Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Pharmaceutics 317 (2006) 19–25
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Inhibitory effects of statins on human monocarboxylate transporter 4

Masaki Kobayashi, Yukio Otsuka, Shirou Itagaki, Takeshi Hirano, Ken Iseki *

Department of Clinical Pharmaceutics & Therapeutics, Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan

Received 23 August 2005; received in revised form 9 January 2006; accepted 24 February 2006
Available online 18 April 2006

Abstract

Human MCT4 (SLC16A3) is responsible for the efflux of L-lactic acid from skeletal muscle cells and is essential for muscle homeostasis. However, the effects of monocarboxylate drugs, such as statins on the MCT4-mediated transport of L-lactic acid have not been elucidated. Inhibition of L-lactic acid transport mediated by MCT4 might to lead to collapse of muscle homeostasis. The aim of this study was to establish an MCT4 transfected cell line and to clarify the transport mechanism of L-lactic acid and the effects of statins on this transport system. Results of Western blot analyses and immunohistochemistry studies indicated that the expression of CD147 and MCT4-FLAG protein were observed and was displayed clear plasma membrane localization in CD147 and MCT4-FLAG co-transfected cell line (cm cells). Uptake of L-lactic acid in cm cells was significantly greater than that in cells transfected with a vector alone. L-lactic acid uptake was concentration-dependent with a K_m value of 28.43 ± 3.87 mM. The results of a previous study showing a K_m value of 28.5 mM in hMCT4-expressed oocytes. Lipophilic statins significantly inhibited [^{14}C] L-lactic acid uptake in a concentration-dependent manner. In contrast, the inhibitory effects of hydrophilic statins were very weak.

Keywords: Monocarbocarboxylate transporter 4; HMG-CoA reductase inhibitor; Transport; Side effect

1. Introduction

The monocarboxylate transporter (MCT) family now comprises 14 members, of which the first four (MCT1–MCT4) subtypes have been demonstrated experimentally to catalyse the proton-linked transport of metabolically important monocarboxylates, such as L-lactic acid, but the detailed kinetic mechanisms of MCT2–MCT4 subtypes have not been elucidated (Halestrap and Meredith, 2004).

At present, there is some evidence indicating that MCTs play a role in the transport of some drugs, such as valproic acid and HMG-CoA reductase inhibitors (statins), that have monocarboxylate structures within the molecules. (Hosoya et al., 2001; Wu et al., 2000; Nagasawa et al., 2000). Nagasawa et al. (2003) reported that lovastatin acid, a statin, by rat mesangial cells is handled by proton-coupled monocarboxylate transporter, and the involvement of MCT4 in the uptake was suggested. However, it has not been determined whether MCT4 contributes to the transport of statins.

Statins are the most widely used cholesterol-lowering agents for prevention of obstructive cardiovascular events (Havel and Rapaport, 1995; Jukema et al., 1995; Downs et al., 1998). However, statins have been shown to induce various forms of skeletal muscle abnormalities ranging from mild myopathy to myositis and occasionally rhabdmyolysis and even death (Evans and Rees, 2002).

MCT4 is the major monocarboxylate transporter isoform present in white skeletal muscle and is responsible for the efflux of L-lactic acid (Manning Fox et al., 2000; Wilson et al., 1998), and it is essential for muscle homeostasis. Since it has been reported that over-accumulation of lactic acid led to intracellular acidification and apoptosis (Jeong et al., 2001), we hypothesized that inhibition of lactic acid transport mediated by MCT4 leads to over-accumulation of lactic acid and induction of intracellular acidification and apoptosis in skeletal muscle.

In order to verify this hypothesis, we established a cell line transfected with MCT4 and to clarify the transport mechanism of L-lactic acid and the effects of statins on this transport system.

* Corresponding author. Tel.: +81 11 706 3770; fax: +81 11 706 3770.
E-mail address: ken-i@pharm.hokudai.ac.jp (K. Iseki).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.02.043

2. Material and methods

2.1. Chemicals

[^{14}C] L-lactic acid sodium salt was purchased from Amersham Life Science (Buckinghamshire, UK). L-lactic acid sodium salt was purchased from ICN Biomedicals Inc. Cerivastatin Na, simvastatin, atorvastatin Ca, lovastatin, rosuvastatin Ca and pravastatin Na were kindly donated by Sankyo (Tokyo, Japan). Fluvastatin Na was kindly supplied by Novartis Pharma (Tokyo, Japan). Simvastatin acid and lovastatin acid were prepared from their lactone form by hydrolysis in a 0.05N NaOH solution, with stirring at 20 °C for 30 min. The hydrolyzed solution was adjusted to pH 7.4 with 0.2N HCl and then stored 4 °C until use. All other reagents were of the highest grade available and used without further purification.

