Increases in Bioavailability of Poorly Absorbed Drug by Acylcarnitine

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ABSTRACT: We examined the effect of acylcarnitines on the *in situ* bioavailability of lucifer yellow (LY) from the loops of small and large intestines of rats. The area under the blood concentration of LY versus time curve (AUC) from the jejunum was significantly increased by the treatments of the loop with 100 μM lauroylcarnitine (LC) or 100 μM palmitoylcarnitine (PC) (fourfold and 17-fold, respectively). No marked change in the expression of claudin-4 protein was observed by the treatments. On the contrary, the expression of P-glycoprotein (P-gp) was decreased by the treatment, more greatly by PC than by LC, suggesting that increases in the bioavailability of LY by LC and PC are associated with the decreased expression of P-gp in jejunum. The increase in the bioavailability was also observed for colon by the treatment of LC, but not that of PC. LC decreased the expression of claudin-4 protein, whereas PC decreased the expression of P-gp in colon. Therefore, LC and PC appear to have different impact on the intestinal transporters depending on the site (i.e., jejunum and colon). © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:3511–3517, 2012

Keywords: lauroylcarnitine; palmitoylcarnitine; bioavailability; rat intestine; lucifer yellow; regional difference; claudin-4; P-glycoprotein

INTRODUCTION

For intestinal absorption of water-soluble and less lipophilic drugs, the paracellular route through the tight junction (TJ) to the lateral intercellular space is considered an effective absorption route.[1] As effective absorption enhancers, lauroylcarnitine (LC) and palmitoylcarnitine (PC) have been reported to enhance the absorption of a hydrophilic compound by widening the TJ on the basis of claudin-4 expression level and dysfunction, including P-glycoprotein (P-gp) expression level,[2–4] as well as by perturbing the brush border membrane.[5] LC and PC were also reported to increase intestinal permeability, inducing claudin-4 depletion and decreasing the electric membrane resistance, resulting in increased permeability of hydrophilic compounds in Caco-2 cell monolayers.[2,6]

Because all of these results were observed in experimental animal and human tissues using *in vitro* sheet and *in vitro* cultured cell systems, we compared the effects of the absorption enhancers LC and PC on the intestinal absorption of lucifer yellow (LY) as a poorly absorbed model compound using the *in situ* loop technique in rat. In particular, the applied concentrations of LC and PC on the basis of their safety in practical use and regional differences in absorption effects were examined.

MATERIALS AND METHODS

Materials

Lauroylcarnitine, PC, and LY were purchased from Sigma Aldrich Company Ltd. (St. Louis, Missouri). All other reagents were of analytical grade or higher.

Animals and Experimental Design

Male Wistar rats (8 weeks old) were purchased from Japan SLC Ltd. (Shizuoka, Japan). All the animal experiments were performed in accordance with the guidelines of Tokyo University of Pharmacy and Life Science. The animals were acclimatized for 2 days before assignment to experimental groups at 7–9 weeks of age (200–300 g), and were housed in wire-bottom cages to avoid contact with fecal excrement, in a clean room maintained at 23 ± 2°C with relative humidity

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of 55 ± 10% and a 12-h light–dark cycle. Rats were allowed free access to regular animal diet and drinking water, with the exception of food deprivation overnight before the experiment. The rats used in this study were handled in accordance with the guidelines of Tokyo University of Pharmacy and Life Sciences.

Absorption Experiments Using the *In Situ* Loop Method

Intestinal absorption was evaluated by the method described in our previous report.[7] Rats were fasted overnight and fixed in a supine position under anesthesia with sodium pentobarbital (50 mg/kg, intraperitoneal). After laparotomy by a middle incision, a 10-cm-long segment from the duodenum to upper jejunum (15 cm length from Treiz's ligament), or from the proximal colon to the distal colon was isolated. The lumen of the segment was washed with prewarmed physiological saline using a syringe, and the remaining saline solution was expelled with air. Both ends were carefully ligated with sutures using polyethylene cannula (outer diameter 0.8 mm, inner diameter 0.5 mm) into the lumen to inject drug solution so as to preserve the vascular supply. Five milliliters of Krebs Henselite bicarbonate buffer (KHBB; 141 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM H_2CO_3, 0.4 mM NaH_2PO_4, K_2HPO_4, 25 mM glucose; pH 7.4) solution containing LY (5 μM) with or without verapamil, LC or PC was administered into the loop. We used verapamil for P-gp inhibitor in this paper because verapamil *in vivo* experiments have been confirmed as a specific inhibitor[8] for several years. The concentrations of LY as a model compound of poorly absorbed marker were measured using a fluorescent spectrophotometer (HITACHI FP-6500; HITACHI, Tokyo, Japan) with excitation and emission wavelengths of 490 and 520 nm, respectively. Blood samples (0.3 mL) were collected every 15 min up to 120 min after the intraloop administration of drug solution, and the intravenous (i.v.) cannula was filled with heparin solution (100 U/mL in physiological saline) during sampling intervals. Blood samples were transferred to tubes containing 5 units of heparin and centrifuged (600 *g*, 15 min). The plasma samples obtained were stored at 4°C until analysis.

