Effects of Labrasol and Other Pharmaceutical Excipients on the Intestinal Transport and Absorption of Rhodamine123, a P-Glycoprotein Substrate, in Rats

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Effects of Labrasol and other pharmaceutical excipients on the intestinal transport and absorption of rhodamine123, a P-glycoprotein substrate (P-gp) were examined. Intestinal transport and absorption studies were examined by an *in vitro* diffusion chamber method and an *in situ* closed loop method. We evaluated the intestinal membrane damage produced by Labrasol by measuring the release of protein and alkaline phosphatase (ALP). Labrasol (0.075—0.1% (v/v)) increased the absorptive transport of rhodamine123 and decreased its secretory transport in the *in vitro* transport studies. However, Labrasol did not change the transport of Lucifer yellow, a non-P-gp substrate, suggesting that the effect of Labrasol on the transport of drugs was specific for rhodamine123. We observed almost no intestinal membrane damage in the presence of Labrasol. These findings suggest that the increase in the absorptive transport of rhodamine123 in the presence of Labrasol may not be due to its intestinal membrane damage. In the *in situ* absorption studies, we found that Labrasol (0.1% (v/v)) significantly enhanced the intestinal absorption of rhodamine123 in rats, although the absorption enhancing effect of Labrasol was much less than that of verapamil. These findings suggest that low concentrations of Labrasol might inhibit the function of P-gp in the intestine, thereby increasing intestinal absorption and bioavailability of P-gp substrates including rhodamine123. However, we may also consider the contribution to the enhanced intestinal absorption of rhodamine123 *via* a passive transport in addition to the inhibitory action of Labrasol for the function of P-gp in the intestine.

Key words intestinal absorption; rhodamine123; P-glycoprotein; Labrasol; pharmaceutical excipient

P-Glycoprotein (P-gp) is a plasma membrane glycoprotein of about 170 kDa that belongs to the superfamily of ATP-binding cassette (ABC) transporters. P-gp is extensively expressed in tumor cells, liver, intestinal brush border membranes, kidney, brain, and adrenal glands.[1] It can actively pump drugs out of cells, thus reducing the oral bioavailability of a wide range of drugs such as digoxin, vinca alkaloids, and β-adrenergic agonists.

It has been reported that some P-glycoprotein inhibitors such as verapamil and cyclosporin A could enhance the bioavailability of P-gp substrate drugs.[2—4] But it is well known that these drugs themselves have pharmacological activities. Therefore, it is important to find a P-gp inhibitor which does not have pharmacological activities. It has been demonstrated that a number of excipients could inhibit the function of P-gp, thereby increasing the absorption of P-gp substrate drugs.[5] These excipients include polyethylene glycols (PEGs), nonionic surfactants, fatty acids and bile salts.[6—16] It has been reported that several excipients such as Cremophor EL, Tween 80, *n*-lauryl-β-D-maltopyranoside (LM), Pluronic P85, vitamin E-TPGS, PEGs, sodium caprate and dimethyl-β-cyclodextrin could inhibit the function of P-gp by *in vitro* and *in vivo* methods.[6—16]

Among these pharmaceutical excipients, Labrasol is one which has been widely used for the solubilization of hydrophobic drugs.[17] Labrasol is obtained from coconut oil and has very low toxicity with an LD_{50} of 22 g/kg for rats. Recently, it was reported that Labrasol had a strong absorption enhancing effect and that intestinal absorption of various poorly absorbable drugs including gentamicin, insulin and vancomycin was enhanced in its presence.[18—20] However, few studies examined the effect of Labrasol on the function of P-gp in the intestine and the intestinal absorption of P-gp substrates. Cornaire *et al.* (2004) examined the effect of Labrasol on the transport of digoxin by an *in vitro* everted sac experiment, and reported that 0.5% Labrasol increased the transport of digoxin in this experiment.[21] However, they demonstrated that 0.5% Labrasol could increase the lactate dehydrogenase (LDH), and so the increase in digoxin transport might be due to the intestinal membrane damage, and not to the inhibition of P-gp function in the intestine. Furthermore, they did not study the different regional effects of Labrasol on the transport of P-gp substrate and *in situ* or *in vivo* intestinal absorption of P-gp substrate with or without Labrasol.

