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Mechanism of the inhibitory effect of zwitterionic drugs (levofloxacin and grepafloxacin) on carnitine transporter (OCTN2) in Caco-2 cells

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Abstract

L-Carnitine plays an important role in lipid metabolism by facilitating the transport of long-chain fatty acids across the mitochondrial inner membrane followed by fatty acid beta-oxidation. It is known that L-carnitine exists as a zwitterion and that member of the OCTN family play an important role in its transport. The aims of this study were to characterize L-carnitine transport in the intestine by using Caco-2 cells and to elucidate the effects of levofloxacin (LVFX) and grepafloxacin (GPFX), which are zwitterionic drugs, on L-carnitine uptake. Kinetic analysis showed that the half-saturation Na^+ concentration, Hill coefficient and K_m value of L-carnitine uptake in Caco-2 cells were 10.3±4.5 mM, 1.09 and 8.0±1.0 μM, respectively, suggesting that OCTN2 mainly transports L-carnitine. LVFX and GPFX have two pK_a values and the existence ratio of their zwitterionic forms is higher under a neutral condition than under an acidic condition. Experiments on the inhibitory effect of LVFX and GPFX on L-carnitine uptake showed that LVFX and GPFX inhibited L-carnitine uptake more strongly at pH 7.4 than at pH 5.5. It was concluded that the zwitterionic form of drugs plays an important role in inhibition of OCTN2 function.

Keywords: L-carnitine; Caco-2; Levofloxacin; Grepafloxacin; OCTN2

1. Introduction

L-Carnitine plays an important role in lipid metabolism by facilitating the transport of long-chain fatty acids across the mitochondrial inner membrane followed by fatty acid beta-oxidation [1,2]. L-Carnitine homeostasis in humans is maintained by acquisition from dietary sources, a modest rate of endogenous biosynthesis and efficient reabsorption [3]. It is known that carnitine deficiency causes cardiomyopathy, muscle weakness, hypoketoic hypoglycemia, Reye's syndrome and sudden infant death [4–6].

Recently, a novel organic cation transporter (OCTN) family has been described, and it has been shown that some OCTNs are capable of transporting L-carnitine [7,8]. OCTN2, a member of the OCTN transporter family, is the most important L-carnitine transporter. It has been reported that OCTN2 transports carnitine Na^+-dependently and that it contributes to L-carnitine homeostasis by transporting L-carnitine in various tissues [7]. Moreover, Elimrani et al. have reported the expression and localization of OCTN2 in Caco-2 cells [9]. It has been also reported that L-carnitine is transported by $ATB^{0,+}$, an amino acid transporter, in a low affinity and high capacity manner [10].

L-Carnitine deficiency is caused by a decrease in carnitine transport. It is known that mutation of OCTN2 decreases L-carnitine transport and that it is one of the causes of primary carnitine deficiency [11–13]. On the other hand, there are some reports of carnitine deficiency being induced by several drugs (valproic acid, cephaloridine, emetine and pivalic acid) [14–17].

L-Carnitine is a highly-hydrosoluble compound and exists *in vivo* as a zwitterion which has a quaternary amine and carboxylate (pK_a 3.8) [18]. OCTN2 is an important transporter for L-carnitine but also transport many cationic drugs, including tetraethylammonium, pyrilamine and verapamil [19]. Moreover, cephaloridine, which is a zwitterion, inhibits transport and

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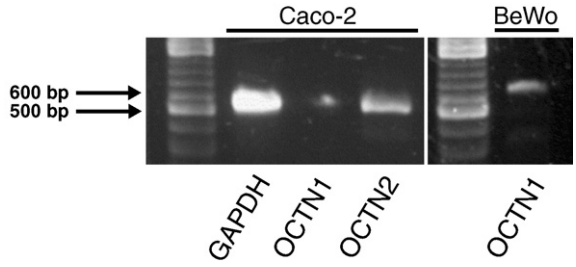

Fig. 1. Expression of OCTN1 and OCTN2 mRNA in Caco-2 cells. The mRNA expression was determined by RT-PCR. Data shown are typical results from three independent experiments.

causes carnitine deficiency [15]. These facts suggest that a compound with an ionic charge is involved in OCTN2 substrate recognition.

Levofloxacin (LVFX) and grepafloxacin (GPFX), a new quinolone antibiotic, are zwitterions that each have a carboxylate and an amine. We previously reported that transport of enoxacin, also a new quinolone antibiotic, is stimulated by diffusion potential in rat BBMVs and that the inhibitory effect of ciplofloxacin on enoxacin absorption differs depending on pH conditions [20,21]. It has also been reported that uptake of LVFX and GPFX in Caco-2 cells under an acidic condition was less than that under a neutral condition [22]. Moreover, quinolones are substrates for the ABC transporter super family [23–25]. However, the transport systems of LVFX and GPFX have not been revealed clearly, and inhibitory effects on transporters in the intestine have not been investigated in detail.