Pharmacokinetic Analysis of Plasma Concentration of LY after i.v. and Intraluminal Administration

Area under the plasma concentration–time curve from 0 min to infinity ($AUC_{0\to\infty}$) was calculated by the trapezoidal method. Absorption ratio (*F*) of LY was obtained by the ratio of $AUC_{0\to\infty}$ after intraluminal administration ($AUC_{0\to\infty,loop}$) to that after i.v. administration ($AUC_{0\to\infty,i.v.}$). The mean residence time (MRT) was also calculated by trapezoidal rule based on the statistical moment theory.[9] The mean absorption time (MAT) was obtained by the difference between MRT of LY after intraluminal administration (MRT_{loop}) and that after i.v. administration ($MRT_{i.v.}$).

Determination of P-gp Level Using Western Blot Analysis

An *in situ* ileum loop (10 cm in length) was prepared in each rat and KHBB solution with or without acylcarnitine (100 μM) (pH 7.4) was administered into the loop (5.0 mL). At 60 min after administration, the crude membrane fractions were prepared from the jejunum using magnesium chloride precipitation.[10,11] Briefly, the luminal contents of jejunum were thoroughly washed out with a sufficient amount of ice-cold saline and the jejunum was divided into several parts of the same length. The jejunal mucosal surface was scraped off with a slide glass. The collected mucosa was homogenized in a buffer containing 0.05 mg/mL phenylmethylsulfonylfluoride (PMSF), 300 mM mannitol, 12 mM Tris, and 5 mM ethylene glycol tetraacetic acid) (pH 7.1) with a tissue homogenizer. An aqueous solution of 10 mM magnesium chloride was added to the homogenate. The homogenate was centrifuged at 3000 *g* for 10 min. The supernatant was then centrifuged at 42,000 *g* for 30 min. The pellet was resuspended in 300 mM mannitol, 20 mM HEPES), 10 mM Tris, and 4 mM magnesium chloride (pH 7.4). The protein expression levels of P-gp in the crude membrane fraction were evaluated by Western blotting. Western blotting using C219 monoclonal antibody (Alexis, San Diego, California) for P-gp was performed as reported previously.[12]

Determination of Claudin Protein Level Using Western Blot Analysis

Crude membrane fractions were homogenized in a storage buffer containing 19 mM KH_2PO_4, 81 mM K_2HPO_4, 1 mM ethylenediaminetetraacetic acid disodium salt, 1 mM dithiothreitol, 20% glycerol, 20 μM butylated hydroxytoluene, and 0.5 mM PMSF using a sonication homogenizer. Claudins in the detergent solubilization samples were detected by Western blot analysis. For the samples solubilized by the detergent, we determined the protein amount by the Lowry method and loaded equal protein amounts in each well. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was conducted in 5%–20% (w/v) gradient polyacrylamide gels and then the separated proteins on the gel were electrophoretically transferred onto polyvinylidene difluoride membranes as described by Tsuji and coworkers.[13] The membranes were blocked with milk protein-based BlockAce™ (Dainippon Pharmaceutical Company, Osaka, Japan) for 20 min with vigorous shaking followed by washing three times with phosphate-buffered saline containing 0.1% Tween 20. They were then incubated with appropriate antibodies overnight at 4°C. After washing, the membranes were incubated with horseradish

peroxidase-conjugated secondary antibodies to detect the primary antibodies. Specific bands were detected using ECplus. The bands were quantified with CS Analyzer (ATTO, Tokyo, Japan).

Lactate Dehydrogenase Assay

Cytotoxicity was examined using a cytotoxicity detection kit (MK401, TaKaRa®; TaKaRa Bio Inc., Shiga, Japan). Effects of LC and PC on the leakage of lactate dehydrogenase (LDH) in jejunal (a) and colonic (b) perfusate were examined. LDH leakage 60 min after collection of the jejunal and colonic epithelial cell membrane after treatment of the system with 2% Triton X 100 (TX-100) is related to the value of 100%, namely, maximum value of leakage.

Statistical Analysis

All the results are expressed as mean value ± standard error (Mean ± SE). Statistical significance between two groups was analyzed using Dunnett's test, and p values less than 0.05 were considered to be statistically significant.