In this study, therefore, we studied the effect of Labrasol on the function of P-gp using two different intestinal absorption experiments. We first adopted an *in vitro* diffusion chamber system using the isolated rat intestinal membrane to evaluate the effects of Labrasol and other pharmaceutical excipients, Gelucire44/14 and Transcutol P on the intestinal transport of rhodamine123, a P-gp substrate. In addition, the intestinal membrane damage of Labrasol was evaluated by measuring the release of alkaline phosphatase (ALP) and protein as biological markers. Finally, we also investigated the effects of Labrasol on the intestinal absorption of rho-

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damine123 by an *in situ* closed loop method.

MATERIALS AND METHODS

Materials Rhodamine123 and Lucifer yellow were purchased from Sigma Aldrich Co. (St. Louis, MO, U.S.A.). Labrasol, Gelucire 44/14 and Transcutol P were obtained from Gattefosse Corp. (Saint-Priest, France). Verapamil hydrochloride was supplied from Nacalai Tesque (Kyoto, Japan). Dimethyl sulfoxide (DMSO), sodium deoxycholate and alkaline phosphatase (ALP) assay kit were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Bicinchoninic acid (BCA) protein assay kit was obtained from Pierce Biotechnology Inc. (Rockford, U.S.A.). All other reagents were of analytical grade.

Preparation of Drug Solution Rhodamine123 and Lucifer yellow were dissolved in Hepes-Tris buffer solution at pH 7.4 to yield a final concentration of 10 μM and 100 μM, respectively. Excipients such as Labrasol, Gelucire 44/14, Transcutol P, (with a series of concentration, v/v) and verapamil (0.3 mM) were added to the drug solution.

Transport of Rhodamine123 across the Intestinal Membranes by an *in Vitro* Diffusion Chamber System The transport of P-gp substrates across the rat intestinal membrane was studied with a diffusion chamber (Corning Coster Corp.).[22] Male Wistar rats, weighing 220—280 g, were fasted overnight and anesthesized with sodium pentobarbital (32 mg/kg body weight i.p.). The studies were carried out in accordance with the guidelines of the animal ethics committee at Kyoto Pharmaceutical University. The intestine was exposed through a midline abdominal incision, removed and washed with PBS. Intestinal segments were isolated and each site was defined as described below. First, 5 cm of the top of the small intestine was regarded as the duodenum. The jejunum was obtained from the next 10 cm portion of the small intestine. The ileum was obtained from the final 10 cm portion of the intestine. The first 3 cm of large intestine was removed and the colon was obtained from the next 5 cm portion of the large intestine. Segments were cut open, the muscle layer was stripped, then intestinal sheets were mounted on the pins of the cell, and the half-cells were clamped together. Drug solution (7 ml) was added to the donor side, and the same volume of drug free buffer was added to the opposite side. The temperature of cells was maintained at 37 °C with a water bath, and solutions from both sides of cells were circulated with a gas lift (95% O_2/5% CO_2). During the transport studies, 0.1 ml aliquots were taken from the receiver side at predetermined time intervals up to 120 min, and immediately replaced by an equal volume of buffer solution. The amount of drug in the receiver side was assayed by fluorescence spectrophotometer. Apparent permeability coefficient (Papp) was calculated as the following equation:

$$\text{Papp} = \text{flux} \times \frac{1}{\text{area}} \times \frac{1}{60} \times \frac{1}{C_0}$$

where Papp is the apparent parameter of permeability (cm/s); the permeability flux is the slope of linear portion of cumulative transport amount to time at the steady state (pmol/min); area is the area of diffusion chamber for transport, 1.78 cm^2; and C_0 is the drug concentration before transport, pmol/ml.

In this study, efflux ratio was used to evaluate the function of P-gp. This ratio was calculated in the following equation:

$$\text{efflux ratio} = \text{Papp sm}/\text{Papp ms}$$

where Papp sm is the average of the intestinal permeability coefficient from serosal to mucosal side and Papp ms is the average of the intestinal permeability coefficient from mucosal to serosal side.

The viability of intestinal membrane during the test period was monitored by measuring the transport of trypan blue dye and electrophysiological parameters. There was no transport of the dye and no remarkable change of the electrophysiological parameters, confirming that the viability of the intestinal membrane was maintained during the transport experiments.

