Temperature-dependent Specific Transport of Levofloxacin in Human Intestinal Epithelial LS180 Cells

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ABSTRACT: It was reported previously that specific levofloxacin uptake in Caco-2 cells was inhibited by nicotine, enalapril, L-carnitine and fexofenadine. The aim of the present study was to characterize the cellular uptake of levofloxacin using another human intestinal cell line, LS180. Levofloxacin uptake in LS180 cells was temperature-dependent and optimal at neutral pH, but was Na^+-independent. The rank order of inhibitory effects of the four compounds on [^{14}C] levofloxacin uptake in LS180 cells was nicotine>enalapril>L-carnitine>fexofenadine, which is consistent with that in Caco-2 cells. The mRNA levels of OATP1A2, 1B1, 1B3 and 2B1 in LS180 cells were markedly different from those in Caco-2 cells, and OATP substrates/inhibitors had no systematic effect on the levofloxacin uptake. The mRNA levels of OCTN1 and 2 in LS180 cells were similar to those in Caco-2 cells. However, the inhibitory effect of nicotine on L-[^{3}H]carnitine uptake was much less potent than that of unlabeled L-carnitine. These results indicate that the specific uptake system for levofloxacin in LS180 cells is identical/similar to that in Caco-2 cells, but that OATPs and OCTNs contribute little to levofloxacin uptake in the human intestinal epithelial cells. Copyright © 2009 John Wiley & Sons, Ltd.

Key words: levofloxacin; OATP; OCTN; LS180 cells; Caco-2 cells

Introduction

Levofloxacin is well absorbed from the intestine, and the bioavailability following oral administration is approximately 100% in humans [1]. Since levofloxacin is a zwitterionic compound, a passive diffusion mechanism may not explain fully the high intestinal absorption [1]. Yamaguchi *et al.* reported that apical uptake of levofloxacin in human intestinal epithelial Caco-2 cells was markedly decreased by lowering the temperature (4°C) and showed concentration-dependent saturation [2]. In order to evaluate the membrane transport responsible for intestinal absorption of levofloxacin, pharmacokinetic analysis of the transcellular transport of levofloxacin across Caco-2 cell monolayers grown on porous membrane filters was performed previously [3]. The apical influx clearance value of levofloxacin was greater than the basolateral influx clearance value in Caco-2 cells [3]. In addition, the apical uptake of [^{3}H]glycylsarcosine (Gly-Sar) in Caco-2 cells was significantly inhibited by Ala-Ala, whereas that of [^{14}C]levofloxacin was not inhibited by Ala-Ala and Gly-Sar [4]. On the other hand, the apical uptake of [^{14}C]levofloxacin in Caco-2 cells was significantly inhibited by several structurally unrelated compounds, such as nicotine, fexofenadine, enalapril and L-carnitine. These results suggested that levofloxacin uptake across the apical membrane in Caco-2 cells was mediated by a specific transport system other than the peptide transport system [4]. The primary aim of the present study was to examine whether the specific transport system for levofloxacin is present in another human intestinal epithelial cell line, LS180, which is a microvillus-expressing cell line

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that possesses characteristics of the small intestine, and has been used as an *in vitro* model of the intestine [5–9]. In addition, the inhibitory effects of the four compounds (nicotine, fexofenadine, enalapril and L-carnitine) on the uptake of [14C]-levofloxacin in LS180 cells were compared with those in Caco-2 cells.

Nicotine consists of a pyridine and an *N*-methyl pyrrolidine ring, thereby being a cyclic tertiary amine; however, there has been limited information about the intestinal transport of nicotine [10]. On the other hand, fexofenadine is a selective histamine H_1 receptor antagonist and a zwitterion which contains a carboxyl group and a piperidino group [11]. Organic anion transporting polypeptide 1A2 (OATP1A2) mediated fexofenadine uptake in single-transfected HeLa cells [12]. Enalapril is an angiotensin-converting enzyme (ACE) inhibitor and a modified Ala-Pro, which consists of a free carboxylic acid moiety and an amide bond [13]. OATP1B1 and OATP1B3 are involved in the transport of enalapril in single-transfected HEK 293 cells, whereas the intestinal peptide transporter (PEPT1) may contribute little to the intestinal absorption of enalapril [14,15]. On the other hand, L-carnitine exists *in vivo* as a zwitterion, and is a highly hydrosoluble compound with a quaternary amine and a carboxylic acid moiety [16]. The uptake of L-carnitine in Caco-2 cells is primarily mediated by the organic cation transporter novel type 2 (OCTN2), and OCTN1 expressed in the human intestine is also capable of transporting L-carnitine [16,17]. The secondary aim of the present study was to investigate the involvement of OATPs and/or OCTNs in the specific transport of levofloxacin in LS180 cells. That is, the effects of OATP substrates/inhibitors and also carboxylate-type compounds on the cellular uptake of [14C] levofloxacin were evaluated in LS180 cells. In addition, the uptake characteristics of L-carnitine in LS180 cells were compared with those of levofloxacin.