In this study, we investigated L-carnitine transport in the intestine by using Caco-2 cells and elucidated the effects of LVFX and GPFX representative zwitterionic drugs on L-carnitine uptake. We also investigated whether the effect of quinolones on L-carnitine uptake is changed by variation of the ionic charge of quinolones.

2. Materials and methods

2.1. Chemicals

L-[^{3}H]-carnitine (80.0 Ci/mmol) was purchased from Amersham Biosciences Corp. Levofloxacin was kindly donated by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Grepafloxacin was kindly supplied by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). All other reagents were of the highest grade available and used without further purification.

2.2. Animals

Male Wistar rats, aged 6 to 7 weeks (300–350 g in weight), were obtained from NRC Haruna (Gunma, Japan). The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the "Guide for the Care and Use of Laboratory Animals".

2.3. Cell Culture

Caco-2 cells obtained from American Type Culture Collection (Rockville, MD) were maintained in plastic culture flasks (Falcon, Becton Dickinson and Co., Lincoln Park, NJ) as described previously [26]. The medium used for growth of Caco-2 cells was Dulbecco's modified Eagle's medium (Gibco Life Technologies, Inc., Grand Island, NY) with 10% fetal bovine serum (ICN Biomedicals, Inc., Aurora, OH), 1% nonessential amino acids (Gibco), 4 mM glutamine (Gibco), and 100 IU/mL penicillin–100 μg/mL streptomycin. Monolayer cultures were grown in an atmosphere of 5% CO_2–95% air at 37 °C. Cells reached confluency after 4–6 days in culture. The cells were harvested with 0.25 mM trypsin and 0.2% EDTA (5 min at 37 °C), resuspended, and seeded into a new flask. Cells between the 48th and 56th passages were used in this study. For the uptake study, Caco-2 cells were seeded at a density of 2×10^5 cells/cm^2 on 24-well plastic plates (Corning Coster Corp., Cambridge, MA). The cell monolayers were fed a fresh growth medium every 2 days and were then used at 4–6 days for the uptake experiments.

2.4. RT-PCR analysis

Total RNA was prepared from Caco-2 cells using an Isogen (Nippon Gene, Tokyo). Single-strand cDNA was made from 2 μg total RNA by reverse transcription (RT) using an Omniscript RT Kit (QIAGEN). PCR was performed using Hot Star Taq PCR (QIAGEN) with OCTN1-, OCTN2- and GAPDH-specific primers through 35 cycles of 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1 min. The primers specific to OCTN1, OCTN2 and GAPDH were designed on the basis of sequence data in the GenBankTM database (accession no.: NM_003059, NM_003060 and NM_002046, respectively). The sequences of the specific primers were as follows: 5′-TGG AAT CTG GTG TGT GAG GA-3′ (sense) and 5′-CCT GAA CAG GTC CAG AAT GA-3′ (antisense) for OCTN1 (609 bp), 5′-CTG TTG GGC TCC TTC ATT TC-3′ (sense) and 5′-TGT TGT GGG ACT GTT GCT TC-3′ (antisense) for OCTN2 (539 bp), 5′-TGG AAA TCC CAT CAC CAT CT-3′ (sense) and 5′-TTC TAG ACG GCA GGT CAG GT-3′ (antisense) for GAPDH (540 bp). A cDNA sample obtained from BeWo cells was used for positive control experiment of the OCTN1 primer. The PCR products were subjected to electrophoresis on a 1% agarose gel and then visualized by ethidium bromide staining.

2.5. Immunocytochemistry

Caco-2 cells were fixed in 10% formaldehyde and permeabilized in 0.1% Triton X-100 for 15 min. The cells were first incubated in a blocking buffer (10% FBS in PBS) for 60 min. Then the cells were incubated overnight at 4 °C with an anti-OCTN2 antibody (dilution of 1:20) (Santa Cruz Biotechnology,

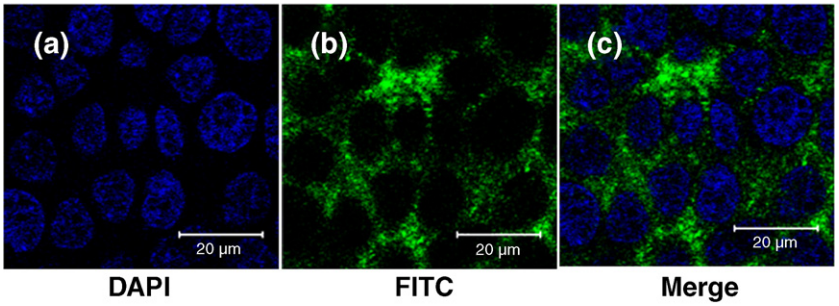

Fig. 2. Immunohistochemistry of OCTN2 in Caco-2 cells. The nucleus was stained by DAPI (blue, a). Expression of OCTN2 was determined using an antibody against OCTN2 (green, b). Fluorescence was visualized by using a confocal microscope at a magnification of ×40.