Assessment of Membrane Damage To evaluate membrane damage, the release of protein and ALP was measured by a diffusion chamber system. In these studies, 0.1% (v/v) Labrasol solution (7 ml) was added to the mucosal side, whereas the same volume of Hepes-Tris buffer was added to the serosal side. Two hours later, 0.5 ml aliquots were taken from the mucosal side. The amount of protein released from the intestinal membranes was measured with a BCA protein assay kit (Pierce Biotechnology Inc.). An ALP assay kit (Wako Pure Chemical Industries, Ltd.) was used to determine the amount of ALP released from the intestinal membranes.

Intestinal Absorption of Rhodamine123 by an *in Situ* Closed Loop Method Intestinal absorption of rhodamine123 was examined by an *in situ* closed loop method, as reported previously.[23—25] Male Wistar rats (250—300 g) were fasted overnight and anesthetized with sodium pentobarbital (32 mg/kg body weight i.p.). The intestine was exposed through the midline abdominal incision. After ligating the bile duct, a segment of ileum (about 20 cm long) was isolated and washed with PBS, then tied off at both ends to form a closed loop. The jugular vein was separated to collect the blood samples. The animal was placed under a heating lamp to maintain the body temperature around 37 °C. Rhodamine123 (0.7 mg/ml) solution, with or without Labrasol (0.075—0.1%, (v/v)) or verapamil (0.3 mM), was introduced directly into the lumen of the intestinal loop (5 mg/kg). Blood samples (*ca.* 0.2 ml) were collected at predetermined time intervals up to 360 min, then rhodamine123 and its metabolite, rhodamine110 were analyzed by HPLC, as described below.

Determination of Drugs The fluorescence intensity of rhodamine123 in the *in vitro* method was determined with a fluorescence spectrophotometer (Spectrafluor Plus, Tecan) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Similarly, Lucifer yellow was measured at an excitation wavelength of 430 nm and an emission wavelength of 535 nm, respectively.

When rhodamine123 in plasma was analyzed in the *in situ* closed loop method, rhodamine123 was metabolized to rhodamine110 by esterase in plasma. Therefore, rhodamine123 and rhodamine110 were separately measured by HPLC (Shimadzu LC-10A System) method, using YMC-Pack ODS reverse phase column (150×4.6 mm, particle size 5 μm) with a fluorescence spectrophotometer (Shimadzu RF-10F) at an excitation wavelength of 485 nm and an emission wavelength of 546 nm. The mobile phase was acetonitrile : 1% triethyl-

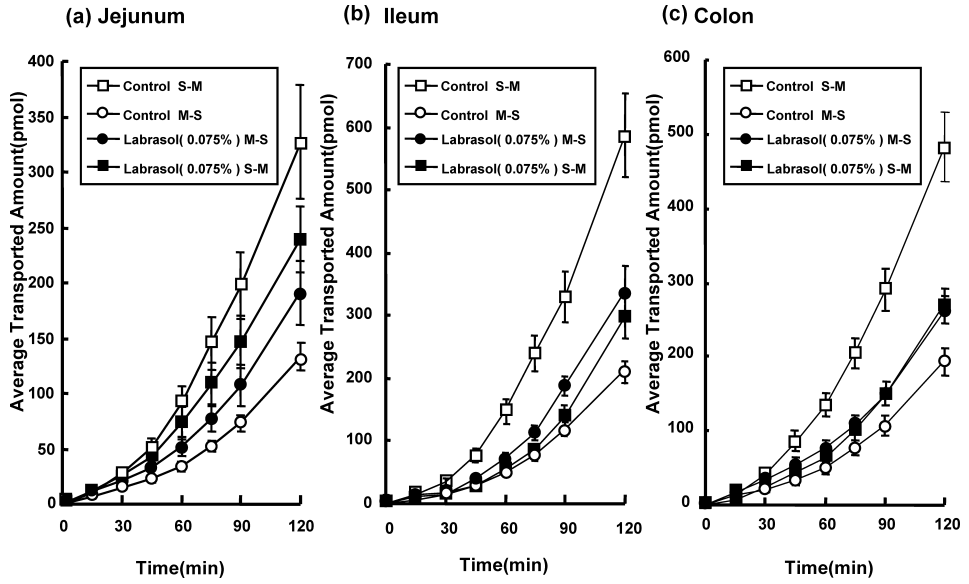

Fig. 1. Time Course of the Mucosal to Serosal (Absorptive) and the Serosal to Mucosal (Secretory) Transport of Rhodamine123 across the Various Rat Intestinal Membranes with or without Labrasol (0.075% (v/v))

Results are expressed as the mean±S.E. of at least three rats keys: (○), absorptive (m to s) transport of control; (□), secretory (s to m) transport of control; (●), absorptive (m to s) transport of rhodamine123 with Labrasol; (■), secretory (s to m) transport of rhodamine123 with Labrasol.

amine (pH=3, phosphate acid)=28 : 72, and run at a rate of 1.0 ml/min following an extraction from plasma with ethyl acetate. The standard curve showed a good linearity over a concentration range of 5 to 500 ng/ml for rhodamine123, and 10 to 200 ng/ml for rhodamine110, respectively.

