uptake rate at low pH_{out} in the presence and absence of a proton gradient across the brush-border membranes. In this series of experiments, the BBMVs were preloaded with 25 mM HEPES/Tris buffer (pH 7.5) or 25 mM MES/Tris buffer (pH 5.5), both containing 270 mM mannitol. The uptake in the presence of an inwardly directed proton gradient ($pH_{in/out}$ = 7.5/5.5), 247 ± 5.74 pmol/mg protein/30 sec (mean ± S.E.M., n = 4), was higher than the uptakes measured in the absence of a proton gradient, 66.6 ± 3.28 or 47.5 ± 4.73 at pH_{in} = pH_{out} 5.5 or 7.5, respectively. At extravesicular pH 5.5, the uptake rate was significantly higher in the presence of an inwardly directed proton gradient than in its absence. Second, the effect of FCCP, a protonophore which reduces the proton gradient across the membrane, on the uptake of [^{14}C]pravastatin was determined. FCCP was added in the presence of an inwardly directed proton gradient ($pH_{in/out}$ = 7.5/5.5) to give a final concentration of 50 μM. As shown in Table 1, FCCP significantly reduced the uptake of [^{14}C]pravastatin, whereas in the absence of a proton gradient FCCP had no effect on [^{14}C]pravastatin uptake (data not shown). Third, the effect of nigericin, an ionophore for sodium, potassium and proton, on the uptake of [^{14}C]pravastatin was studied. Nigericin acts as a potassium (or sodium)-proton exchanger and generates an inwardly directed proton gradient across the membrane in the presence of an inwardly directed potassium or sodium gradient. Nigericin was added in the absence of a proton gradient ($pH_{in/out}$ = 7.5/7.5), but in the presence of an inwardly directed potassium gradient ($[K^+]_{in}/[K^+]_{out}$ = 0/145 mM). As shown in Table 1, the uptake of [^{14}C]pravastatin in the presence of nigericin was significantly increased.

Table 1. Effect of FCCP and Nigericin on [^{14}C]Pravastatin Uptake[a]

$pH_{in/out}$	Ionophore	Uptake rate (pmol/mg protein/10 sec)
7.5/5.5	Control	339 ± 21.5
7.5/5.5	+FCCP	233 ± 23.3[b]
7.5/7.5	Control	21.4 ± 5.17
7.5/7.5	+Nigericin	47.5 ± 3.57[b]

[a] The preloaded condition of the membrane vesicles was the same as described in the legend for Fig. 1. Ten microliters of the membrane vesicles was preincubated for 10 min at 37°C with 10 μl of 25 mM HEPES/Tris buffer (pH 7.5) containing 270 mM mannitol and either 100 μM FCCP or 20 μM nigericin for the studies on the effect of FCCP or nigericin, respectively. For the study on the FCCP effect, the uptake of [^{14}C]pravastatin (0.20 mM) was measured for 10 sec at 37°C by incubating it in 80 μl of 25 mM MES/Tris buffer (pH 5.5), containing 270 mM mannitol with 50 μM FCCP. For the study of the effect of nigericine, the uptake of [^{14}C]pravastatin (0.20 mM) was measured for 10 sec at 37°C by incubating it in 80 μl of 25mM HEPES/Tris buffer (pH 7.5) containing 145 mM KCl and 10 μM of nigericin. Final concentrations of FCCP, nigericin and ethanol were 50 μM, 10 μM and 0.5%, respectively. In the absence of ionophores (control), ethanol was added at a final concentration of 0.5%. Each value represents the mean ± S.E.M. of four experiments.

[b] Significantly different ($p < 0.05$) when compared with the uptake of each control uptake.

Effect of Monovalent Cation and Bicarbonate Ion Gradients and Membrane Potential on [^{14}C]Pravastatin Uptake

In order to examine the driving forces other than a proton gradient for pravastatin transport, the uptake of [^{14}C]-pravastatin was measured in the presence of an inwardly directed monovalent cation gradient of sodium or potassium and outwardly directed bicarbonate gradient. Distinct from the significantly enhanced uptake in the presence of a proton gradient (296 ± 15.4 pmol/mg protein/10 sec, mean ± S.E.M., n = 4), the imposition of a sodium (10.2 ± 0.980) or potassium (9.06 ± 4.71) gradient had no effect compared with the control uptake (8.07 ± 5.83) in the absence of proton gradient at pH 7.5. An outwardly directed bicarbonate gradient which was reported previously as one of the driving-forces for the transport of acetic acid (3) did not stimulate the initial uptake of [^{14}C]pravastatin either at extravesicular pH of 5.5 or 7.5 (data not shown).