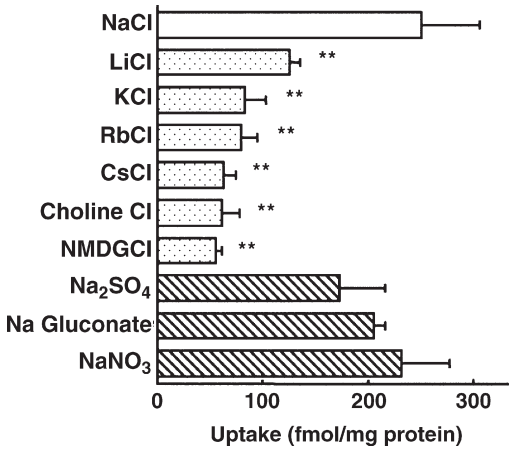

Fig. 3. Effect of replacement of Na^+ with other cations on L-carnitine uptake. L-[^{3}H]-carnitine (5 nM) uptake was measured for 30 min. Na^+ or Cl^- in the buffer was replaced with other cations or anions. Each point represents the mean with S.D. of three determinations. **Significantly different from NaCl at $p<0.01$.

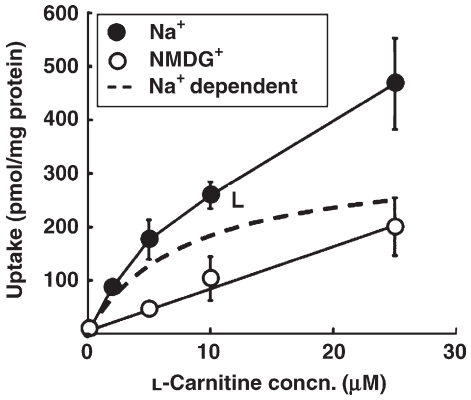

Fig. 5. Concentration dependence of L-carnitine uptake by Caco-2 cells. L-[^{3}H]-carnitine uptake by Caco-2 cells at various concentrations of L-[^{3}H]-carnitine from 5 nM to 25 μM was measured for 30 min. The L-[^{3}H]-carnitine uptake was measured in a buffer containing Na^+ (●) or $NMDG^+$ (○). The dashed line shows the Na^+-dependent component of L-[^{3}H]-carnitine uptake, determined by subtracting $NMDG^+$ from Na^+ at each concentration of L-[^{3}H]-carnitine uptake.

Santa Cruz, CA). The cells were subsequently incubated for 1 h at room temperature with an FITC-conjugated donkey anti-goat secondary antibody (Santa Cruz Biotechnology) at a dilution of 1:200. Nuclei were stained with DAPI and the cells were mounted in VECTASHIELD Mounting Medium (Vector Laboratories, Burlingame, CA). The OCTN2 protein was visualized by using a confocal microscope (Zeiss LSM-510; Carl Zeiss Inc., Thornwood, NY).

2.6. Uptake experiment

Uptake experiments using Caco-2 cells were carried out as described previously [26]. The L-carnitine uptake was linear for 60 min. Therefore, the L-carnitine uptake was determined at uptake time for 30 min in all uptake experiments. The cells were washed two times with Hanks' balanced salt solution (HBSS) containing 137 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.3 mM KH_2PO_4, 0.4 mM NaH_2PO_4, 4.2 mM $NaHCO_3$, 25 mM D-glucose, and 10 mM HEPES and then equilibrated in HBSS at 37 °C for approximately 10 min. L-[^{3}H]-Carnitine (0.25 μCi/mL) premixed with or without inhibitors in warm HBSS was added to the cells in a shaking hot box (37 °C). When required, Na^+ was isosmotically substituted by *N*-methyl-D(−)-glucamine ($NMDG^+$) or other ions. The dosing solutions were then aspirated, and the cells were washed with ice-cold HBSS two times followed by addition of 0.5 mL of lysing solution (1% SDS in 0.2 N NaOH), and shaking of the plate in the hot box was continued. Samples of the lysate were then collected for scintillation spectrometry (1600 TR, Packard Instruments, Meriden, CT) and protein assay by Lowry's method with bovine serum albumin as the standard.

2.7. Oral administration experiment

Wistar rats were fasted overnight (16 h) prior to oral administration experiment using L-[^{3}H]-carnitine with a dose of 204 ng/kg (81 μCi/kg) in saline solution. Blood samples (0.1 mL) were collected into tubes from a cervical vein at 15, 30, 45, 60 and 90 min after administration of L-[^{3}H]-carnitine. Following centrifugation (3,000 rpm, 10 min), 20 μL of serum was used for scintillation spectrometry (1600TR, Packard). In the case of inhibitory experiments, LVFX was dissolved in L-[^{3}H]-carnitine solution to a final concentration of 62.5 mM.