Statistical Analyses Results are expressed as the mean±S.E. of at least three experiments. Statistical significance was assessed using ANOVA and Bonferroni-type multiple *t*-test (parametric, Ver 1.1, 1998—2003), with $p<0.05$ as the minimal level of significance.

RESULTS

Effect of Labrasol on the Transport of Rhodamine123 across the Intestinal Membranes by an *in Vitro* Diffusion Chamber System Figure 1 shows the time course of mucosal to serosal (absorptive) and serosal to mucosal (secretory) transport of rhodamine123 across the various intestinal membranes in the presence or absence of Labrasol (0.075% (v/v)). As shown, in the absence of Labrasol, the secretory transport of rhodamine123 across the various intestinal regions was much greater than its absorptive transport, indicating that the net movement of rhodamine123 across the intestinal membranes was preferentially in the secretory direction. When Labrasol (0.075% (v/v)) was added to the mucosal side, the absorptive transport was enhanced, whereas the secretory transport was reduced in all the intestinal regions. We observed almost no polarized transport of rhodamine123 in the presence of Labrasol (0.075% (v/v)).

In a previous study, we found that the absorptive transport of rhodamine123 was enhanced and its secretory transport was inhibited in the presence of verapamil (0.3 mM) or cyclosporin A (20 μM) using this *in vitro* transport model.[15,16] Therefore, we confirmed that this system was useful to evaluate the effect of some adjuvant on the function of P-gp in the intestine.

Table 1 shows the effect of different concentrations of Labrasol on the transport of rhodamine123 across the various intestinal membranes of rats by the *in vitro* diffusion chamber system. As shown in Table 1, in jejunum, Labrasol (0.075% (v/v)) significantly increased the absorptive transport of rhodamine123, whereas it had no significant effect on the secretory transport of rhodamine123. On the other hand, we found no significant difference in the absorptive and secretory transport of rhodamine123 with 0.05 and 0.1% (v/v) of Labrasol. The efflux ratio of rhodamine123 was remarkably reduced in the case of 0.075% (v/v) of Labrasol, but its ratios were not so much changed in the presence of 0.05 and

Table 1. Effect of Labrasol on Apparent Permeability Coefficients of Rhodamine123 across the Various Intestinal Membranes of Rats Determined by an *in Vitro* Diffusion Chamber Method

	Papp ($\times10^{-6}$ cm/s)		S→M/M→S (ratio)
	M→S	S→M	
Jejunum			
Control	1.56±0.15	3.67±0.59	2.35
0.05% Labrasol	1.39±0.19†	2.41±0.32†	1.73
0.075% Labrasol	2.22±0.34*	2.61±0.24†	1.18
0.10% Labrasol	1.57±0.16†	2.38±0.40†	1.52
Ileum			
Control	2.58±0.19	6.89±0.48	2.67
0.025% Labrasol	3.18±0.46†	4.47±0.90*	1.40
0.05% Labrasol	3.54±0.40*	4.80±0.70*	1.36
0.075% Labrasol	4.29±0.56**	3.87±0.55*	0.90
0.10% Labrasol	4.19±0.50**	3.98±0.60**	0.95
10.0% Labrasol	0.53±0.09**	3.53±0.59**	6.66
20.0% Labrasol	0.43±0.10**	3.56±0.84**	8.28
Colon			
Control	2.27±0.21	5.37±0.52	2.37
0.05% Labrasol	2.38±0.44†	5.13±0.26†	2.16
0.075% Labrasol	2.90±0.18*	3.30±0.23*	1.14
0.10% Labrasol	2.21±0.17†	3.71±0.42*	1.68

Results are expressed as the mean±S.E. of at least three experiments. *$p<0.05$, compared with the control. **$p<0.01$, compared with the control. †No significant difference compared with the control.