RESULTS

Possibility of LY as a P-gp Substrate

In Figure 1, the absorption of LY after intestinal administration was examined in the presence of verapamil and its absence. The concentration of LY used in this study was 5 μM. Although the plasma concentration of LY was very low in the absence of verapamil, the coadministartion of 25 μM verapamil remarkably increased the plasma concentration of LY. These results suggest that LY is a substrate of P-gp.

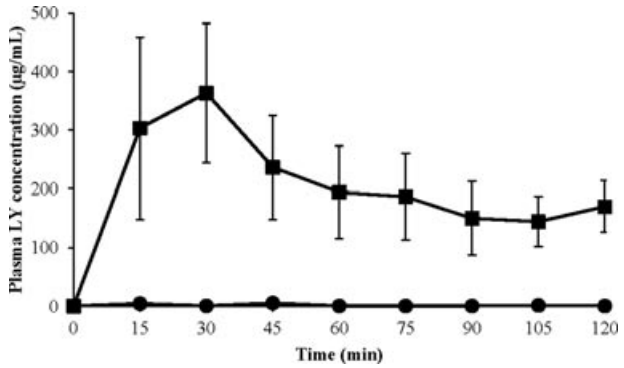

Figure 1. Plasma concentration of lucifer yellow (LY) after jejunal administration in the presence of verapamil (closed square) and its absence (closed circle). The luminal concentration of LY and verapamil is 5 and 25 μM, respectively. Each point with a bar represents the mean and standard error (SE) of 4–6 experiments.

Table 1. Pharmacokinetic Parameters of Lucifer Yellow Administered Intravenously to Acylcarnitine-Treated Rats

Parameters	Control	LC	PC
$AUC_{0\to\infty,i.v.}$ (mg mL^{-1} min)	129 ± 15	107 ± 5	144 ± 48
$MRT_{i.v.}$ (min)	3.28 ± 0.92	3.11 ± 0.52	3.98 ± 1.22

LC, lauroylcarnitine; PC, palmitoylcarnitine.
The concentration of acylcarnitines used in this study was 100 μM.
$AUC_{0\to\infty,i.v.}$ was calculated by the trapezoidal method.
Each value represents the mean ± SE of six determinations.

Status after i.v. Administration

Plasma concentration–time profiles following i.v. administration of LY were shown in Figure 2 and the pharmacokinetic (PK) parameters are summarized in Table 1. No marked change in PK parameters of LY in LC- and PC-treated rats was observed when compared with the control group.

PK Profile of LY after Treatment by Absorption Enhancer in Jejunum

Time courses of the plasma LY concentration in the control and absorption enhancer-treated rats after intrajejunal loop administration of LY are shown in Figure 3. Plasma LY levels in 100 μM LC-treated rats were higher than those of the control rats at 15, 30, and 45 min sampling points (Fig. 3). On the contrary, plasma LY levels in 100 μM PC-treated rats were higher up to 90 min (Fig. 3). Table 2 shows the PK parameters of LY in the three groups. In rats treated with LC and PC, there were about fourfold and 17-fold increases in the $AUC_{0\to\infty,loop}$, respectively, and concurrent increases in the value of F compared with those of control rats (Table 2). Decreases in MAT were observed in the presence of LC and PC (Table 2).

Table 2. Pharmacokinetic Parameters of Lucifer Yellow Administered to the Jejunal Loop in the Control, LC- or PC-Treated Rats

Parameters	Control	LC	PC
$AUC_{0\to\infty,loop}$ (mg mL^{-1} min)	1.14 ± 0.17	4.60 ± 0.86*	18.9 ± 4.8*,***
F (%)	0.89 ± 0.11	4.29 ± 0.69*	13.2 ± 3.2*,***
MRT_{loop} (min)	17.2 ± 5.7	12.2 ± 1.2	11.3 ± 2.0
MAT (min)	13.9 ± 4.6	9.13 ± 1.00**	7.30 ± 1.70**,***

LC, lauroylcarnitine; PC, palmitoylcarnitine.
The concentration of acylcarnitines used in this study was 100 μM.
$AUC_{0\to\infty,loop}$ was calculated by the trapezoidal method. F (absorption ratio) was obtained using $AUC_{0\to\infty,loop}/AUC_{0\to\infty,i.v.}$.
MAT was obtained as the difference between MRT_{loop} and $MRT_{i.v.}$.
Each value represents the mean ± SE of 4–10 determinations.
*$p < 0.05$ versus control.
**$p < 0.01$versus control.
***$p < 0.05$ versus LC-treated rats.