2.8. Analytical procedures

Student's *t*-test was used to determine the significance of differences between two group means. For kinetic studies, the Michaelis–Menten constant and the stoichiometry between L-carnitine and Na^+ were fitted to the following equations by a nonlinear least-squares regression analysis using Origin® (version 6.1J):

$$v = V_{max}S/(K_m + S) + Kd\ S, \tag{1}$$

$$\log[v/(V_{max} - v)] = n\ \log[Na^+] - \log K_{Na}, \tag{2}$$

where v and S are the uptake rate and concentration of the substrate, respectively, and K_m, V_{max}, K_{Na} and n are the half-saturation concentration

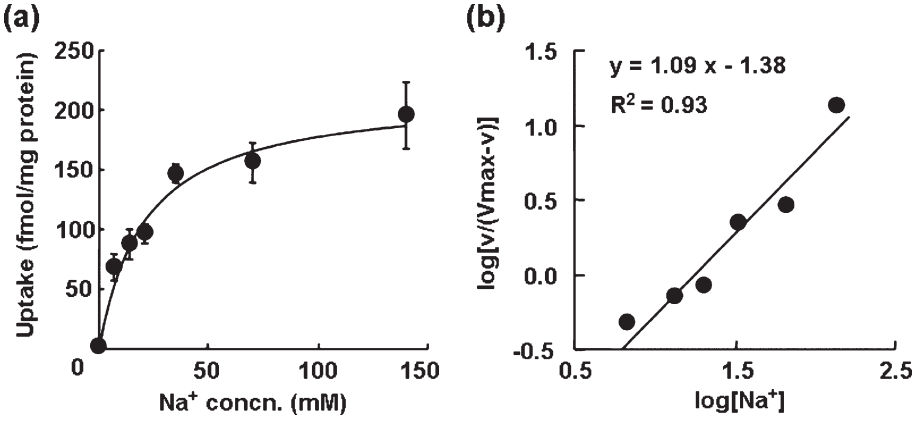

Fig. 4. Na^+-dependent L-carnitine uptake (a) and Hill plot analysis (b). L-[^{3}H]-carnitine (5 nM) uptake was measured at Na^+ concentrations from 0 to 140 mM for 30 min (a). L-[^{3}H]-carnitine uptake is shown by a Hill plot (b). Na^+ was replaced with $NMDG^+$ to maintain osmolarity.

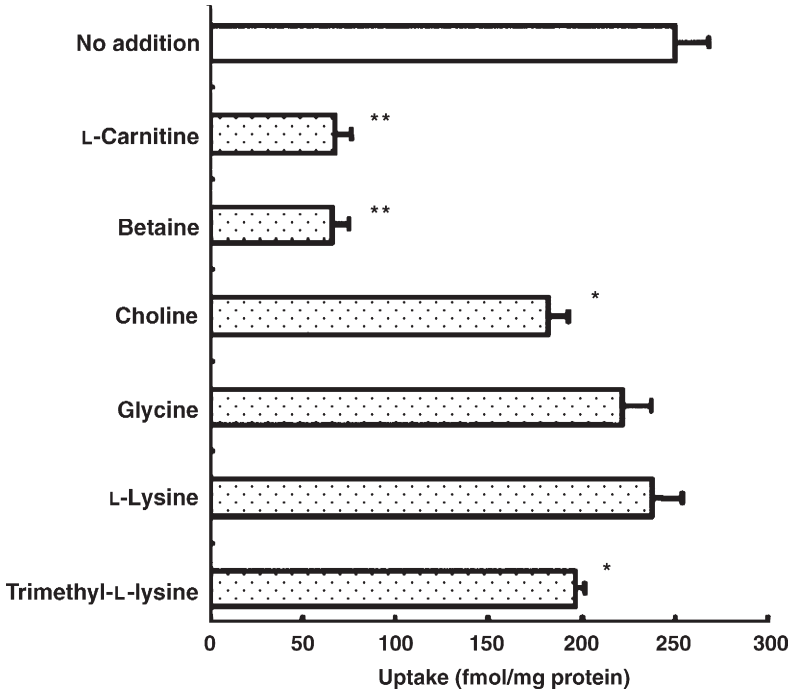

Fig. 6. Inhibitory effect of structure homologue on L-carnitine uptake. L-[^{3}H]-carnitine (5 nM) uptake was measured for 30 min in the presence of 5 mM of each structure homologue of L-carnitine except for unlabeled L-carnitine (0.5 mM). *Significantly different from no addition at $p<0.05$, **$p<0.01$.

(Michaelis constant), maximum transport rate, half-saturation concentration of Na^+ and Hill coefficient, respectively.

3. Results

3.1. Expression of OCTN family members in Caco-2 cells

In the first part of this study, we investigated the mRNA expression levels of OCTN family members by RT-PCR analysis using total RNA isolated from Caco-2 cells and specific primers of OCTN1 and OCTN2. OCTN2 and GAPDH mRNA were detected on size for 540 bp clearly, but there was no band of OCTN1 mRNA (Fig. 1). Furthermore, we performed immunocytochemistry to investigate the expression of OCTN2 protein in Caco-2 cells. A green signal (FITC, OCTN2) was observed around a blue signal (DAPI, nucleus), suggesting that OCTN2 is expressed at the plasma membrane (Fig. 2).