Table 2. Effect of Labrasol on Apparent Permeability Coefficient of Lucifer Yellow across the Ileal or Colonic Membranes of Rats Determined by an *in Vitro* Diffusion Chamber Method

	Papp ($\times 10^{-6}$ cm/s)		S→M/M→S (ratio)
	M→S	S→M	
Ileum			
Control	8.47±0.62	9.97±0.61	1.18
0.05% Labrasol	10.29±0.99†	11.29±0.94†	1.10
0.075% Labrasol	10.53±1.59†	11.62±0.89†	1.10
0.1% Labrasol	11.68±1.05*	11.65±0.69†	1.00
Colon			
Control	11.12±0.56	11.12±0.58	1.00
0.075% Labrasol	12.94±0.80†	11.26±0.54†	0.87

Results are expressed as the mean±S.E. of at least three experiments. * $p<0.05$, compared with the control. † No significant difference compared with the control.

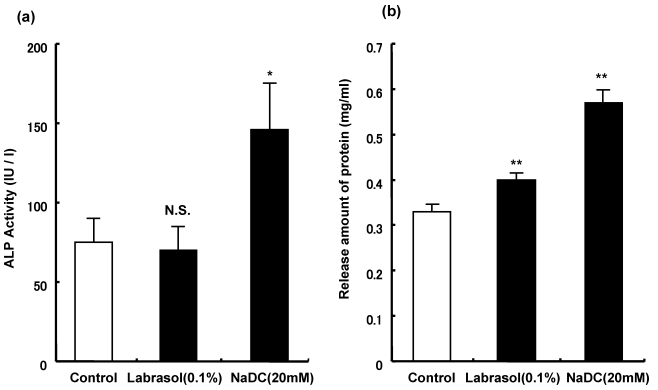

Fig. 2. Effect of Labrasol on the Release of Alkaline Phosphatase (ALP) and Protein from the Ileal Membranes by an *in Vitro* Diffusion Chamber Method

The concentration of Labrasol was 0.1% (v/v). Results are expressed as the mean±S.E. of 5—7 experiments. (*) $p<0.05$, (**) $p<0.01$, N.S., not significantly different compared with the control.

Table 3. Effect of Gelucire44/14, Transcutol P on Apparent Permeability Coefficients of Rhodamine123 in Ileum Determined in an *in Vitro* Diffusion Chamber Method

	Papp ($\times 10^{-6}$ cm/s)		S→M/M→S (ratio)
	M→S	S→M	
Gelucire44/14			
Control	2.25±0.22	6.15±0.66	2.73
0.01%	3.27±0.65†	5.22±0.97†	1.60
0.05%	2.99±0.62†	3.32±0.39*	1.11
0.10%	1.76±0.28†	3.13±0.51*	1.79
1.00%	0.69±0.09**	3.00±0.32**	4.35
10.00%	0.28±0.03**	1.69±0.42**	6.04
Transcutol P			
Control	2.24±0.18	6.60±0.56	2.95
0.05%	1.97±0.32†	5.22±0.82†	2.65
0.1%	2.99±0.62†	5.10±1.48†	1.71
1.0%	2.99±0.46†	7.73±0.29†	2.59
10%	2.32±0.39†	5.28±0.30†	2.28
20%	3.09±0.44†	7.11±0.98†	2.30

Results are expressed as the mean±S.E. of at least three experiments. * $p<0.05$, compared with the control. ** $p<0.01$, compared with the control. † No significant difference compared with the control.

0.1% (v/v) of this excipient.

In the ileum, Labrasol enhanced the absorptive transport of rhodamine123 and inhibited its secretory transport in a concentration dependent manner over the concentration range of 0.025—0.1% (v/v). In the presence of these concentrations of Labrasol, the efflux ratio of rhodamine123 decreased as the concentrations of Labrasol increased and we observed almost no polarized transport of rhodamine123 with Labrasol. However, the absorptive transport of rhodamine123 was remarkably reduced in the presence of high concentrations (10—20% (v/v)) of this substance.

In the colon, Labrasol at a concentration of 0.075% (v/v) enhanced the absorptive transport of rhodamine123 while decreasing the secretory transport. However, we found almost no remarkable effect of Labrasol on the intestinal transport of rhodamine123 at its different concentrations in the colon, although we observed a significant decrease in the secretory transport in the presence of 0.1% (v/v) of Labrasol. Overall, the inhibitory effect of Labrasol on the function of P-gp was greatest in the ileum of all the intestinal regions examined in this study.