Materials and Methods

Materials

[14C]Levofloxacin (2.43 MBq/mg) was kindly donated by Daiichi Pure Chemicals Co. (Ibaraki, Japan). L-[*N*-Methyl-3H]carnitine hydrochloride (L-[3H]carnitine) (3145 GBq/mmol) and [3H]mannitol (740 GBq/mmol) were purchased from American Radiolabeled Chemicals Inc. (St Louis, MO, USA). [14C]Mannitol (1.96 GBq/mmol) was purchased from Moravek Biochemicals Inc. (Brea, CA, USA). Levofloxacin hydrochloride was purchased from LKT Laboratories (St Paul, MN, USA). L-Carnitine hydrochloride and Dulbecco's modified Eagle's medium (DMEM) were acquired from Nacalai Tesque (Kyoto, Japan). Fetal bovine serum (FBS) was acquired from Biowest Inc. (Nuaille, France). All other chemicals were of the finest grade available.

Cell culture

LS180 cells at passage number 38 were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were maintained by serial passage in plastic dishes with DMEM supplemented with 10% heat-inactivated FBS, 100 μM nonessential amino acids, 100 units/ml penicillin G and 100 μg/ml streptomycin in an atmosphere of 5% CO_2–95% air at 37°C [8]. The cell lines were seeded at a density 5×10^5 cells/cm^2 on a 3.8 cm^2 plastic dish using a Falcon® multiwell™ plate (BD Bioscience, Bedford, MA, USA), and maintained for 7 days. All experiments were conducted with LS180 cells between passages 54 to 69.

Caco-2 cells at passage number 43 were obtained from the Riken Bioresource Center (Tsukuba, Japan). To determine mRNA expression of transporters in Caco-2 cells, the cells were maintained for 21 days [6]. All experiments were conducted with Caco-2 cells between passages 62 to 66.

Real-time PCR assay of solute carrier transporter mRNA in Caco-2 and LS180 cells

Total RNA was isolated from Caco-2 and LS180 cells using an RNA extraction kit (RNeasy® Mini Kit, Qiagen, Valencia, CA, USA) according to the manufacturer's instructions [8]. Reverse transcription of extracted total RNA was performed as described previously [8]. Real-time PCR was carried out on an Mx3000P® QPCR System (Stratagene, La Jolla, CA, USA) using SYBR® Premix Ex Taq™ (TaKaRa, Shiga, Japan) according

to the manufacturer's instructions. The sequences of the specific primers have been reported elsewhere [12,18,19]. Cycling conditions were 1 cycle for 30 s at 95°C, followed by 45 cycles of 5 s denaturation at 95°C, 20 s annealing at 60°C and 15 s extension at 72°C [8]. The mRNA level of transporters was normalized according to the GAPDH mRNA level, and the ratio is presented using a common logarithm.

Uptake of levofloxacin in LS180 cells

The cellular uptake of [^{14}C]levofloxacin was examined using LS180 cells grown on plastic dishes of multiwell plates. The composition of the incubation medium was as follows: 125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM CaCl$_2$, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4 \cdot$7H$_2$O and 25 mM HEPES (pH 6.0), and the pH of the medium was adjusted with a solution of NaOH. The cells were first pre-incubated for 15 min at 37°C with 2 ml incubation medium containing unlabeled 20 μM levofloxacin to equilibrate the drug concentration. After the pre-incubation period, the incubation medium was replaced with 0.5 ml fresh incubation medium containing [^{14}C]levofloxacin (0.1 μCi/well). After the cells were incubated with [^{14}C]levofloxacin for another 5–30 min at 37°C, they were immediately washed with ice-cold phosphate buffer and collected. Radioactivities in the cells were determined using a liquid scintillation counter and normalized against the initially applied doses. To estimate the extracellular trapping of the radio-labeled drug, the amount of [^{3}H]mannitol in the cells was also determined [4].