2.2. Establishment of human MCT4-transfected cells

LLC-PK1 cells were cultured in medium 199 with 10% fetal bovine serum and 1% penicillin–streptomycin in an atmosphere of 5% CO_2. Since CD147 interacts with MCT4 and assists MCT4 expression at the cell surface, we established a CD147 and MCT4 co-transfected cell line. Firstly, human CD147 cDNA was ligated into a pcDNA3.1/zeo(+) vector (pcDNA-CD147). After culture for 1 day, LLC-PK1 cells were transfected with pcDNA-CD147 and pSV2, a blasticidin S-resistance marker, using the lipofection method (LLC-CD147 cells). As a control, a pcDNA3.1/zeo(+) vector and pSV2 were transfected in the same way (LLC-pcDNA cells). The cells were cultured in the above-described medium for another 2 days, and then the culture medium was changed to the above-described medium containing 2.0 μg/mL blasticidin S for up to 3–4 weeks and blasticidin S-resistant colonies were isolated. For expression in LLC-PK1 cells, it was decided to append a FLAG epitope to the 5′ terminus and c-myc, a His epitope, to the 3′ terminus of MCT4 to enable detection of expression by Western blotting and immunofluorescence microscopy. The MCT4-FLAG insert was prepared using PCR with primers flanking the MCT4-FLAG insert and containing suitable restriction sites for insertion into the pSF-1 vector. Following successful PCR amplification, the product was ligated into the pSF-1 vector digested with the same restriction enzymes (pSF-FLAG-MCT4). The identity of the product was confirmed by sequencing. LLC-CD147 cells and LLC-pcDNA cells were transfected with pSF-FLAG-MCT4 using the lipofection method (LLC-CD147/MCT4-FLAG cells and LLC-pcDNA/MCT4-FLAG cells). As a control, the pSF-1 vector were transfected in the same way (LLC-CD147/pSF-1 cells and LLC-pcDNA/pSF-1 cells). These cells were cultured in the above-described medium for another 2 days, and then the culture medium was changed to the above-described medium containing 0.8 mg/mL G418 for up to 3–4 weeks and G418-resistant colonies were isolated. LLC-CD147/MCT4-FLAG cells (cm cells), LLC-pcDNA/MCT4-FLAG cells (vm cells), LLC-CD147/pSF-1 cells (cv cells) and LLC-pcDNA/pSF-1 cells (vv cells) were used for the experiments.

2.3. Western blot analysis

Total protein extracts were prepared from cm, vm, cv and vv cells. The cells were scraped and centrifuged at 4000 rpm for 1 min at 4 °C. The pellet was suspended in 1 mL of PBS and centrifuged at 4000 rpm for 1 min at 4 °C. The resulting pellet was suspended in a lysis buffer containing 1.0% Triton X-100, 0.1% SDS and 4.5 M urea. The suspension was allowed to stand for 5 min and was sonicated for 15 min at 4 °C. The suspension was then centrifuged at 12,000 rpm for 15 min at 4 °C, and the protein concentration in the clear supernatant was determined by the method of Lowry et al. (1951). The samples were denatured at 100 °C for 3 min in a loading buffer containing 50 mM Tris–HCl, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.002% BPB and 3.6 M urea and separated on 4.5% stacking and 10% SDS polyacrylamide gels. Proteins were transferred electrophoretically onto nitrocellulose membranes (Trans-Blot; BIO-RAD) at 15 V for 90 min. The membranes were blocked with PBS containing 0.05% Tween 20 (PBS/T) and 10% non-fat dry milk for 1 h at room temperature. After being washed with PBS/T, the membranes were incubated with mouse anti-FLAG antibody (Sigma–Aldrich) (diluted 1:1000) or goat anti-CD147 antibody (Santa Cruz Biotechnology) (diluted 1:500) for 1 h at room temperature and washed three times with PBS/T for 10 min each time. The membranes were subsequently incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Santa Cruz Biotechnology) or donkey anti-goat secondary antibody (Santa Cruz Biotechnology) at a dilution of 1:4000 and washed three times with PBS/T for 10 min each time. The bands were visualized by enhanced chemiluminescence according to the instructions of the manufacturer (Amersham).