The effect of an electrical membrane potential across the brush-border membrane was studied after preloading the BBMVs with 100 mM potassium-gluconate in the presence or absence of valinomycin (10 μM). In the presence of valinomycin, which increases intravesicular negativity, the proton gradient dependent uptake of [^{14}C]pravastatin at 60 sec was significantly depressed from 673 ± 37.4 pmol/mg protein (mean ± S.E.M., n = 4) to 367 ± 26.5 ($p < 0.05$). On the other hand, valinomycin had no effect on pravastatin uptake in the absence of potassium (data not shown), confirming that the valinomycin effect can be ascribed to change of membrane potential rather than to other nonspecific effects.

Time Course of [^{14}C]Pravastatin Uptake

The time courses for the uptake of [^{14}C]pravastatin by BBMVs in the presence and absence of a proton gradient were measured as shown in Fig. 2. Intravesicular pH was kept at 7.5 and the uptake was carried out at an extravesicular pH of 5.5 or 7.5. In the absence of a proton gradient ($pH_{in/out}$ = 7.5/7.5), [^{14}C]pravastatin uptake was very slow. In contrast, [^{14}C]pravastatin uptake was significantly accelerated in the presence of an inwardly directed proton gradient ($pH_{in/out}$ = 7.5/5.5) and showed an overshoot phenomenon with the maximum uptake at 60 sec.

Concentration Dependence of Pravastatin Uptake

When the initial uptake of pravastatin was measured as a function of the substrate concentration over the range of 0.20 to 40 mM in the presence of an inwardly directed proton gradient ($pH_{in/out}$ = 7.5/5.5), apparently saturable uptake was observed at 37°C (data not shown). The uptake at 4°C was significantly less than that observed at 37°C (a partial result was shown in Fig. 1) and increased linearly with increases in the concentration of substrate, indicating that the uptake at 4°C was regarded as nonmediated simple diffusion. Therefore the uptake at 4°C was used for the estimation of k_d and was analyzed as described under Materials and Methods. In this way, the following apparent kinetic parameters were obtained: J_{max} was 10.6 ± 1.21 nmol/mg protein/10 sec (mean ± S.D.), K_t was 15.2 ± 3.2 mM and k_d was 0.054 ± 0.006 nmol/mg protein/10 sec/mM. A partial result of the

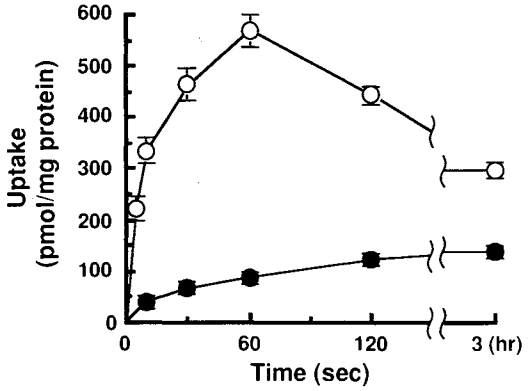

Fig. 2. Time course for the uptake of [^{14}C]pravastatin in the presence and absence of proton gradient. The preloaded condition of membrane vesicles was the same as described in the legend for Fig. 1. The uptake of [^{14}C]pravastatin (0.20 mM) was measured at 37°C by incubating the membrane vesicles either in MES/Tris buffer (pH 5.5, open circle) or 25 mM HEPES/Tris buffer (pH 7.5, closed circle) containing 270 mM mannitol. Each point represents the mean ± S.E.M. of four experiments.

saturable transport of pravastatin was shown in Fig. 3 as the Lineweaver-Burk plot.