In order to evaluate the Na$^+$ dependence of the cellular uptake of [^{14}C]levofloxacin, incubation medium containing various Na$^+$ concentrations was prepared by replacing NaCl with *N*-methyl-D-glucamine hydrochloride, and the pH of the medium was adjusted to 6.0 by the addition of KOH. When uptake was measured at an acidic or alkaline pH (pH 5.0–8.0), the pH of the medium was adjusted using HCl or NaOH.

The effect of various compounds on the uptake of [^{14}C]levofloxacin in LS180 cells on plastic dishes of a multiwell plate was evaluated at 37°C in the presence of unlabeled 10–100 μM levofloxacin. The cells were first pre-incubated for 15 min at 37°C with 2 ml incubation medium. The incubation medium was replaced with 450 μl fresh incubation medium supplemented with various compounds at 5 min before the addition of 50 μl solution of [^{14}C]levofloxacin (0.1 μCi/well). After the cells were incubated with [^{14}C]levofloxacin for 15 min at 37°C, the amount of [^{14}C]levofloxacin in the cells was determined as described above.

Uptake of L-carnitine in LS180 cells

In order to evaluate the cellular uptake of L-[^{3}H]carnitine in LS180 cells, the cells were first pre-incubated for 15 min at 37°C with 2 ml incubation medium containing unlabeled 1 μM L-carnitine. The incubation medium was replaced with 0.5 ml fresh incubation medium containing L-[^{3}H]carnitine (0.1 μCi/well). After the cells were incubated with L-[^{3}H]carnitine for another 15 min at 37°C, the amount of L-[^{3}H]carnitine in the cells was determined as described above. To estimate the extracellular trapping of the radio-labeled drug, the amount of [^{14}C]mannitol in the cells was also determined.

In order to evaluate the Na$^+$ dependence of the cellular uptake of L-[^{3}H]carnitine, incubation medium containing various Na$^+$ concentrations was prepared by replacing NaCl with *N*-methyl-D-glucamine hydrochloride, and the pH of the medium was adjusted to 6.0 by the addition of KOH. When uptake was measured at an acidic or alkaline pH (pH 5.0–8.0), the pH of the medium was adjusted using HCl or NaOH.

The effect of various compounds on the uptake of L-[^{3}H]carnitine in LS180 cells was evaluated at 37°C in the presence of unlabeled 1–10 μM L-carnitine. The incubation medium was replaced with 450 μl fresh incubation medium supplemented with various compounds at 5 min before the addition of the 50 μl solution of L-[^{3}H]carnitine (0.1 μCi/well). After the cells were incubated with L-[^{3}H]carnitine for 15 min at 37°C, the amount of L-[^{3}H]carnitine in the cells was determined as described above.

Statistical analysis

Values are expressed as the mean±SE. In all figures, when error bars are not shown, they are smaller than the symbol. Multiple comparisons

were performed using Scheffé's test following one-way ANOVA provided that the variances of groups were similar. If this was not the case, the Scheffé-type test was applied following Kruskal-Wallis analysis. A value of $p < 0.05$ was considered to be statistically significant.

Results

Expression of OATPs and OCTNs mRNA in Caco-2 and LS180 cells

The mRNA levels of OATPs and OCTNs in LS180 cells were compared with those in Caco-2 cells by real-time PCR (Figure 1). In addition, the mRNA expression of PEPT1 was measured for reference, because the specific transport of Gly-Sar was observed in Caco-2 cells [4]. The expression of PEPT1 mRNA in LS180 cells was much lower than that in Caco-2 cells, and the expression pattern of OATP mRNAs in LS180 cells was markedly different from that in Caco-2 cells (Figure 1). That is, the expression of OATP1A2 mRNA in LS180 cells was much lower than that in Caco-2 cells, and OATP1B1 was not significantly expressed in the two cell lines. The expression of OATP1B3 mRNA in LS180 cells

was higher than that in Caco-2 cells, whereas the expression of OATP2B1 mRNA in LS180 cells was lower than that in Caco-2 cells. On the other hand, the mRNA for OCTN1 and 2 was moderately expressed in both Caco-2 and LS180 cells, and the OCTN1/2 mRNA expression ratio in LS180 cells was similar to that in Caco-2 cells (Figure 1).