3.2. L-Carnitine uptake into Caco-2 cells

To study the effects of various cations on specificity of L-carnitine uptake in Caco-2 cells, Na^+ in the transport medium was replaced with various cations. Fig. 3 shows that the level of L-carnitine uptake was highest in the presence of Na^+ and

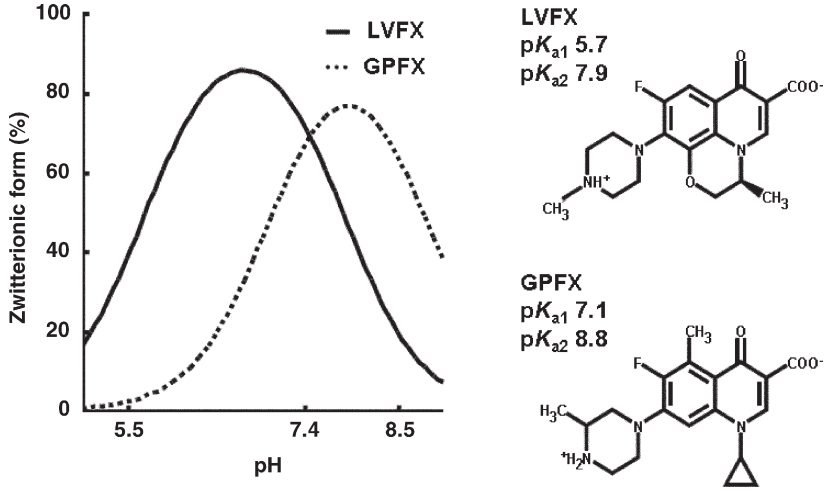

Fig. 7. Theoretical ratio of zwitterionic forms of LVFX and GPFX under various pH conditions. The ratios of zwitterionic forms of LVFX and GPFX were calculated by using the Henderson–Hasselbalch equation.

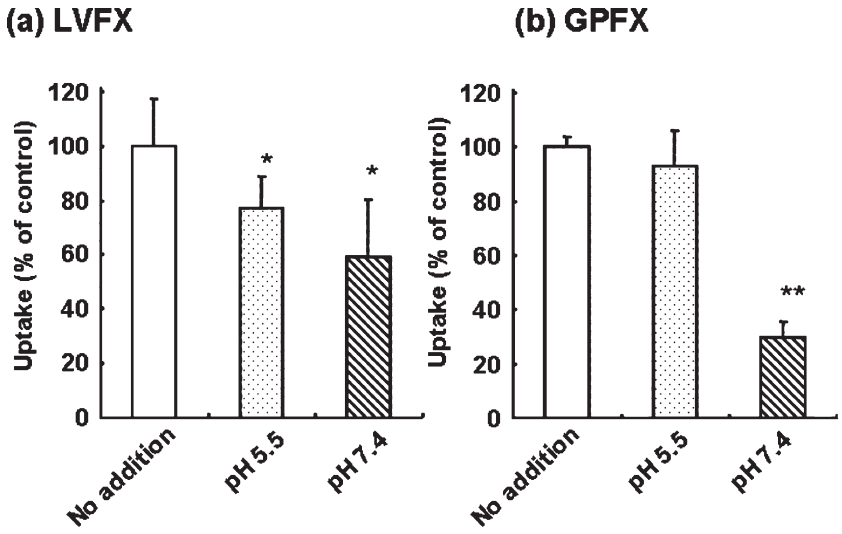

Fig. 8. Inhibitory effects of LVFX (a) and GPFX (b) on L-carnitine uptake at pH 5.5 and pH 7.4. L-[^{3}H]-carnitine (5 nM) uptake at pH 5.5 or 7.4 was measured for 30 min in the presence of 5 mM of LVFX or 2 mM of GPFX. *Significantly different from no addition at $p<0.05$, **$p<0.01$.

decreased significantly in the presence of other cations. Furthermore, replacement of Cl^- with other anions had no effect on L-carnitine uptake.

Since L-carnitine transport by OCTN2 has been shown to be Na^+-dependent [27], we examined Na^+-dependent uptake of L-carnitine in Caco-2 cells (Fig. 4). It was found that L-carnitine uptake is saturable, and nonlinear least-squares analysis showed a half- saturation Na^+ concentration of 10.3±4.5 mM (Fig. 4a). Moreover, Hill plot analysis showed that the stoichiometry of transport of L-carnitine and Na^+ was 1.09, suggesting that one Na^+ molecule is associated with the transport of one molecule of L-carnitine in Caco-2 cells (Fig. 4b).