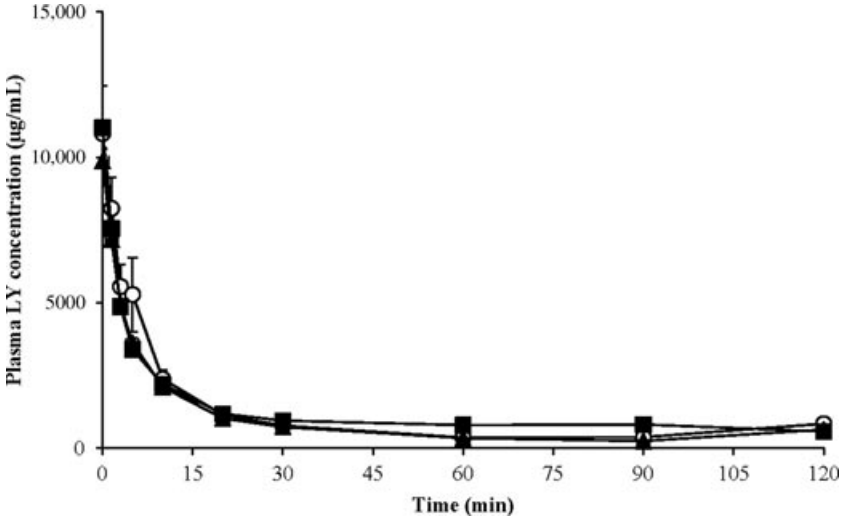

Figure 2. Plasma concentration–time curve of LY after intravenous administration to control (open circle), lauroylcarnitine-treated rats (closed triangle) and palmitoylcarnitine-treated rats (closed square). The concentration of lauroylcarnitine and palmitoylcarnitine used in this study was 100 μM. Each point with a bar represents the mean and SE of 4–10 experiments.

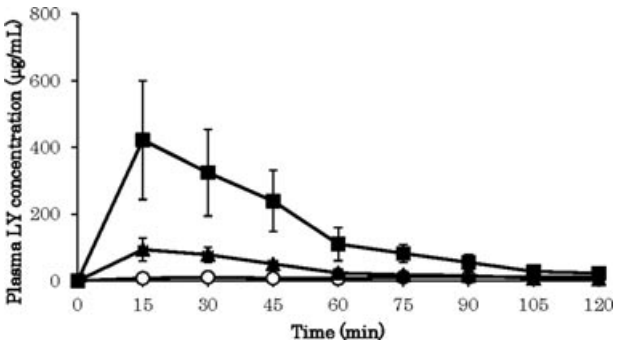

Figure 3. Plasma concentration–time curve of LY after administration to jejunal loop in the control (open circle), lauroylcarnitine-treated rats (closed triangle) and palmitoylcarnitine-treated rats (closed square). The concentration of lauroylcarnitine and palmitoylcarnitine used in this study was 100 μM. Each point with a bar represents the mean and SE of 4–10 experiments.

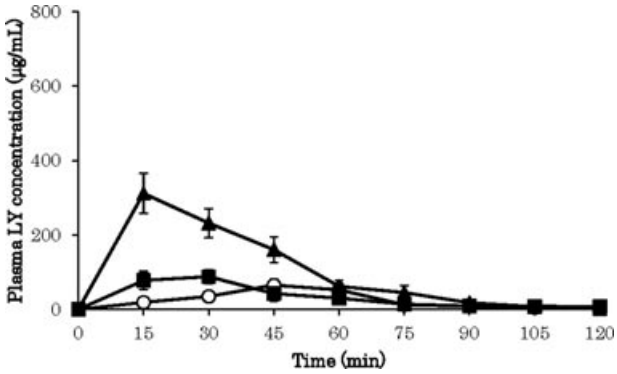

Figure 4. Plasma concentration–time curve of LY after administration to colonic loop in the control (open circle), lauroylcarnitine-treated rats (closed triangle) and palmitoylcarnitine-treated rats (closed square). The concentration of lauroylcarnitine and palmitoylcarnitine used in this study was 100 μM. Each point with a bar represents the mean and SE of 4–10 experiments.

PK Profile of LY after Treatment by Absorption Enhancer in Colon

Time courses of the plasma LY concentration in the control and absorption enhancer-treated rats after intracolon loop administration of LY are shown in Figure 4. Plasma LY levels in 100 μM LC-treated rats were higher than those of the control rats at 15, 30, and 45 min sampling points (Fig. 4). By contrast, values for 100 μM PC-treated rats were higher than those of the control rats just at 15 and 30 min (Fig. 4). Table 3 shows the PK parameters of LY in the three groups. In rats treated with LC and PC, there were about 4.2-fold and 1.4-fold increases in the $AUC_{0\rightarrow\infty,loop}$, respectively (Table 3). The enhancing effect of LC on LY absorption was larger than that of PC in the colon but not in the jejunum (Tables 2 and 3). In rats treated with LC, a tendency to decrease was observed but the difference was not statistically significant in MAT compared with that in the control rats (Table 3). No marked changes in MAT were observed in the presence of PC (Table 3).