Characterization of levofloxacin uptake in LS180 cells

To investigate the existence of a specific transport system for levofloxacin in LS180 cells, the time-dependent uptake of [^{14}C]levofloxacin in LS180 cells was measured. The uptake of [^{14}C]levofloxacin at medium pH 6.0 and 37°C was 1.19%, 1.51% and 1.68% of the applied dose (20 μM) at 5, 15 and 30 min, respectively, which suggested that the uptake had almost reached a steady state at 15 min after the start of incubation. Then the effects of extracellular Na$^+$ and pH on the 15 min uptake of [^{14}C]levofloxacin were examined in LS180 cells (Figure 2). The uptake of [^{14}C]levofloxacin at 37°C was not Na$^+$-dependent in the concentration range of 25–125 mM. On the other hand, [^{14}C]levofloxacin uptake in LS180 cells was 1.3-fold higher at physiological pH 7.4 than at acidic pH 5.0 ($p < 0.05$). In addition, the cellular uptake was markedly decreased at 4°C (Figure 2). These results indicated that the uptake of levofloxacin in LS180 cells was mediated by
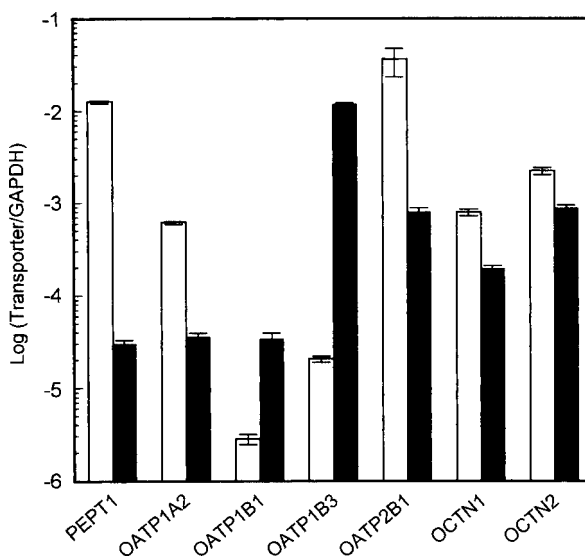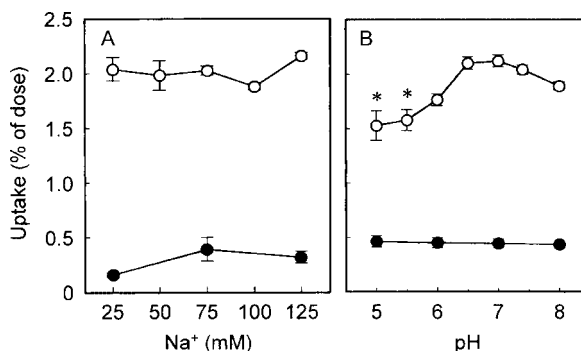

Figure 1. Relative mRNA expression of several SLC transporters in Caco-2 (open columns) and LS180 (closed columns) cells. Each column represents the mean±SE for three measurements

Figure 2. Na$^+$ (A) and pH (B) dependence of cellular uptake of levofloxacin in LS180 cells. Cells were incubated with [^{14}C]levofloxacin for 15 min at 37°C (open circles) or 4°C (closed circles) in the presence of 20 μM unlabeled levofloxacin. Each point represents the mean±SE for 4–14 measurements. *$p < 0.05$: significantly different from pH 7.4 at 37°C

a temperature-dependent specific transport system.

The inhibitory effects of nicotine, enalapril, L-carnitine, fexofenadine, and also fluoroquinolones, on the uptake of [14C]levofloxacin were evaluated in LS180 cells (Figure 3). Fexofenadine, unlabeled levofloxacin and lomefloxacin decreased the uptake of [14C]levofloxacin to 63%, 44% and 37% of the control value, respectively. L-Carnitine, enalapril and nicotine significantly decreased the uptake of [14C]levofloxacin to 25%, 19% and 17% of the control value, respectively (Figure 3). The rank order of inhibitory effects of the four compounds (nicotine>enalapril> L-carnitine>fexofenadine) in LS180 cells was consistent with that in Caco-2 cells [4], which indicated that the specific transport system responsible for the levofloxacin uptake in LS180 cells is identical or similar to that in Caco-2 cells.