To clarify L-carnitine uptake into Caco-2 cells in detail, the concentration dependence of L-carnitine was studied. We analyzed Na^+-dependent L-carnitine uptake by subtracting the Na^+-independent component from total L-carnitine uptake (Fig. 5). The Na^+-dependent component of L-carnitine was saturable. Nonlinear least-squares analysis showed a K_m value of 8.0±1.0 μM.

3.3. Effects of structural homologues and quinolones on L-carnitine uptake

The effects of structural homologues on L-carnitine uptake were studied. As shown in Fig. 6, betaine and choline significantly inhibited L-carnitine uptake. On the other hand, glycine and L-lysine had no effect on L-carnitine uptake. However, a significant inhibitory effect on L-carnitine uptake was observed in a presence of trimethyl-L-lysine.

Since both LVFX and GPFX have a carboxylate and an amine, they have two pK_a values: 5.7 and 7.9 for LVFX and 7.1 and 8.8 for GPFX, respectively. We calculated the ratio of zwitterions of these two quinolones under each pH buffer condition. According to theoretical calculations, about 75% of the total amount of LVFX and 64% of GPFX exist as zwitterionic form under a pH 7.4 buffer condition. In contrast, only 2% of the total amount of GPFX exists as a zwitterionic form under a pH 5.5 buffer condition, while about 39% of LVFX exists as a zwitterionic form under the same condition (Fig. 7).

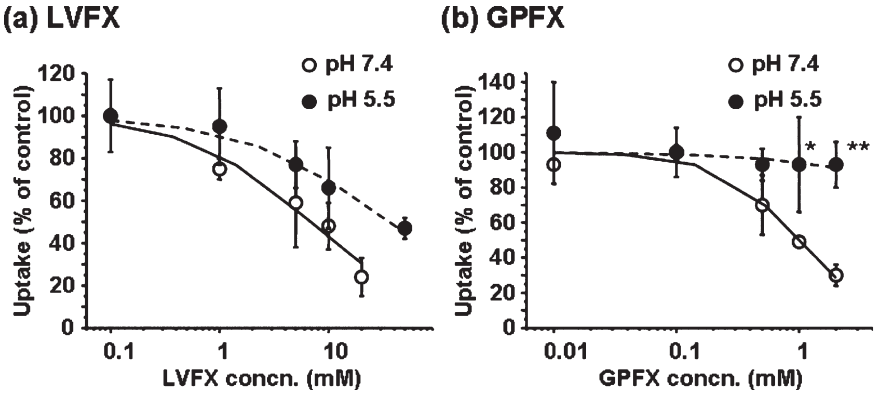

Fig. 9. Inhibitory effects of various concentrations of LVFX (a) and GPFX (b) on L-carnitine uptake. L-[^{3}H]-carnitine (5 nM) uptake at pH 5.5 or 7.4 was measured for 30 min in the presence of LVFX at concentrations of 0.1–50 mM (a) or in the presence of GPFX at concentrations of 0.01–2 mM (b). *Significantly different from pH 7.4 at $p<0.05$, **$p<0.01$.

Table 1
Ratio of zwitterionic form and IC_{50} value of quinolones on L-carnitine uptake under each pH condition

		Ratio of zwitterionic form (%)	IC_{50}
LVFX	pH 5.5	39	36.1 mM
	pH 7.4	75	6.8 mM
GPFX	pH 5.5	2	–
	pH 7.4	64	1.0 mM

Inhibitory effects of LVFX and GPFX on L-carnitine uptake under pH 5.5 and pH 7.4 buffer conditions were studied. L-Carnitine uptake in the presence of 5 mM LVFX was decreased to 60–80% of the control level under both buffer conditions. On the other hand, while 2 mM GPFX inhibited L-carnitine uptake significantly under a pH 7.4 buffer condition, it had no effect on L-carnitine uptake under a pH 5.5 buffer condition (Fig. 8).

To elucidate the effect of pH buffer condition on the inhibitory mechanism of quinolones, we examined the inhibitory effects of various concentrations of LVFX and GPFX on L-carnitine uptake under both pH 5.5 and 7.4 buffer conditions. LVFX inhibited L-carnitine uptake at pH 7.4 more strongly than that it did at pH 5.5 (Fig. 9a), and the IC_{50} value of LVFX at pH 5.5 was about 5-fold higher than that at pH 7.4 (Table 1). The inhibitory effect of GPFX was also studied. The difference between the inhibitory effects of GPFX under pH 5.5 and pH 7.4 buffer conditions was more apparent than that of LVFX, and the inhibitory effect of GPFX at pH 5.5 on L-carnitine uptake was significantly weaker than that at pH 7.4 (Fig. 9b). Furthermore, we investigated the kinetic nature of the inhibitory effect of LVFX on L-carnitine uptake. Eadie–Hofstee plot analysis showed that LVFX inhibits L-carnitine in a non-competitive manner (Fig. 10). Nonlinear least-squares analysis showed a K_i value of 9.0 mM.