Effects of Various Compounds on [^{14}C]Pravastatin Uptake

The inhibitory effects of various organic anions on the initial uptake of [^{14}C]pravastatin were examined in the presence of a proton gradient. These inhibitory experiments were carried out under the condition of voltage clamped in order to avoid the influence of membrane potential change during the uptake by the inhibitors. As shown in Table 2, monocarboxylic acids, such as acetic acid, benzoic acid, lactic acid, mevalonic acid and nicotinic acid as well as structural analogues lovastatin acid and simvastatin acid were inhibitory, whereas di- or tri-carboxylic acids and acidic amino acid did not reduce the pravastatin uptake. Furthermore, anion exchange inhibitor DIDS, which modifies lysine residue of membrane proteins reduced the uptake of [^{14}C]-pravastatin, whereas modification of SH-groups of membrane proteins by *p*-chloromercuribenzene sulfonic acid (PCMBS) had no effect.

In order to know the inhibitory mechanism of monocarboxylic acids, kinetical analysis of the inhibitions by acetic acid and nicotinic acid was examined, with the results shown as the Lineweaver-Burk plots in Fig. 3. Both of two monocarboxylic acids competitively inhibited pravastatin uptake, and their inhibition constants were estimated to be 12.5 mM and 5.74 mM, respectively.

Countertransport of [^{14}C]Pravastatin Uptake

The uptake of [^{14}C]pravastatin was examined at pH 5.5 in the absence of a proton gradient. The preloading of 20 mM pravastatin into BBMVs enhanced the [^{14}C]pravastatin uptake from 100 ± 14.7 pmol/mg protein/30 sec (mean ± S.E.M., n = 4) to 142 ± 18.3, showing a countertransport phenomenon.

Table 2. Inhibitory Effects of Various Compounds on the Initial Uptake of [^{14}C]Pravastatin[a]

Inhibitors	Concentration (mM)	Relative uptake[b] (% of control)
Acetic Acid	10	72.8 ± 4.4[d]
Benzoic Acid	10	74.1 ± 5.9[d]
Lactic Acid	10	83.8 ± 4.1[d]
Lovastatin Acid	0.5	82.4 ± 4.1[c,d]
Mevalonic Acid	10	56.2 ± 1.6[d]
Nicotinic Acid	10	55.7 ± 6.3[d]
Simvastatin Acid	1	60.7 ± 8.5[c,d]
Oxalic Acid	10	102.7 ± 8.23
Phthalic Acid	10	98.1 ± 17
Citric Acid	10	113.5 ± 13
Glutamic Acid	10	97.0 ± 12
DIDS	1	74.9 ± 3.81[d]
PCMBS	0.1	104.8 ± 10.7

[a] BBMVs were preloaded with 25 mM HEPES/Tris buffer (pH 7.5) containing 100 mM mannitol and 100 mM K-gluconate. The uptake of [^{14}C]pravastatin (0.14 mM) was measured for 10 sec at 37°C by incubating the membrane vesicles in 25 mM MES/Tris buffer (pH 5.5) containing 100 mM mannitol, 100 mM K-gluconate, 10 μM valinomycin, 0.5% ethanol and indicated inhibitor.

[b] Each value represents the mean ± S.E.M. of three to four experiments. Control value was 117.6 ± 2.7 (pmol/mg protein/10 sec).

[c] Control study on the effect of simvastatin acid and lovastatin acid includes DMSO (0.2%) used to dissolve them, and control value for them was 89.5 ± 6.2 (pmol/mg protein/10 sec).

[d] Significantly different ($p < 0.05$) when compared with the uptake and control.

Effect of Pravastatin on the Uptakes of Various Monocarboxylic Acids

In order to confirm that the inhibitory effects shown in Table 2 are specific, the inhibitory effect of pravastatin on several monocarboxylic acid uptakes was examined. The results are shown in Table 3. The initial uptake rates of radiolabeled acetic acid, lactic acid, mevalonic acid, and nicotinic acid were reduced in the presence of pravastatin at a concentration of 20 mM.