2.4. Immunohistochemistry studies

Cells were washed once with PBS and fixed with 4% paraformaldehyde for 5 min. The fixed cells were washed twice with PBS and then 1% Triton X-100 was added and the cells were incubated for 5 min. The cells were then reacted with primary antibodies (1:100 dilution) for 1 h at 37 °C. Then, cells were washed three times with PBS and treated with secondary antibodies (Santa Cruz Biotechnology) (1:200 dilution) against CD147 (FITC) and FLAG (Rhodamine) for 1 h at 37 °C in the dark. After the cells had been washed three times with PBS and once with water, they were incubated with PBS containing 90% glycerol and DABCO. Then, the cells were observed under a confocal laser scanning microscope (Carl Zeiss).

2.5. Uptake experiments

Uptake of [^{14}C] L-lactic acid was performed as described previously (Kobayashi et al., 2004). After removal of the growth medium, cells were washed with HEPES (pH 7.4) buffer (25 mM D-glucose, 137 mM NaCl, 5.37 mM KCl, 0.3 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 4.17 mM $NaHCO_3$, 1.26 mM $CaCl_2$, 0.8 mM

$MgSO_4$ and 10 mM HEPES) and preincubated at 37 °C for 10 min with 0.5 mL of HEPES (pH 7.4) buffer. Uptake was initiated by applying MES (pH 5.5–6.5) buffer (25 mM D-glucose, 137 mM NaCl, 5.37 mM KCl, 0.3 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 4.17 mM $NaHCO_3$, 1.26 mM $CaCl_2$, 0.8 mM $MgSO_4$ and 10 mM MES) or HEPES (pH 7.0–8.0) buffer containing [^{14}C] L-lactic acid (0.2 μCi/mL). The uptake study was performed at 37 °C. After a predetermined time period, uptake was terminated by suctioning off the applied solution and immersing the plates in ice-cold HEPES (pH 7.4) buffer.

To quantify the radioactivity of [^{14}C] L-lactic acid taken up by the cells, the cells were solubilized in 1% SDS/0.2N NaOH. The remainder of the sample was mixed with 8 mL of scintillation cocktail (ASCII, Amersham) to measure the radioactivity in a liquid scintillation counter (Packard, 1600TR).

The cellular protein content was determined by the method of Lowry et al. with bovine serum albumin as a standard.

2.6. Data analysis

For kinetic studies, the Michaelis–Menten constant (K_m) and maximum uptake rate (V_{max}) of L-lactic acid were estimated from the follwing equation using a nonlinear least-squares regression analysis:

$$v = V_{max} \cdot S/(K_m + S),$$

where V and S are uptake rate of L-lactic acid and concentration of L-lactic acid, respectively.

Unless otherwise indicated, all data are expressed as means ± S.D. of the mean. Student's t-test was used to determine the significance of differences between two group means. Statistical significance among means of more than two groups was determined by one-way analysis of variance (ANOVA). Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Expression of CD147 and MCT4-FLAG in LLC-PK1 cells

To confirm the expression of CD147 and MCT4-FLAG in transfected cells, we performed Western blot analyses and immunohistochemistry studies. As shown in Fig. 1 (a), CD147 protein was detected at about 40 kDa in cm and cv cells. On the other hand, MCT4-FLAG protein was detected at about 50 kDa in cm and vm cells. No expression of CD147 or MCT4-FLAG was observed in vv cells. As shown in Fig. 1 (b), CD147 and MCT4-FLAG were stained near the nuclei of cv or vm cells, respectively. On the other hand, cm cells showed intense CD147 and MCT4-FLAG staining and plasma membrane localization. Moreover, MCT4-FLAG was located apical membrane (data not shown). No staining of CD147 and MCT4-FLAG was observed in vv cells.

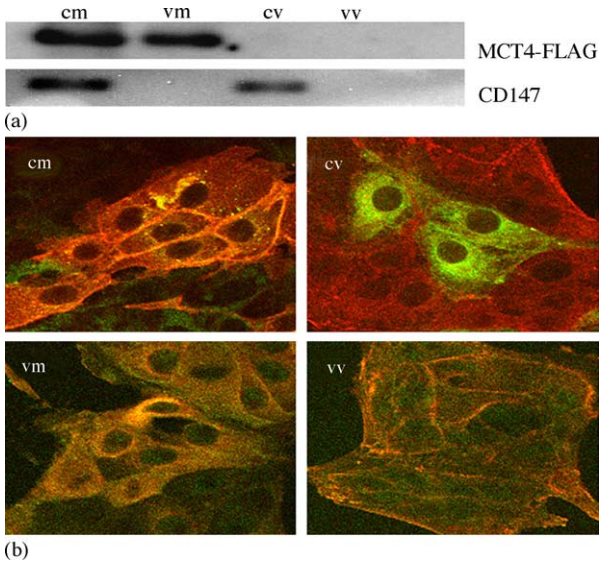
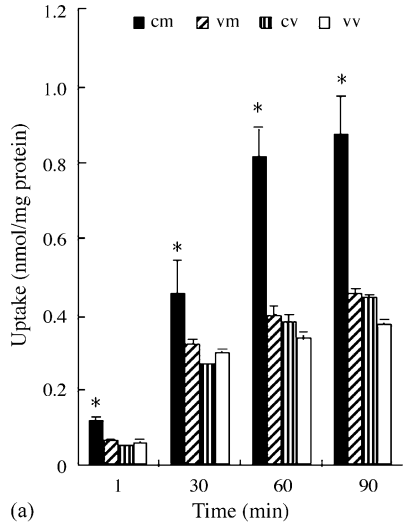