3.4. Effect of LVFX on L-carnitine absorption in rats

We investigated whether LVFX has an inhibitory effect on L-carnitine absorption from the rat intestine. L-Carnitine and LVFX were administrated together as described in Materials and Methods. Coadministration of LVFX had no effect on C_{max} of L-carnitine, but T_{max} of L-carnitine was delayed significantly from 45 min to 90 min by LVFX coadministration (Fig. 11).

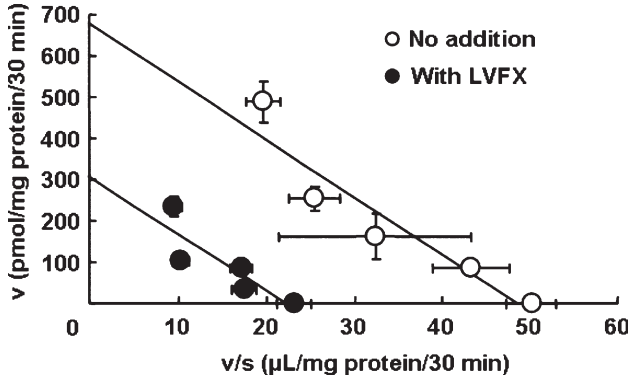

Fig. 10. Eadie–Hofstee plot analysis of the effect of LVFX on L-carnitine uptake. L-[^{3}H]-carnitine (5 nM) uptake at pH 7.4 was measured for 30 min in the absence (○) or presence (●) of LVFX at 10 mM.

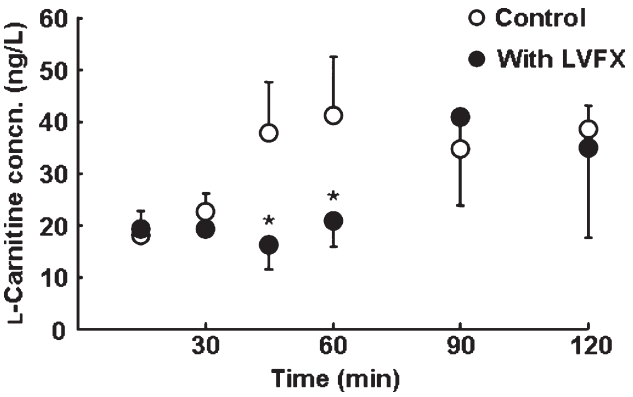

Fig. 11. Inhibitory effect of LVFX on L-carnitine absorption from the rat intestine. Serum concentration of L-carnitine was determined after a single p.o. administration (204 ng/kg) in the absence (○) or presence (●) of LVFX at 62.5 mM. *Significantly different from control at $p<0.05$.

4. Discussion

Although L-carnitine is synthesized by methionine and lysine in humans, its amount is not sufficient to maintain homeostasis [28]. Therefore, L-carnitine absorption from dietary sources in the intestine plays an important role in the maintenance of L-carnitine homeostasis [3]. It has been reported that L-carnitine absorption from the intestine is Na^+-dependent, suggesting that OCTN2 transports L-carnitine mainly in the intestine [29,30]. Recently, the expression and characteristics of intestinal OCTN2 in Caco-2 cells were reported [9]. However, the mechanism of the substrate recognition and inhibitory mechanism of OCTN2 have not been investigated in detail.

In the first part of this study, we investigated the mRNA expression levels of OCTN family member and characterized the L-carnitine transport mechanism in Caco-2 cells. RT-PCR analysis showed the expression of OCTN2 mRNA. On the other hand, OCTN1 mRNA was not detected (Fig. 1). Although it has been reported that OCTN1 is expressed in Caco-2 cells [31], our results suggested that the expression level of OCTN1 is much lower than that of OCTN2 in Caco-2 cells which we used. OCTN1 is also known to transport L-carnitine in other tissues [32,33], but the results of our study suggested that OCTN1 had little contribution to L-carnitine transport. Moreover, while Elimrani et al. have reported that OCTN2 expression level is highest in mature Caco-2 cells, the expression of OCTN2 protein was observed clearly by immunocytochemistry (Fig. 2).

Since OCTN2 transports L-carnitine Na^+-dependently, we investigated the uptake of L-carnitine in transport buffer containing Na^+ or other cations. While replacement of Cl^- with other anions had no effect on L-carnitine uptake, replacement of Na^+ with other cations significantly decreased L-carnitine uptake (Fig. 3). Kinetic analysis showed that the half-saturation Na^+ concentration, Hill coefficient and K_m value were 10.3± 4.5 mM, 1.09 and 8.0±1.0 μM, respectively (Figs. 4 and 5). It

has been reported that $ATB^{0,+}$ is also a candidate for L-carnitine transport; however, the K_m value of $ATB^{0,+}$ has been reported to be 0.83±0.08 mM [10], suggesting that $ATB^{0,+}$ does not contribute to L-carnitine transport in our study. Furthermore, these characteristics and parameter values are similar to those in studies using HEK293 cells transiently expressing human OCTN2 [27]. Taking together, these facts suggesting that OCTN2 contributes greatly to L-carnitine transport in Caco-2 cells.