Inhibitory effect of various OATP substrates/ inhibitors on levofloxacin uptake in LS180 cells

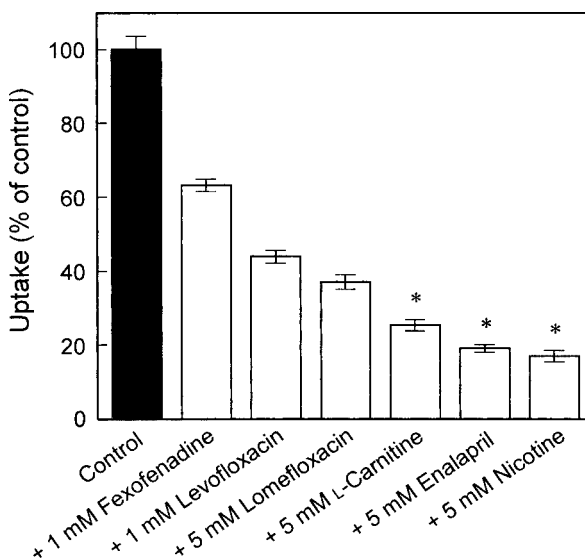To evaluate the involvement of OATPs in the levofloxacin uptake in LS180 cells, the effects of various OATP inhibitors and also carboxylate-type compounds on the cellular uptake of [14C]-levofloxacin were further examined. An inhibitor of OATP1B1 and 1B3, rifampicin, decreased the uptake of [14C]levofloxacin to 76% of the control value, whereas other inhibitors of OATP1B1 and 1B3 (bosentan and taurocholic acid) did not inhibit the uptake of [14C]levofloxacin (Figure 4). OATP2B1 inhibitors (probenecid and DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid disodium salt)) and OATP1A2 inhibitors (hesperidin and naringin) had no significant effect on the uptake of [14C]levofloxacin. A carboxylate-type compound, methotrexate, decreased the uptake of [14C]levofloxacin to 62% of the control value, whereas other carboxylate-type compounds (folic acid and sulfasalazine) did not inhibit the uptake of [14C]levofloxacin (Figure 4). These results indicated that OATPs did not contribute significantly to the uptake of levofloxacin in LS180 cells.

Comparison of cellular uptake of L-carnitine and levofloxacin in LS180 cells

To evaluate whether levofloxacin uptake in LS180 cells is mediated by OCTNs, the uptake profile of

Figure 3. Inhibitory effect of various compounds on cellular uptake of levofloxacin in LS cells. Cells were incubated with [14C]levofloxacin for 15 min in the presence of 100 μM unlabeled levofloxacin and various compounds. Closed column represents the mean±SE for 15 measurements, and open columns represent the mean±SE for 6–8 measurements. *p<0.05: significantly different from the control
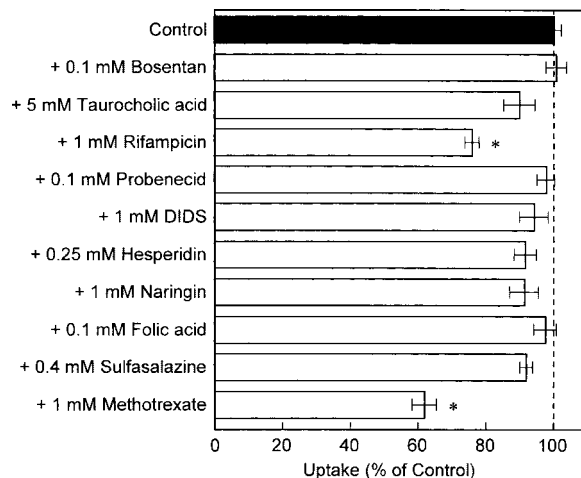
Figure 4. Inhibitory effect of various compounds, including OATP inhibitors, on cellular uptake of levofloxacin in LS180 cells. Cells were incubated with [14C]levofloxacin for 15 min in the presence of 100 μM unlabeled levofloxacin and various compounds. Closed column represents the mean±SE for 15 measurements, and open columns represent the mean±SE for 6–8 measurements. *p<0.05: significantly different from the control
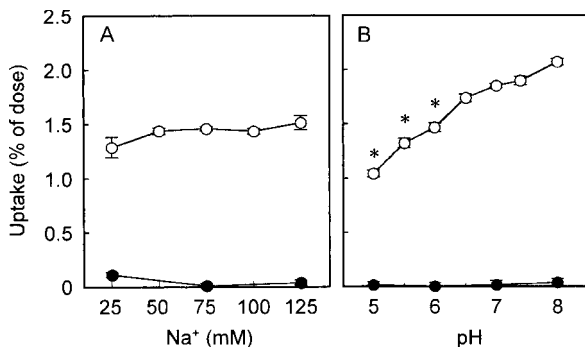
Figure 5. Na$^+$ (A) and pH (B) dependence of cellular uptake of L-[^{3}H]carnitine in LS180 cells. Cells were incubated with L-[^{3}H]carnitine for 15 min at 37°C (open circles) or 4°C (closed circles) in the presence of 1 μM unlabeled L-carnitine. Each point represents the mean ± SE for 6–8 measurements. *$p < 0.05$: significantly different from pH 7.4 at 37°C