Effect of Labrasol on the Transport of Lucifer Yellow, a Non-P-gp Substrate across the Intestinal Membranes by an *in Vitro* Diffusion Chamber System To confirm whether the effect of Labrasol on the intestinal transport of drugs was specific for the substrates of P-gp, we next examined its effect on the intestinal transport of Lucifer yellow, which is transported by passive diffusion *via* a paracellular pathway and is used as a model of non-P-gp substrate. As shown in Table 2, we found almost no significant differences in the absorptive and secretory transport of Lucifer yellow with or without Labrasol in the ileum and the colon, although 0.1% (v/v) Labrasol slightly increased its absorptive transport in the ileum. In addition, the efflux ratio of Lucifer yellow in the ileum and the colon was not changed by the addition of Labrasol. Together, these findings suggest that the effect of Labrasol on the intestinal transport of drugs was specific for rhodamine123, a substrate of P-gp.

Effect of Labrasol on the Ileal Membrane Damage Determined by Measuring the Release of Alkaline Phosphatase and Protein We also examined the effect of Labrasol on the ileal membrane damage by measuring the release of alkaline phosphatase and protein. We used 0.1% (v/v) of Labrasol for evaluating its intestinal membrane damage, since this concentration was effective for reducing the efflux ratio of rhodamine123 across the ileal membrane as described above. The results are shown in Fig. 2. Sodium deoxycholate, a positive control, significantly increased the release of these two markers from the intestinal membranes. However, we observed almost no membrane damage in the presence of Labrasol, although it slightly increased the amount of protein released from the intestinal membranes. These findings suggest that the increase in the absorptive transport of rhodamine123 in the presence of Labrasol is probably not due to the membrane damage it causes.

Effects of Gelucire44/14 and Transcutol P on P-Glycoprotein Function by *in Vitro* Diffusion Chamber System We next examined the effects of other pharmaceutical excipients, Gelucire44/14 and Transcutol P on the transport of rhodamine123 by the diffusion chamber system. The reason for the selection is that they are typical pharmaceutical excipients and are frequently used as solubilizing agents. As shown in Table 3, Gelucire44/14 significantly reduced the secretory

Table 4. Pharmacokinetic Parameters of Rhodamine123 and Rhodamine110 after Intestinal Administration of Rhodamine123 with or without Labrasol Determined by an *in Situ* Closed Loop Method

Parameters	C_{max} ($ng \cdot ml^{-1}$)	T_{max} (min)	$AUC_{0—360}$ ($ng \cdot ml^{-1} \cdot min$)	Fa (%)
Rhodamine123				
Control	19.16±1.53	80.00±10.00	4719.01±565.08	13.30
Verapamil (0.3 mM)	43.18±1.91**	80.00±10.00†	11449.3±536.43**	32.00
Labrasol (0.075%, v/v)	20.71±0.91†	120.00±24.50†	5988.31±436.97†	17.03
Labrasol (0.1%, v/v)	24.35±3.16†	67.50±7.50†	6608.80±465.16*	22.82
Rhodamine110				
Control	72.02±5.42	70.00±10.00	17286.58±1071.87	—
Verapamil (0.3 mM)	65.23±7.66†	50.00±10.00†	16864.14±735.35†	—
Labrasol (0.075%, v/v)	62.34±1.87†	120.00±30.00†	18853.97±400.83†	—
Labrasol (0.1%, v/v)	77.14±6.88†	67.50±7.50†	19562.59±358.76†	—

The dosage of rhodamine123 was 5 mg/kg. Date are expressed as the means of 3—4 rats±S.E. $*p<0.05$, compared with the control. $**p<0.01$, compared with the control. † No significant difference compared with the control.