Effects of LC and PC on the Protein Components of TJ

The opening of TJ is primarily dependent on the composition of the TJ components, especially the components responsible for the barrier function, such as claudin-4. Thus, we particularly focused on the effects of 60 min treatment of LC and PC on the protein expression level of claudin-4 (Fig. 5). The treatment

Table 3. Pharmacokinetic Parameters of Lucifer Yellow Administered to the Colon in the Control, LC- or PC-Treated Rats

Parameters	Control	LC	PC
$AUC_{0\to\infty,loop}$ (mg mL^{-1} min)	2.99 ± 0.73	12.6 ± 3.8*	4.25 ± 1.09
F (%)	2.33 ± 0.55	11.7 ± 3.4*	2.95 ± 0.73
MRT_{loop} (min)	14.9 ± 1.0	12.7 ± 5.8	16.5 ± 1.3
MAT (min)	11.6 ± 0.8	9.60 ± 5.00	12.5 ± 1.1

LC, lauroylcarnitine; PC, palmitoylcarnitine.
The concentration of acylcarnitines used in this study was 100 μM.
$AUC_{0\to\infty,loop}$ was calculated by the trapezoidal method. *F* (absorption ratio) was obtained using $AUC_{0\to\infty,loop}/AUC_{0\to\infty,i.v.}$. MAT was obtained as the difference between MRT_{loop} and $MRT_{i.v.}$.
Each value represents the mean ± SE of 4–10 determinations.
*$p < 0.05$ versus control.
**$p < 0.01$ versus control.

with LC had a great impact on the distribution of claudin-4 in colon (Fig. 5b). This protein expression level of the colonic brush border membrane fraction dramatically decreased (Fig. 5b). Unlike the claudin-4 in the presence of LC in colon, there was no significant difference in claudin-4 both in the LC treatment in jejunum (Fig. 5a) and in the presence of PC in both jejunum (Fig. 5a) and colon (Fig. 5b).

Effects of LC and PC on the Protein Expression Level of P-gp

We further examined changes in the expression level of P-gp by treatment with LC and PC for 60 min (Fig. 6). Significant decrease was found in P-gp in the presence of PC in jejunum and the level of P-gp in the presence of LC in jejunum showed a tendency to decrease (Fig. 6a). LC had no effect on the level of P-gp in colon (Fig. 6b). On the contrary, PC decreased its expression level in colon (Fig. 6b).

Effects of LC and PC on Leakage of LDH in Perfusate

To demonstrate the effects of LC and PC on cytotoxicity, the leakage of LDH from jejunal and colonic membranes was examined. No marked change in LDH release was observed for less than 100 μM LC and PC (Fig. 7).

DISCUSSION

In this study, LY was used as a probe compound for poorly absorbed drug, because LY is not metabolized by hepatic enzyme after entry to the systemic circulation. Also, LY is known as a permeable compound through the paracellular route. In this study, the possibility that LY is a P-gp substrate was examined using *in situ* method (Fig. 1). The plasma concentration after intestinal administration of LY (luminal concentration, 5 μM) was very low. However, the coadministartion of verapamil (luminal concentration, 25 μM), a representative P-gp substrate, remarkably enhanced the plasma concentration of LY,