Figure 6. Inhibitory effect of various compounds on cellular uptake of L-carnitine in LS180 cells. Cells were incubated with L-[^{3}H]carnitine for 15 min in the presence of 1 μM unlabeled L-carnitine and 100 μM various compounds. Closed column represents the mean ± SE for 12 measurements, and open columns represent the mean ± SE for 5–6 measurements. *$p < 0.05$: significantly different from the control

L-carnitine in LS180 cells was compared with that of levofloxacin. The change in extracellular Na$^+$ concentration between 25 and 125 mM had no significant effect on the uptake of L-[^{3}H]carnitine in LS180 cells at 37°C (Figure 5A). Raising the pH of the medium from 5.0 to 8.0 increased the uptake of L-[^{3}H]carnitine at 37°C (Figure 5B). On the other hand, the uptake of L-[^{3}H]carnitine at 4°C was very low (Figure 5). Next, the study evaluated the inhibitory effects of various compounds, which inhibited the levofloxacin uptake, on the uptake of L-[^{3}H]carnitine in LS180 cells. Since the uptake of L-[^{3}H]carnitine in LS180 cells was almost completely inhibited by 1 mM fexofenadine (data not shown), the inhibitory effects of the compounds were tested at a concentration of 100 μM (Figure 6). Fexofenadine, levofloxacin and unlabeled L-carnitine decreased significantly the uptake of L-[^{3}H]carnitine to 77, 34, and 8% of the control value, respectively; however, lomefloxacin, enalapril and nicotine had little effect on the uptake of L-[^{3}H]carnitine (Figure 6). In addition, choline chloride, which is structurally related to L-carnitine, decreased L-[^{3}H]carnitine uptake to 78% and 24% of the control value at 5 and 50 mM, respectively (Figure 7A). However, the uptake of [^{14}C]levofloxacin was only slightly decreased to 91% of the control value even in the presence of 50 mM choline chloride (Figure 7B). These findings suggested that OCTNs contributed little to the levofloxacin uptake in LS180 cells.
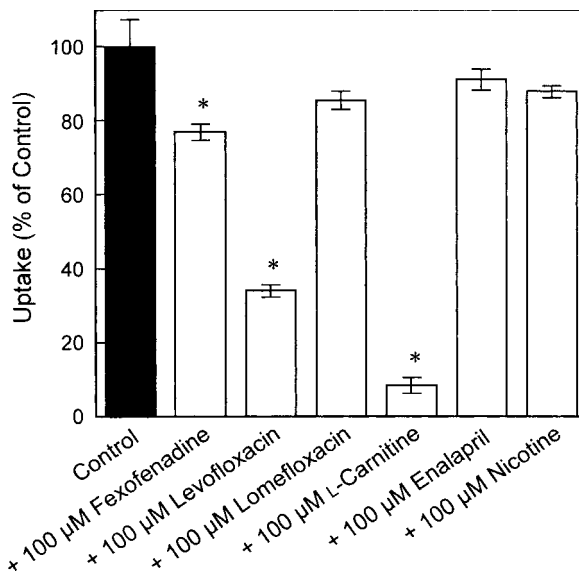
Inhibitory effect of angiotensin-converting enzyme inhibitors on levofloxacin uptake in LS180 cells