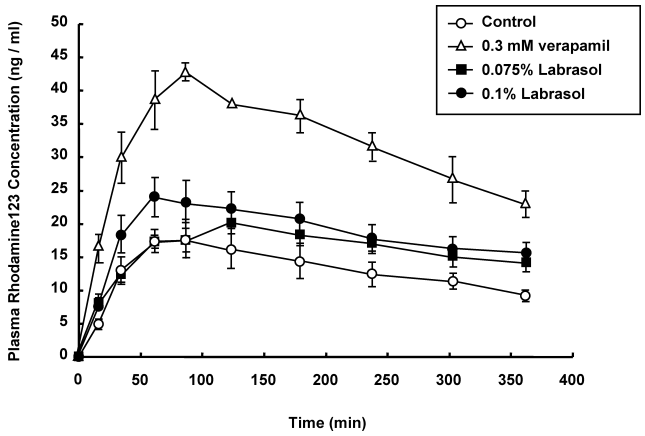

Fig. 3. Effect of Labrasol on Plasma Concentration–Time Profiles of Rhodamine123 after Its Administration into the Ileal Loops of Rats

The concentrations of Labrasol and verapamil were 0.075—0.1% (v/v) and 0.3 mM, respectively. Keys: (○), control; (△), 0.3 mM verapamil; (■), 0.075% (v/v) Labrasol; (●), 0.1% (v/v) Labrasol. Results are expressed as the mean±S.E. of 3—5 experiments.

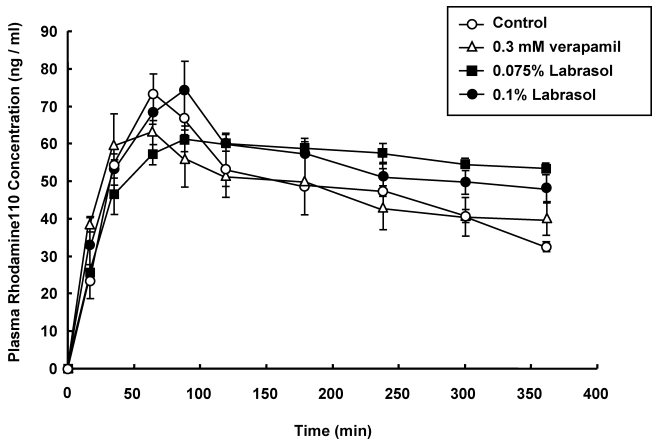

Fig. 4. Effect of Labrasol on Plasma Concentration–Time Profiles of Rhodamine110 after Administration of Rhodamine123 into the Ileal Loops of Rats

The concentrations of Labrasol and verapamil were 0.075—0.1% (v/v) and 0.3 mM, respectively. Keys: (○), control; (△), 0.3 mM verapamil; (■), 0.075% (v/v) Labrasol; (●), 0.1% (v/v) Labrasol. Results are expressed as the mean±S.E. of 3—5 experiments.

transport of rhodamine123 in the ileum in a concentration dependent manner, although over the concentration range of 0.01—0.1% (v/v) it did not affect the absorptive transport. On the other hand, the absorptive transport of rhodamine123 was significantly reduced in the presence of high concentrations (1.0—10.0% (v/v)) of Gelucire44/14; we observed the smallest efflux ratio of rhodamine123 in the presence of 0.05% (v/v). On the other hand, Transcutol P at various concentrations did not change the absorptive and secretory transport of rhodamine123 in the ileum. Therefore, these findings indicated that the inhibitory action of Gelucire44/14 for P-gp function in the intestine was observed like Labrasol, although its effect was not as clearly observed. It was also found that Transcutol P had no inhibitory effect on the function of P-gp in the intestine. Based on these findings, Labrasol had the strongest inhibitory function effect on P-gp among these pharmaceutical excipients used in this study.

Effect of Labrasol on the Intestinal Absorption of Rhodamine123 by an *in Situ* Loop Method Finally, we examined the effect of Labrasol on the intestinal absorption of rhodamine123 by an *in situ* loop method. Figure 3 and Table 4 show plasma concentration–time profiles and pharmacokinetic parameters of rhodamine123 after its intestinal administration in the presence or absence of Labrasol. It was found that Labrasol significantly enhanced the intestinal absorption of rhodamine123, although the effect was less than that of verapamil, a typical P-gp inhibitor. The bioavailability % of rhodamine123 in the control was 13.30%, but was improved to 17.03% and 22.82% with 0.075% (v/v) and 0.1% (v/v) Labrasol, respectively. On the other hand, we also determined the plasma concentration of rhodamine110, a metabolite of rhodamine123, after administration of rhodamine123 to the ileal loops of rats. As shown in Fig. 4 and Table 4, there was no significant difference in the plasma concentration of rhodamine110 and its pharmacokinetic parameters after administration of rhodamine123 with or without Labrasol.