Fig. 1. Expression (a) and localization (b) of CD147 and MCT4-FLAG in cm, vm, cv and vv cells. (a) Proteins were separated by SDS-PAGE, and Western blotting was performed with antibodies against CD147 and FLAG. (b) Cells were treated with antibodies against CD147 (FITC-secondary) and FLAG (rhodamine-secondary).

3.2. Characterization of MCT4-mediated L-lactic acid uptake

To clarify whether MCT4-FLAG retains the fundamental characteristics of MCT4, we examined transport activity. The time profile of the uptake of [^{14}C] L-lactic acid via MCT4 is shown in Fig. 2 (a). The intracellular accumulation of [^{14}C] L-lactic acid was significantly greater in cm cells than that in vm, cv and vv cells. The [^{14}C] L-lactic acid uptake by cm cells was linear for 60 min. Fig. 2 (b) shows the concentration-dependence of the uptake of [^{14}C] L-lactic acid by cm cells. The uptake was saturated at higher [^{14}C] L-lactic acid concentrations. The K_m and V_{max} values were found to be 28.43 ± 3.87 mM and 28.01 ± 1.80 nmol/mg protein/min, respectively. On the other hand, the uptake by vm, cv, and vv cells was not saturated at higher [^{14}C] L-lactic acid concentrations. Moreover, the uptake of [^{14}C] L-lactic acid by vm, cv and vv cells at pH 6.0 and that at pH 7.4 were not significantly different (data not shown). Fig. 3 shows the effects of a substrate and inhibitors of MCT4 on [^{14}C] L-lactic acid uptake by cm cells. α-Cyano-4-hydroxycinnamate (CHC) and phloretin, typical MCT4 inhibitors, significantly inhibited [^{14}C] L-lactic acid uptake. Moreover, CHC inhibited [^{14}C] L-lactic acid uptake in a concentration-dependent manner. On the other hand, MCT4 inhibitors had a little effect on [^{14}C] L-lactic acid uptake by vm, cv and vv cells. However, the inhibitory effect of MCT4 inhibitors by vm, cv and vv cells was lower than that by cm cells (data not shown). These observations (Figs. 1–3) demonstrate that CD147 and MCT4 expressed in this study retain the fundamental characteristics of MCT4 and are suitable for functional studies of MCT4.



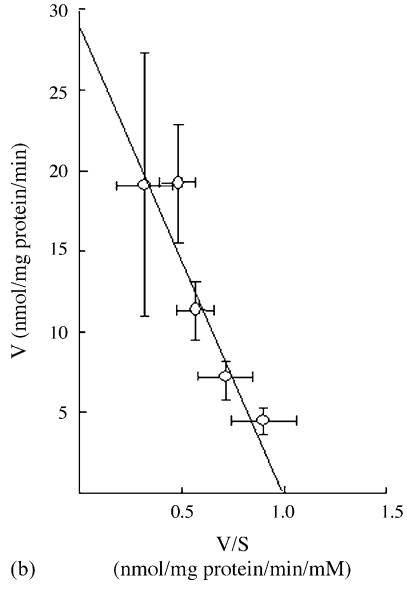
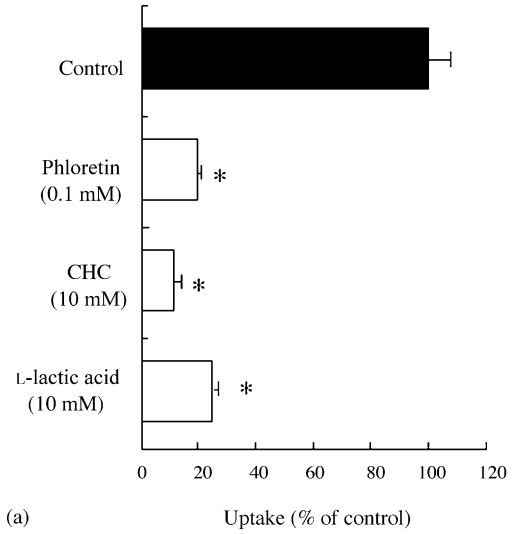