DISCUSSION

In the present study it was shown that pravastatin is transported in intestinal BBMVs by a carrier-mediated mechanism rather than by simple diffusion. The uptake of pravastatin took place by an apparently saturable mechanism at 37°C in the presence of an inwardly directed proton gradient, whereas at a lower temperature, 4°C, the uptake decreased markedly, and saturability was not observed. Furthermore, countertransport by preloading of an unlabeled pravastatin into BBMVs was observed. These results strongly indicate the existence of a carrier-mediated mechanism for the intestinal brush-border transport of pravastatin. The uptake of pravastatin increased at acidic extravesicular pH, with the proton gradient most likely the driving force for the transport. We have already demonstrated that the intestinal transport of monocarboxylic acids including acetic acid (2,3) nicotinic acid (4), benzoic acid (5) and salicylic acid (6) is performed by extravesicular pH-dependent and carrier-mediated transport mechanisms by using small

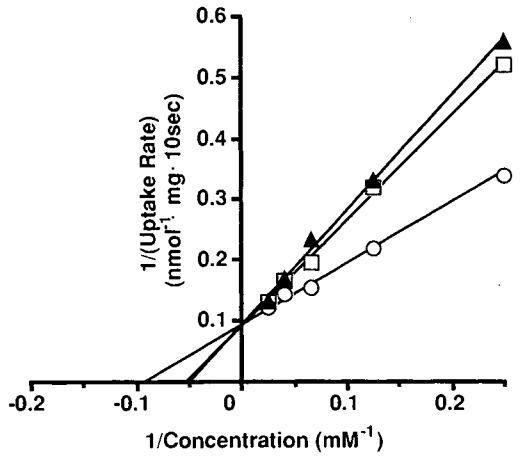

Fig. 3. Lineweaver-Burk plots for pravastatin uptake showing inhibitions by acetic acid and nicotinic acid. The preloaded condition of membrane vesicles was the same as described in the legend for Fig. 1. The uptake of pravastatin was measured for 10 sec by incubating the membrane vesicles in 25 mM MES/Tris buffer (pH 5.5) containing 270 mM mannitol in the presence of acetic acid (25 mM, closed triangle) or nicotinic acid (10 mM, open square) and in the absence of these anions (open circle). Each point represents the mean of three to four experiments after subtraction of nonsaturable uptake estimated from the uptake at 4°C. The lines were obtained by the linear regression of each case.

intestinal BBMVs and the intestinal epithelial cell line Caco-2. Since pravastatin is structurally classified as a monocarboxylic acid, the effect of extravesicular pH on the uptake of [^{14}C]pravastatin was examined. As shown in Fig. 1 the uptake of pravastatin increased markedly with lowering pH_{out}, indicating that the uptake of pravastatin is dependent on the pH_{out}. Apparently pH-dependent uptake cannot be ascribed to pH-dependent binding to the membranes, since the uptake at pH 5.5 was comparable to that at pH 7.5 in the absence of a proton gradient. The time course for the uptake of [^{14}C]-pravastatin in the presence of an inwardly directed proton gradient ($pH_{in/out}$ = 7.5/5.5) showed a clear overshoot phenomenon (Fig. 2). The proton gradient dependence of pravastatin uptake was confirmed by the study using ionophores, FCCP and nigericin (Table 1). Therefore, the enhanced uptake at acidic pH_{out} is ascribed to the inwardly directed proton gradient rather than acidic pH *per se*. and pravastatin is supposed to be co-transported with proton. In our previous studies, it was demonstrated that the carrier-mediated transport system for monocarboxylic acids includes not only a proton-cotransport mechanism but also a bicarbonate exchange transport mechanism which is sensitive to pH (3). However, in the present study, no stimulative effect of a bicarbonate gradient on the uptake of [^{14}C]pravastatin was observed, suggesting that the transport of pravastatin is not driven by a bicarbonate gradient, which is distinct from the features of acetic acid transport (3).