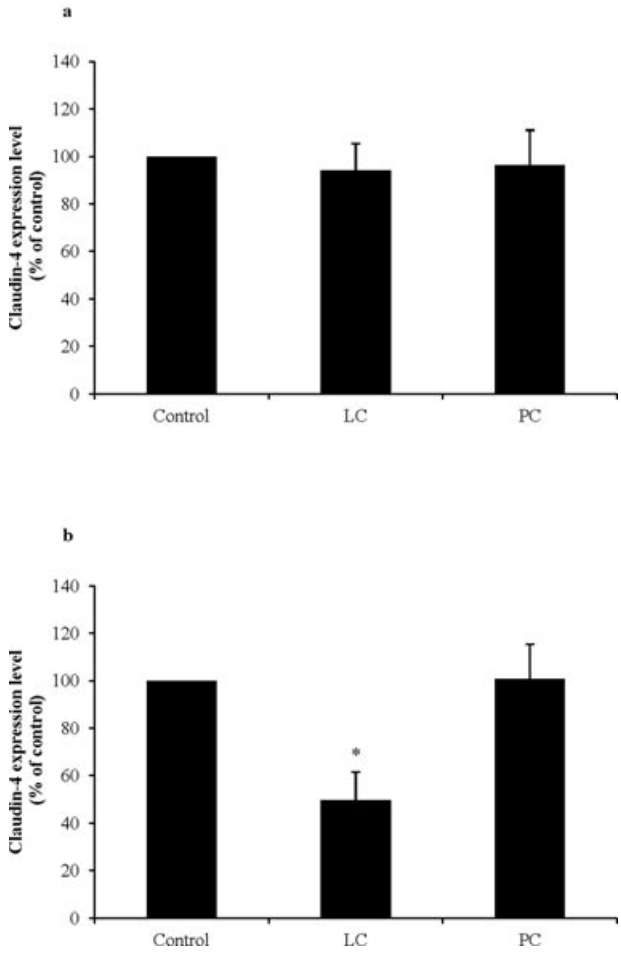

Figure 5. The level of claudin-4 protein in jejunal (a) and colonic (b) epithelial brush border membrane fractions determined by Western blotting. Values shown represent the mean and SE for three experiments per group. *Significantly different from the data for control ($p < 0.05$).

suggesting LY to be a substrate of efflux transporting systems, especially P-gp. Also in our previous studies, the basal-to-apical (secretary) transport of LY (10 μM) was six times greater than the apical-to-basal (absorptive) transport in Caco-2 cells,[4] where P-gp is overexpressed. These *in situ* and *in vitro* results show that LY can be used a P-gp substrate, a permeable compound through the transcellular route as well as the paracellular route.

The plasma concentration and $AUC_{0\to\infty,i.v.}$ of LY after i.v. administration did not change significantly after intraluminal administration of LC and PC when compared with the control condition (Fig. 2 and Table 1). No effect of LC and PC on the status of LY after i.v. administration was found.

In the present study, it was demonstrated that the *F* of LY from jejunal epithelial membrane was increased by about fourfold and 17-fold when compared with $AUC_{0\to\infty,loop}$, in the presence of LC and PC, respectively (Fig. 3 and Table 2). However, PC in colon

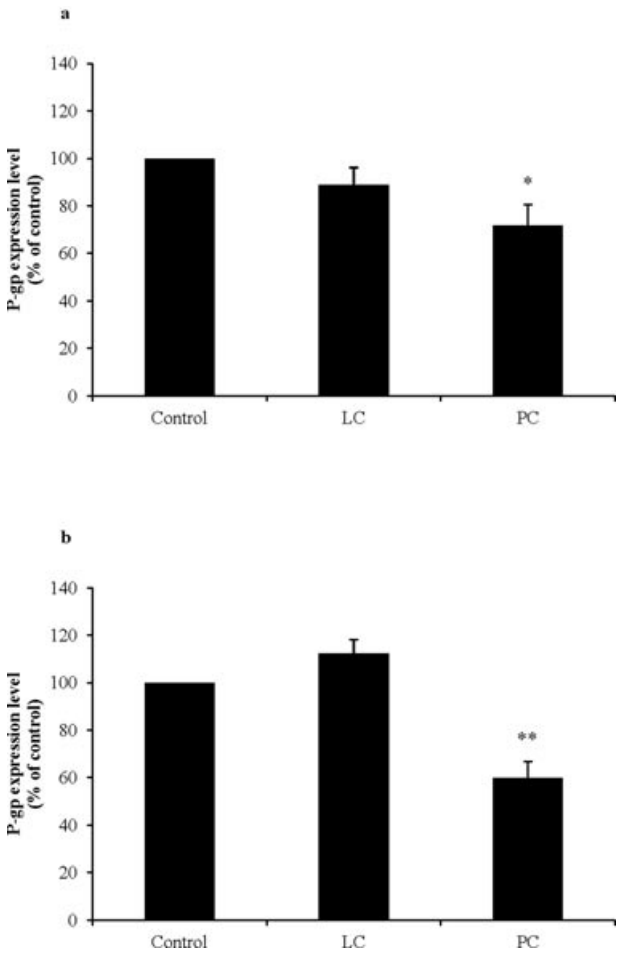

Figure 6. The level of P-gp protein in jejunal (a) and colonic (b) epithelial brush border membrane fractions determined by Western blotting. Values shown represent the mean and SE for three experiments per group. *Significantly different from the data for control ($p < 0.05$). **Significantly different from the data for control ($p < 0.01$).