Enalapril significantly inhibited the uptake of levofloxacin in LS180 cells (Figure 3). In order to evaluate the substrate recognition characteristics of the transporter for levofloxacin, the study further evaluated the effect of several ACE inhibitors (1 mM) on the uptake of [^{14}C]levofloxacin in LS180 cells (Figure 8). Perindopril and lisinopril had no significant effect on the uptake of [^{14}C]levofloxacin in LS180 cells. On the other hand, temocapril, quinapril, captopril and enalapril decreased the uptake of [^{14}C]levofloxacin to 59%, 54%, 51% and 50% of the control value, respectively (Figure 8).

Discussion

Maeda *et al.* evaluated the levofloxacin uptake activity of Caco-2 subclones, and selected candidate transporter genes functioning for influx transport of levofloxacin [1]. Based on functional
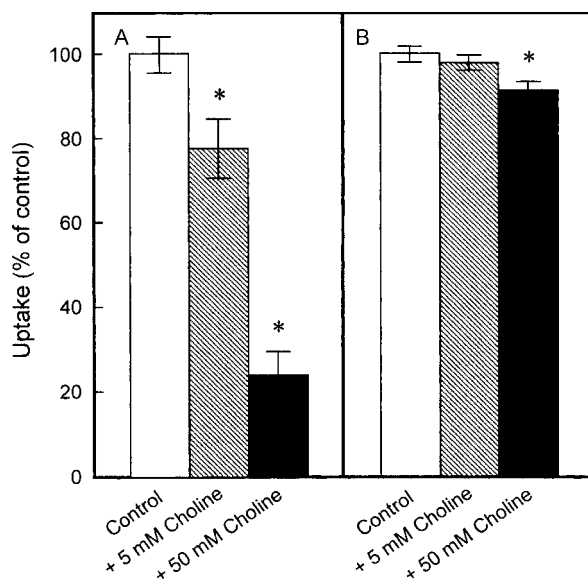
Figure 7. Inhibitory effect of choline chloride on cellular uptake of L-carnitine and levofloxacin in LS180 cells. (A) Cells were incubated with L-[^{3}H]carnitine for 15 min in the presence of 10 μM unlabeled L-carnitine and choline chloride. (B) Cells were incubated with [^{14}C]levofloxacin for 15 min in the presence of 10 μM unlabeled levofloxacin and choline chloride. Each column represents the mean±SE for six measurements. *$p < 0.05$: significantly different from the control
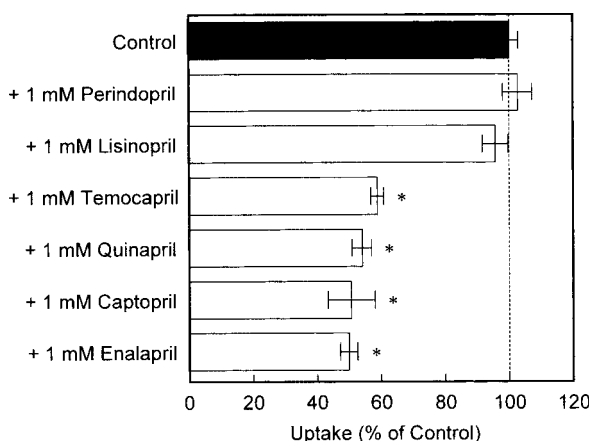

Figure 8. Inhibitory effect of ACE inhibitors on cellular uptake of levofloxacin in LS180 cells. Cells were incubated with [^{14}C]levofloxacin for 15 min in the presence of 100 μM unlabeled levofloxacin and 1 mM ACE inhibitors. Closed column represents the mean±SE for 10 measurements, and open columns represent the mean±SE for 5–7 measurements. *$p < 0.05$: significantly different from the control

analysis of each transporter gene for which a good correlation was found between the mRNA

expression level and levofloxacin transport activity in Caco-2 subclones, OATP1A2 was concluded to transport levofloxacin. When OATP1A2 was expressed in Xenopus oocytes, levofloxacin uptake was essentially pH-independent. OATP1A2-mediated uptake of levofloxacin showed a K_m value of 136 μM [1]. On the other hand, the apparent uptake of levofloxacin by Caco-2 cells showed high- and low-affinity components with K_m values of 0.489 and 14.6 mM, respectively. Accordingly, plural transporters are functional for the transport of levofloxacin in Caco-2 cells, and OATP1A2 is likely to function as a high-affinity transporter [1]. However, it is still unclear which transporter is responsible for the lower-affinity (high-capacity) uptake of levofloxacin in Caco-2 cells.