It has been reported that some zwitterionic drugs inhibit L-carnitine transport [33]. It has therefore been suggested that substrate recognition by ionic charge of a drug is one of the mechanisms by which OCTN2 functions. First, we investigated the inhibitory effects of structural homologues on L-carnitine uptake (Fig. 6). Betaine, which is a zwitterionic compound, strongly inhibited L-carnitine uptake, but glycine and L-lysine had no inhibitory effect despite the fact that they are zwitterions. However, trimethyl-L-lysine inhibited L-carnitine uptake. Furthermore, choline, which is a cationic compound, significantly inhibited L-carnitine uptake. L-carnitine, betaine, choline and trimethyl-L-lysine each have a quaternary amino group in their cationic structures. but glycine and L-lysine do not. These facts suggest that the ionic charge of a compound is important for substrate recognition of OCTN2, but the structure of the cationic region of a compound is also involved in the substrate recognition mechanism of OCTN2.

We elucidated the effects of two quinolones (LVFX and GPFX), which are zwitterions and each have a higher amino group (tertiary for LVFX and secondary for GPFX) in their structures, on L-carnitine uptake. LVFX and GPFX have different pK_a values, and the existence ratios of zwitterionic forms were also found to be different under each pH buffer condition (Fig. 7). It has been reported that the inhibitory effect of quinapril (angiotensin-converting enzyme inhibitor) on PepT1 is changed by its ratio of zwitterionic form [34]. Moreover, Yamaguchi et al. reported that uptake of LVFX and GPFX in Caco-2 cells under an acidic condition was less than that under a neutral condition [22]. We therefore hypothesized that the ratio of zwitterionic form of quinolones is involved in the inhibitory effect on L-carnitine uptake by OCTN2. Figs. 8 and 9 show the inhibitory effects of LVFX and GPFX on L-carnitine uptake under pH 5.5 and 7.4 buffer conditions. It was clear that both quinolones inhibited L-carnitine uptake more strongly at pH 7.4 than at pH 5.5. Especially GPFX had no effect on L-carnitine uptake at pH 5.5. The ratios of zwitterionic forms of LVFX and GPFX were about 65–75% in a neutral condition but decreases to 39% for LVFX and 2% for GPFX under an acidic condition (Fig. 7), suggesting that quinolones need to exist as zwitterions for inhibiting OCTN2 function and that the inhibitory effect of the cationic form, if any, is weak (Table 1). It has also been shown that the transport activity of OCTN2 decreases under an acidic condition [35]. We also investigated the pH dependence of L-carnitine uptake in Caco-2 cells and found that the amount of L-carnitine uptake at pH 5.5 was decreased to about 60–70% of the control level (data not shown). However, K_m values at pH 7.4 and 5.5 were 8.0±1.0 μM and 15.5±2.0 μM, respectively, suggesting that pH conditions had little effect on transport activity of OCTN2 in these inhibitory experiments. We investigated the kinetic nature of the inhibitory effect of LVFX at pH 7.4. It was clearly shown that LVFX inhibits L-carnitine transport in a non-competitive manner (Fig. 10). Some beta-lactam antibiotics have been suggested to be transported by OCTN2 [36]. However, it has been reported that GPFX transport in Caco-2 cells is Na+-independent [22], suggesting that quinolones are not transported by OCTN2. Ohashi et al. proposed that OCTN2 has both anion and cation binding sites for zwitterionic compounds [37]. Our results suggest quinolones bind to those site and have an inhibitory effect on L-carnitine transport. However, because of their more bulky structures than the structure of L-carnitine, inhibitory effect of quinolones might not compete to recognition of L-carnitine by OCTN2. Moreover, an L-carnitine absorption study using rat intestines showed that LVFX inhibited L-carnitine absorption and that T_{max} of L-carnitine was delayed significantly (Fig. 11). Since the acidic condition of the intestine is maintained *in vivo*, these results suggest that drugs exist as zwitterions in a higher ratio under an acidic condition and that a stronger inhibitory effect on L-carnitine transport might occur. However, GPFX inhibited L-carnitine uptake more strongly than did LVFX, while the ratio of zwitterionic forms of both quinolones are similar under a neutral buffer condition. Although the results of this study suggested that the ratio of zwitterionic form and amino group structure are important for substrate recognition of OCTN2, further investigation is needed to clarify the difference in affinity of those drugs to OCTN2.

In summary, L-carnitine was found to be mainly transported by OCTN2 in Caco-2 cells. LVFX and GPFX inhibited L-carnitine transport via OCTN2 in the ratio of zwitterionic forms depending manner, suggesting that chemical structures, ionic charge and structure of cationic region of drugs or compounds play important roles in the inhibition of L-carnitine transport in the intestine.

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