Fig. 2. Uptake of L-lactic acid (3.3 μM) by cm, vm, cv and vv cells (a). Eadie-Hofstee plot of uptake of L-lactic acid by cm cells (b). (a) [^{14}C] L-lactic acid uptake by cm, vm, cv and vv cells was measured at pH 6.0. Each column represents the mean with S.D. of three–six determinations. Asterisk (*): significantly different from vm, cv and vv cells at $P < 0.05$. (b) Uptake values in the absence of an inwardly directed H^+-gradient (extracellular pH 7.4) were subtracted from those in the presence of an inwardly directed H^+-gradient (extracellular pH 6.0) for 30 min. Each point represents the mean ± S.D. of three determinations.



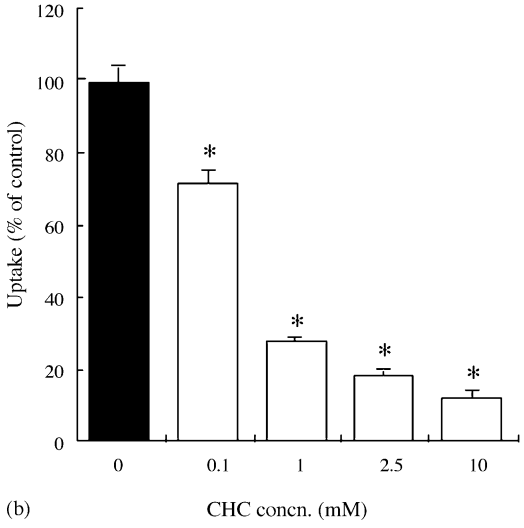


Fig. 3. Inhibitory effects of a substrate and inhibitors of MCT4 (a) and concentration-dependent inhibitory effect of CHC (b) on L-lactic acid (3.3 μM) uptake by cm cells. The [^{14}C] L-lactic acid uptake by cm cells was measured at pH 6.0 for 30 min. Each column represents the mean with S.D. of three–eight determinations.

3.3. Inhibitory effects of statins on MCT4-mediated uptake of L-lactic acid

To clarify whether there are interactions of L-lactic acid and statins with MCT4, the effects of statins that have monocarboxylate structures within the molecules on [^{14}C] L-lactic acid uptake by cm cells was investigated. As shown in Fig. 4 (a), lipophilic statins, fluvastatin, atorvastatin, lovastatin acid, simvastatin acid and cerivastatin, significantly inhibited [^{14}C] L-lactic acid uptake in a concentration-dependent manner and exhibited strong potency with IC_{50} values of 32.4 ± 3.19, 32.6 ± 2.09, 44.2 ± 9.70, 79.4 ± 2.54 and 96.0 ± 5.51 μM, respectively. On the other hand, as shown in Fig. 4 (b), the inhibitory effects of hydrophilic statins, rosuvastatin and pravastatin, were very weak (Table 1).

Table 1
IC_{50} values of statins on the uptake of L-lactic acid (3.3 μM) by cm cells

Statins	IC_{50} values (μM)
Fluvastatin	32.4 ± 3.19
Atorvastatin	32.6 ± 2.09
Lovastatin acid	44.2 ± 9.70
Simvastatin acid	79.4 ± 2.54
Cerivastatin	96.0 ± 5.51
Rosuvastatin	>100
Pravastatin	>1000

Data were taken from Fig. 4. The effects of lipophilic and hydrophilic statins on the uptake of [^{14}C] L-lactic acid by cm cells were examined.