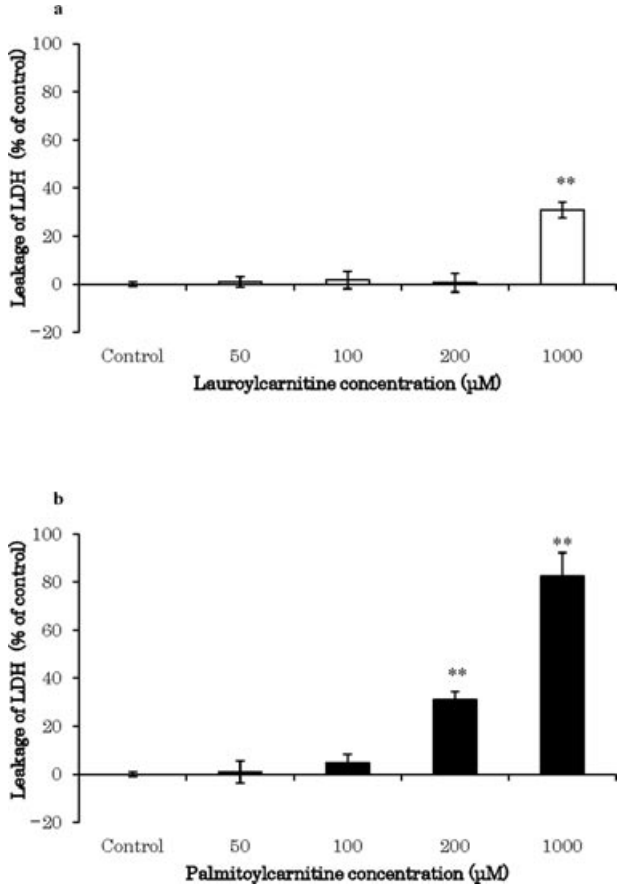

Figure 7. Effects of lauroylcarnitine and palmitoylcarnitine on the leakage of lactate dehydrogenase (LDH) in jejunal (a) and colonic perfusate. LDH leakage 60 min after collection of the jejunal and colonic epithelial cell membrane after treatment of the system with 2% Triton X 100 is related to the value of 100%, namely, maximum value of leakage. Data represent means and SE ($n = 6$–8 for each condition). **Significantly different from the data for control ($p < 0.01$).

did not induce any significant change in MRT_{loop} and MAT, including LY bioavailability (Fig. 4 and Table 3). These findings indicate that the applications of LC and PC in jejunum and LC in colon have marked effects on the improvement of bioavailability of LY as a poorly absorbed compound.

In the present study, it was demonstrated that the MAT of LY was decreased by LC and PC in jejunum (Table 2). Using the same concentration of LC and PC as in this study, we recently reported that LC and PC increase the permeation clearance of LY in the apical to basal direction through TJ opening and/or dysfunction, as well as reduction of the expression level of P-gp in Caco-2 cell monolayers.[2,4]

On the contrary, the rat intestine that expresses the claudin family is widely used for the study of TJs including claudin-4.[14] In the present study, we employed rat intestinal loop technique to examine the relationship in the *in vivo* system between the absorption-enhancing effect of LC and PC and the expression level of claudin-4. We examined the expression level of TJ proteins in plasma membrane by solubilization using TX-100 as a nonionic detergent. The applied concentration of LC and PC was fixed to 100 μM for 60 min to achieve TJ opening. Under the addition of LC, the expression level of claudin-4 in colon decreased significantly (Fig. 5b). In the previous study, LC and PC induced the displacement of claudin-4 from lipid raft containing insoluble fraction in Caco-2 cell monolayers.[4] In the present study, the effects of LC and PC on the expression level of proteins varied in a manner dependent on the kind of proteins: the effects of LC in colon tended to be greater for claudin-4 (Fig. 5b). In particular, claudin-4 can be considered to be affected by the F of LY induced by LC in colon (Fig. 5b). As for the effect of LC treatment in colon, claudin-4 was considered to be decreased from

plasma membrane (Fig. 5b). From these results, it can be considered that the mechanism of action of LC in colon is similar to that of LC in Caco-2 cell monolayers regarding the protein levels of claudin-4 and TJ function.

Previously, restoration of the barrier function by replenishment of TJ proteins in plasma membranes indicated that the LC and PC effect on the barrier function is reversible in Caco-2 cell monolayers.[4] These observations suggest the close relationship between barrier function of TJs and claudin-4 (Fig. 5). Because LC had significant effects in terms of decreasing the level of protein of claudin-4 in colon but not in jejunum, the detailed site-specific action of LC requires further investigation.