Table 3. Inhibitory Effects of Pravastatin on the Initial Uptake of Various Monocarboxylic Acids[a]

Monocarboxylic acids (μM)	Pravastatin (mM)	Uptake rate[b]
[^{3}H]Acetic Acid	(0)	280 ± 11[c]
(2.0)	(20)	190 ± 18[c,e]
[^{14}C]Lactic Acid	(0)	12.6 ± 0.62[d]
(7.5)	(20)	5.89 ± 0.25[d,e]
[^{3}H]Mevalonic Acid	(0)	92.0 ± 6.0[c]
(0.15)	(20)	60.0 ± 4.5[c,e]
[^{14}C]Nicotinic Acid	(0)	282 ± 5.5[d]
(50)	(20)	64.9 ± 2.0[d,e]

[a] BBMVs were preloaded with 25 mM HEPES/Tris buffer (pH 7.5) containing 100 mM mannitol and 100 mM K-gluconate. The uptake of monocarboxylic acids (at the concentration indicated in parentheses) was measured at 37°C except for acetic acid, measured at 27°C, by incubating the membrane vesicles in 25 mM MES/Tris buffer (pH 6.0) except for mevalonic acid, incubated at pH 5.5 containing 100 mM mannitol, 100 mM K-gluconate, 10 μM valinomycin and 0.5 % ethanol.

[b] Each value represents the mean ± S.E.M. of three to four experiments.

[c] (fmol/mg protein/10 sec).

[d] (pmol/mg protein/10 sec).

[e] Significantly different ($p < 0.05$) when compared with the uptake in the absence of pravastatin.

The nature of the transport mechanism involved in the pravastatin uptake was assessed by the inhibitory effect of various compounds (Table 2 and Fig. 3). All of the monocarboxylic acids examined in the present study reduced significantly the uptake of [^{14}C]pravastatin, but di- or tricarboxylic acids and acidic amino acid had no inhibitory effect. In addition, competitive-type inhibitory effects of acetic acid and nicotinic acid were observed. Inhibition constant (K_i) obtained for nicotinic acid was 5.7 mM. The estimated K_i value of nicotinic acid is close to K_t of nicotinic acid (4.43 mM) reported previously in rats (4), suggesting that pravastatin and nicotinic acid share a common transport carrier. Inhibition by an anion exchange inhibitor (DIDS) was similar to the result in the inhibition on the uptake of [^{14}C]pravastatin by isolated rat hepatocytes, whereas the absence of inhibition by SH-reagent (PCMBS) was different from the results by isolated rat hepatocytes (9), indicating that the substrate binding site of the carrier protein in the intestine is different from that of liver.

As already reported, the pharmacological effect of pravastatin is more specific to liver than more lipid soluble HMG-CoA reductase inhibitors and the specificity was suggested to be attributable to the specific distribution of pravastatin into liver (7). The specific uptake of pravastatin by liver comes from its hydrophilic nature that makes pravastatin less permeable through cell membrane by passive diffusion and the presence of a carrier-mediated transport mechanism functioning at the hepatic sinusoidal membrane. More lipophilic derivatives are also anticipated to distribute into liver as well as other tissues by passive diffusion. Furthermore, the fact that pravastatin is associated with little sleep disturbance, a recognized side effect of HMG-CoA reductase inhibitors, is suggested to be due to its different permeability through the blood-brain barrier as compared to other more lipophilic HMG-CoA reductase inhibitors (13). Although a similar monocarboxylic acid transport mechanism was likely to function at the blood-brain barrier with the intestinal brush-border membrane, the affinity of pravastatin to the brain transporter was very low, resulting in only negligible permeability into brain (13). Taken together with the

permeabilities and the mechanisms of HMG-CoA reductase inhibitors among these tissues, it may be possible to deliver drugs to the target organ specifically including intestinal absorption in the case of water-soluble HMG-CoA reductase inhibitors.

In conclusion, it was demonstrated that the transport of pravastatin across the intestinal brush-border membrane from rabbits occurs by a pH dependent and carrier-mediated mechanisms. The transporter for pravastatin was suggested to be common with that for anionic compounds such as nicotinic acid and acetic acid. Therefore, pH-dependent intestinal absorption of weak organic acidic drugs may be partially ascribed to pH-dependent and carrier-mediated transport mechanisms as well as passive diffusion. The present result provides a new insight into the enhanced oral delivery of weak acidic drugs by the modification of the parent drugs to have not only an increased lipophilicity but also a higher affinity to the specific carrier systems in the plasma membrane which can be utilized for the drug transport.

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