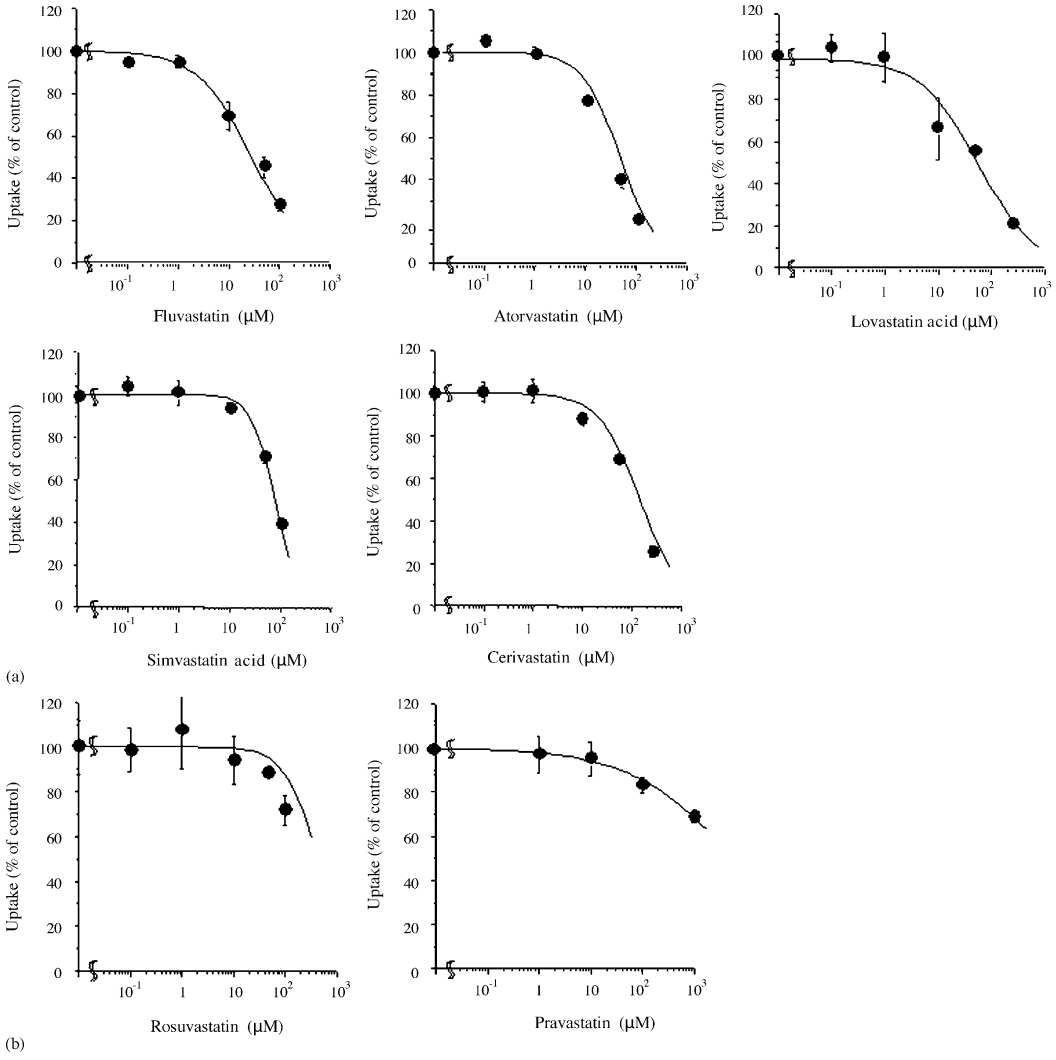


Fig. 4. Inhibitory effects of lipophilic (a) and hydrophilic (b) statins on L-lactic acid (3.3 μM) uptake by cm cells. [^{14}C] L-lactic acid uptake by cm cells was measured at pH 6.0 for 30 min. Each point represents the mean ± S.D. of three–six determinations.

4. Discussion

In the present study, we investigated the interactions of L-lactic acid and statins with MCT4. Firstly, since CD147 interacts with MCT4 and assists MCT4 expression at the cell surface, we established a CD147 and MCT4 co-transfected cell line. Western blot analyses and immunohistochemistry studies indicated that the expression of CD147 and MCT4-FLAG protein were observed and was displayed clear plasma membrane localization in CD147 and MCT4-FLAG co-transfected cell line (cm cells) (Fig. 1). The uptake of [^{14}C] L-lactic acid into cm cells was significantly greater than that into vm, cv and vv cells. Although [^{14}C] L-lactic acid uptake by cm cells was linear for 60 min, this equilibrium time seems to be late as compared to other MCT family. Further studies are needed to determine the reasons. We examined pH- and temperature- dependence of L-lactic acid uptake by cm cells. The effect of extracellular pH on [^{14}C] L-lactic acid uptake by cm cells was examined over the pH range of 5.5–7.4. The uptake of L-lactic acid markedly increased with a decrease in extracellular pH. The [^{14}C] L-lactic acid uptake at acidic pH at 37 °C was greater than that at 4 °C, supporting the speculation that [^{14}C] L-lactic acid uptake by cm cells is dependent on pH (data not shown). The uptake by cm cells was saturated at higher [^{14}C] L-lactic acid concentrations. The K_m and V_{max} values were found to be 28.43 ± 3.87 mM and 28.01 ± 1.80 nmol/mg protein/min, respectively (Fig. 2b). These results are consistent with the results of a previous study showing that the K_m value in hMCT4-expressed oocytes was 28.5 mM (Manning Fox et al., 2000). Moreover, α-cyano-4-hydroxycinnamate and phloretin, typical MCT4 inhibitors, significantly inhibited [^{14}C] L-lactic acid uptake. These observations (Figs. 1–3) demonstrate that CD147 and MCT4 expressed in this study retain the fundamental characteristics of MCT4 and are suitable for functional studies of MCT4.