In the present study, we examined the relationship between the increases in *F* of LY and the profile of expressed P-gp in rat jejunal and colonic membranes in the presence of LC and PC. The expression of P-gp significantly influences the *F* of LY through jejunal absorption induced by LC and PC (Fig. 6a). The decrease of claudin-4 expression level by LC and PC treatment was not observed in jejunum (Fig. 5a), suggesting that LC and PC have common mechanisms of action to modulate the expression level of P-gp. However, our data indicated significant influence of PC treatment on the decrease of P-gp expression level in colon (Fig. 6b). The difference of the influence on P-gp expression level is considered to be related to the difference of critical micellar concentration between LC and PC (1.2 and 0.075 mM, respectively). Our present data can be explained partly by the contribution of paracellular transport and transcellular transport by secretory transporter in the intestine. The inhibitory action of verapamil is not limited to P-gp. Therefore, acylcarnitines is considered to have some effects on modulation of the function of P-gp or other efflux transporters. Although it is considered that adenosine triphosphate-binding cassette transporter such as P-gp makes a substantial contribution to the secretory transport, the contribution of the other secretory transporters and absorptive transporters, which have not been identified yet, can be well considered.

In the present experiments, no changes in leakage of LDH were observed for less than 100 μM concentrations of LC and PC in jejunum and colon (Fig. 7). This is because the concentration of LC and PC used as absorption enhancers in this study is expected to be closely related to the clinically safe and necessary amount of these compounds.

CONCLUSION

Our results demonstrate that absorption enhancement of LC involves down-regulation of a specific TJ protein, claudin-4, in colon. On the contrary, P-gp function and expression level are down-regulated by PC in jejunum. These findings offer profound insight into the detailed mechanism of action of acylcarnitines on the TJ proteins and efflux transporters such as P-gp. Those results indicated, the clinical use of LC and PC as excipients for the inhibition of intestinal P-gp and other efflux transporters is useful in the improvement of the oral bioavailability.

REFERENCES

1. Powell DW. 1981. Barrier function of epithelia. Am J Physiol 241:G275–G288.
2. Doi N, Tomita M, Hayashi, M. 2011. Absorption enhancement through displacement of tight junction proteins and cholesterol by acylcarnitines in Caco-2 cell monolayers. Drug Metab Pharmacokinet 26:1–9.
3. Shimazaki T, Tomita M, Sadahiro S, Hayashi M, Awazu S. 1998. Absorption-enhancing effects of sodium caprate and palmitoylcarnitine in rat and human colons. Dig Dis Sci 43:641–645.
4. Tomita M, Doi N, Hayashi M. 2010. Effects of acylcarnitines on efflux transporting system in Caco-2 cell monolayers. Eur J Drug Metab Pharmacokinet 35:1–7.
5. LeCluyse EL, Appel LE, Sutton SC. 1991. Relationship between drug absorption enhancing activity and membrane perturbation effects of acylcarnitine. Pharm Res 8:84–87.
6. Sugibayashi K, Onuki Y, Takayama K. 2009. Displacement of tight junction proteins from detergent-resistant membrane domains by treatment with sodium caprate. Eur J Pharm Sci 36:246–253.
7. Tomita M, Watanabe A, Fujinaga I, Yamakawa T, Hayashi M. 2010. Nonlinear intestinal absorption of methylprednisolone caused by absorptive and secretory transporters. Int J Pharm 387:1–6.
8. Iida A, Tomita M, Matsuura Y, Takizawa Y, Hayashi M. 2006. Improvement of intestinal absorption of P-glycoprotein substrate by D-tartaric acid. Drug Metab Pharmacokinet 21:424–428.
9. Yamaoka K, Nakagawa T, Uno T. 1978. Statistical moments in pharmacokinetics. J Pharmackinet Biopharm 6:547–558.
10. Tomita M, Kishimoto H, Takizawa Y, Hayashi M. 2009. Effect of intestinal ischemia/reperfusion on P-glycoprotein mediated biliary and renal excretion of rhodaimne123 in rat. Drug Metab Pharmacokinet 24:428–437.
11. Tomita M, Takizawa Y, Kishimoto H, Hayashi M. 2009. Effect of intestinal ischemia/reperfusion on P-glycoprotein mediated ileal excretion of rhodamine123 in rat. J Pharm Pharmacol 61:1319–1324.
12. Hsing S, Gatmaitan Z, Arias IM. 1992. The function of Gp170, the multidrug-resistance gene product, in the brush border of rat small intestinal mucosa. Gastroenterology 102:879–885.
13. Oku T, Itoh S, Ishii R, Suzuki K, Nauseef WM, Toyoshima S, Tsuji T. 2005. Homotypic dimerization of the actin-binding protein p57/coronin-1 mediated by a leucine zipper motif in the C-terminal region. Biochem J 387:325–331.
14. Van Itallie CM, Anderson JM. 2004. The molecular physiology of tight junction pores. Physiology (Bethesda) 19:331–338.