In the present study, the cellular uptake of levofloxacin was characterized in LS180 cells. Levofloxacin uptake in LS180 cells was temperature-dependent and optimal at neutral pH, but was Na$^+$-independent (Figure 2). The rank order of the inhibitory effects of the four compounds on levofloxacin uptake in LS180 cells was nicotine>enalapril>L-carnitine>fexofenadine (Figure 3), which is consistent with that in Caco-2 cells [4]. The findings indicated that the specific transport system responsible for the levofloxacin uptake in LS180 cells was identical or similar to that in Caco-2 cells. The expression level of OATP1A2 mRNA in LS180 cells was much lower than that in Caco-2 cells (Figure 1). In addition, OATP1A2 inhibitors (hesperidin and naringin) did not inhibit the uptake of levofloxacin in LS180 cells (Figure 4). The results also suggested that the uptake of levofloxacin in LS180 cells as well as Caco-2 cells is mainly mediated by a specific transport system other than OATP1A2.

OATP1B1, 1B3 and 2B1 are also expressed in the human intestine [12]. The study further examined whether these OATPs were involved in the specific transport of levofloxacin in LS180 cells. The mRNA levels of OATP1B1, 1B3 and 2B1 in LS180 cells were markedly different from those in Caco-2 cells (Figure 1), and OATP substrates/ inhibitors had no systematic inhibitory effect on the uptake of levofloxacin in LS180 cells (Figure 4). In addition, Yamaguchi et al. examined the effect of organic anions on the uptake of levofloxacin in Caco-2 cells, and demonstrated

that probenecid and DIDS did not inhibit the uptake of levofloxacin in the cells [2]. These findings suggested that levofloxacin uptake in intestinal epithelial cells is mediated by a specific transport system distinct from OATP1B1, 1B3 and 2B1.

OCTN2 is a physiologically important carnitine transporter, and is expressed in the human intestine [16]. Hirano *et al.* investigated the inhibitory effect of levofloxacin on the uptake of L-carnitine in Caco-2 cells, and showed that levofloxacin inhibited the apical uptake of L-carnitine in a non-competitive manner [20]. In the present study, it was found that the characteristics of levofloxacin uptake in LS180 cells were different from those of L-carnitine. That is, nicotine and enalapril inhibited the levofloxacin uptake significantly (Figure 3); however, the inhibitory effect of nicotine on L-[^{3}H]carnitine uptake was much less potent than that of unlabeled L-carnitine (Figure 6). Furthermore, choline chloride inhibited the uptake of L-carnitine, but not of levofloxacin (Figure 7). These findings suggested that the uptake transporter of levofloxacin is distinct from that of L-carnitine in intestinal epithelial cells.

Many ACE inhibitors are well absorbed from the intestine despite their low lipophilicities, and display absorption rates of 30% to 100% of a dose [15,21]. However, it is unclear which transporter is involved in the intestinal absorption of ACE inhibitors [15]. The present study additionally examined the effect of several ACE inhibitors on levofloxacin uptake in LS180 cells (Figure 8). In addition to enalapril, temocapril, quinapril and captopril inhibited the uptake of levofloxacin in LS180 cells (Figure 8). To our knowledge, this is the first report demonstrating that the uptake of levofloxacin is affected by several ACE inhibitors in the intestinal epithelial cells. Further systematic studies will be needed to clarify whether levofloxacin was transported by the influx transporter responsible for the intestinal absorption of ACE inhibitors.

In conclusion, the present results indicated that the specific transport system responsible for the levofloxacin uptake in LS180 cells is identical or similar to that in Caco-2 cells, but that OATPs and OCTNs contributed little to the uptake of levofloxacin in the human intestinal epithelial cells.

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