Next, to clarify the inhibitory effects of statins on [^{14}C] L-lactic acid transport mediated by MCT4, we examined the inhibitory effects of statins on [^{14}C] L-lactic acid uptake by cm cells. Lipophilic statins, fluvastatin, atorvastatin, lovastatin acid, simvastatin acid and cerivastatin, significantly inhibited [^{14}C] L-lactic acid uptake in a concentration-dependent manner

and exhibited strong potency with IC_{50} values of 32.4 ± 3.19, 32.6 ± 2.09, 44.2 ± 9.70, 79.4 ± 2.54 and 96.0 ± 5.51 μM, respectively. Lipophilic statins had little effect on [^{14}C] L-lactic acid uptake by vv cells. Although lipophilic statins significantly inhibited [^{14}C] L-lactic acid uptake by cm cells, the uptake of lipophilic statins by cm cells and that by vv cells were not significantly different (data not shown).

On the other hand, the inhibitory effects of hydrophilic statins, rosuvastatin and pravastatin, were very weak (Fig. 4, Table 1). We previously reported that L-lactic acid efflux is mediated by MCT4 in RD cells, a prototypic embryonal rhabdomyosarcoma cell line (Kobayashi et al., 2005). We examined the effects of statins on the efflux of L-lactic acid from RD cells. The efflux of L-lactic acid from RD cells was significantly inhibited by lipophilic statins. On the other hand, hydrophilic statins had little effect (data not shown). These results are consistent with the results shown in Fig. 4.

Statins are the most widely used cholesterol-lowering agents for prevention of obstructive cardiovascular events (Havel and Rapaport, 1995; Jukema et al., 1995; Downs et al., 1998). However, severe adverse events, including myopathy and rhabdmyolysis, associated with lipophilic statins sometimes limit the lipid-lowering therapy with these agents (Hodel, 2002; Thompson et al., 2003). It was previously reported that lipophilic statins caused cell injury, while hydrophilic paravastatin had no effect (Kubota et al., 2004; Matzno et al., 2003; Matsuyama et al., 2002). Accordingly, we speculate that statin-induced cytotoxicity is associated with the inhibitory effects of statins on L-lactic acid transport mediated by MCT4. However, Takeda et al. (2004) previously reported that therapeutically relevant plasma concentration of fluvastatin and simvastatin are 2.30 and 0.5 μM. Therapeutically relevant plasma concentration of drugs are defined as five-fold of steady-state maximal plasma concentration of drugs (Zhang et al., 2000). The therapeutically relevant plasma concentrations of fluvastatin and simvastatin were approximately 14- and 160-fold lower than IC_{50} values of these statins for human MCT4. Further studies are required to reveal the reason.

Now, we showed that statin-induced cytotoxicity is associated with intracellular acidification (submitted to a journal). Investigations to determine whether intracellular L-lactic acid content is involved in statin-induced apoptosis and to determine the inhibitory effects of statins on L-lactic acid transport mediated by MCT4 are in progress.

In the present study, we have determined the transport mechanism of L-lactic acid via hMCT4 and identified the potent inhibitors, lipophilic statins. The rank order of inhibitory effects of MCT4-mediated transport was fluvastatin $\geqq$ atorvastatin > lovastatin acid > simvastatin acid > cerivastatin. On the other hand, hydrophilic statins, rosuvastatin and pravastatin, had very weak effects on MCT4-mediated transport.

Acknowledgement

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

Downs, J.R., Clearfield, M., Weis, S., Whitney, E., Shapiro, D.R., Beere, P.A., Langendorfer, A., Stein, E.A., Kruyer, W., Gotto Jr., A.M., 1998. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. JAMA 279, 1615–1622.

Evans, M., Rees, A., 2002. Effects of HMG-CoA reductase inhibitors on skeletal muscle: are all statins the same? Drug Saf. 25, 649–663.

Halestrap, A.P., Meredith, D., 2004. The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch. 447, 619–628.

Havel, R.J., Rapaport, E., 1995. Management of primary hyperlipidemia. New Engl. J. Med. 332, 1491–1498.

Hodel, C., 2002. Myopathy and rhabdomyolysis with lipid-lowering drugs. Toxicol. Lett. 128, 159–168.

Hosoya, K., Kondo, T., Tomi, M., Takanaga, H., Ohtsuki, S., Terasaki, T., 2001. MCT1-mediated transport of L-lactic acid at the inner blood-retinal barrier: a possible route for delivery of monocarboxylic acid drugs to the retina. Pharm. Res. 18, 1669–1676.

Jeong, D., Kim, T.S., Lee, J.W., Kim, K.T., Kim, H.J., Kim, I.H., Kim, I.Y., 2001. Blocking of acidosis-mediated apoptosis by a reduction of lactate dehydrogenase activity through antisense mRNA expression. Biochem. Biophys. Res. Commun. 289, 1141–1149.

Jukema, J.W., Bruschke, A.V., van Boven, A.J., Reiber, J.H., Bal, E.T., Zwinderman, A.H., Jansen, H., Boerma, G.J., van Rappard, F.M., Lie, K.I., 1995. Effects of lipid lowering by pravastatin on progression and regression of coronary artery disease in symptomatic men with normal to moderately elevated serum cholesterol levels. The Regression Growth Evaluation Statin Study (REGRESS). Circulation 91, 2528–2540.

Kubota, T., Fujisaki, K., Itoh, Y., Yano, T., Sendo, T., Oishi, R., 2004. Apoptotic injury in cultured human hepatocytes induced by HMG-CoA reductase inhibitors. Biochem. Pharmacol. 67, 2175–2186.

Kobayashi, M., Fujita, I., Itagaki, S., Hirano, T., Iseki, K., 2005. Transport mechanism for L-lactic acid in human myocytes using human prototypic embryonal rhabdomyosarcoma cell line (RD cells). Biol. Pharm. Bull. 28, 1197–1201.

Kobayashi, M., Itagaki, S., Hirano, T., Iseki, K., 2004. Mechanism of L-lactic acid transport in L6 skeletal muscle cells. Drug Metab. Pharmacokinet. 19, 363–368.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Manning Fox, J.E., Meredith, D., Halestrap, A.P., 2000. Characterisation of human monocarboxylate transporter 4 substantiates its role in lactic acid efflux from skeletal muscle. J. Physiol. 529, 285–293.

Matsuyama, K., Nakagawa, K., Nakai, A., Konishi, Y., Nishikata, M., Tanaka, H., Uchida, T., 2002. Evaluation of myopathy risk for HMG-CoA reductase inhibitors by urethane infusion method. Biol. Pharm. Bull. 25, 346–350.

Matzno, S., Tazuya-Murayama, K., Tanaka, H., Yasuda, S., Mishima, M., Uchida, T., Nakabayashi, T., Matsuyama, K., 2003. Evaluation of the synergistic adverse effects of concomitant therapy with statins and fibrates on rhabdomyolysis. J. Pharm. Pharmacol. 55, 795–802.

Nagasawa, K., Muraki, Y., Matsuda, T., Ohnishi, N., Yokoyama, T., 2000. Inhibitory effect of statins on fetal bovine serum-induced proliferation of rat cultured mesangial cells and correlation between their inhibitory effect and transport characteristics. J. Pharm. Sci. 89, 1594–1604.

Nagasawa, K., Nagai, K., Ishimoto, A., Fujimoto, S., 2003. Transport mechanism for lovastatin acid in bovine kidney NBL-1 cells: kinetic evidences imply involvement of monocarboxylate transporter 4. Int. J. Pharm. 262, 63–73.

Takeda, M., Noshiro, R., Onozato, M.L., Tojo, A., Hasannejad, H., Huang, X.L., Narikawa, S., Endou, H., 2004. Evidence for a role of human organic anion transporters in the muscular side effects of HMG-CoA reductase inhibitors. Eur. J. Pharmacol. 483, 133–138.

Thompson, P.D., Clarkson, P., Karas, R.H., 2003. Statin-associated myopathy. JAMA 289, 1681–1690.

Wilson, M.C., Jackson, V.N., Heddle, C., Price, N.T., Pilegaard, H., Juel, C., Bonen, A., Montgomery, I., Hutter, O.F., Halestrap, A.P., 1998. Lactic acid efflux from white skeletal muscle is catalyzed by the monocarboxylate transporter isoform MCT3. J. Biol. Chem. 273, 15920–15926.

Wu, X., Whitfield, L.R., Stewart, B.H., 2000. Atorvastatin transport in the Caco-2 cell model: contributions of P-glycoprotein and the proton-monocarboxylic acid co-transporter. Pharm. Res. 17, 209–215.

Zhang, L., Gorset, W., Washington, C.B., Blaschke, T.F., Kroetz, D.L., Giacomini, K.M., 2000. Interactions of HIV protease inhibitors with a human organic cation transporter in a mammalian expression system. Drug Metab. Dispos. 28, 329–334.