Moreover, we also examined the plasma concentration of rhodamine123 after its intravenous administration with or without 0.075% (v/v) and 0.1% (v/v) Labrasol (data not shown). There was no significant difference in the plasma concentration–time profiles of rhodamine123 after these intravenous administrations as compared with control.

DISCUSSION

In the present study, we demonstrated that low concentra-

tions of Labrasol could probably inhibit the function of P-gp in the ileum and colon, thereby increasing intestinal absorption of rhodamine123 in rats. We used rhodamine123 as a P-gp substrate, since it was easily assayed and widely used for evaluating the function of P-gp in various research fields.[26] Moreover, this compound is also useful for screening and selecting the effective pharmaceutical excipients that can inhibit the function of P-gp in the intestine.

As shown in Fig. 1 and Table 1, the secretory transport of rhodamine123 was reduced and its absorptive transport was enhanced in the presence of Labrasol (0.075% (v/v)) in the ileum and the colon. In addition, this study indicated that the Papp sm/Papp ms ratio of rhodamine123 was clearly reduced in the presence of Labrasol. Our previous studies confirmed that verapamil (0.3 mM) and cyclosporin A (20 μM), typical P-gp inhibitors, significantly reduced the Papp sm/Papp ms ratio of rhodamine123.[15,16] Therefore, this *in vitro* diffusion chamber method is applicable for evaluating the effect of pharmaceutical excipients (P-gp inhibitors) on the function of P-gp in the intestine. In addition, Labrasol might inhibit the function of P-gp, thereby decreasing the Papp sm/Papp ms ratio of rhodamine123 in the intestine.

We observed a concentration-dependent and regional-different effect of Labrasol on the intestinal transport of rhodamine123 (Table 1). The results were consistent with the previous study of Cornaire *et al.* (2004),[21] although they did not examine the different regional effects of Labrasol and used different substrates of P-gp (digoxin) and a different transport model (everted sac). The inhibitory effect of Labrasol on the function of P-gp was more clearly observed in the ileum and colon, whereas we saw no inhibitory effect of Labrasol on the secretory transport of rhodamine123 across the jejunal membranes. Mouly and Paine (2003) have already reported that the expression level of P-gp in the intestine was dependent on the region of the intestine.[27] They reported that P-gp expression increased from the proximal to distal regions of the small intestine. Therefore, such different expression levels of P-gp in the intestine might be one of the reasons for the different regional effect of Labrasol on the intestinal transport of rhodamine123 across the intestinal membranes. Moreover, we unexpectedly found that the transport of rhodamine123 decreased in the presence of high concentrations (10—20%) of Labrasol. Although the reason is still unclear, the viscosity of drug solution increased with these high concentrations and the diffusion and transport of rhodamine123 might be inhibited in this viscous solution. Alternatively, it may be plausible that Labrasol at high concentrations formed micelles and the amount of rhodamine123 entrapped in these micelles may increase with this increasing concentration.

In this study, we found almost no significant effect of Labrasol on the intestinal transport of Lucifer yellow, a model drug of non-P-gp substrate transported by passive diffusion in both absorptive and secretory direction, although 0.1% (v/v) Labrasol slightly increased the absorptive transport of Lucifer yellow in the ileum. In addition, Labrasol did not change the efflux ratio of Lucifer yellow in the ileum and colon. Consequently, the effect of this substance on the intestinal transport of drugs was specific for rhodamine123, a P-gp substrate. On the other hand, we found an increase in the absorptive transport of Lucifer yellow by Labrasol with relatively high concentration (0.1% (v/v)), and this might be attributed to opening the tight junction of cells caused by Labrasol. However, Koga *et al.* (2002) reported that Labrasol (0.01—0.5% (v/v)) had no significant effect on the intestinal membrane resistance (R_m), an index of loosing the tight junction *via* a paracellular route.[28] Therefore, further studies are necessary to elucidate the mechanism whereby Labrasol increased the intestinal transport of Lucifer yellow, a typical paracellular marker compound.

In the membrane toxicity studies, it was found that Labrasol was much less effective on the release of protein and ALP from the intestinal membranes than sodium deoxycholate, a typical toxic absorption enhancer. The release of protein is a good index to evaluate the intestinal membrane toxicity, since many toxic substances could stimulate the release of protein, one of the components of biological membranes from the intestinal membranes.[29,30] ALP, a marker enzyme of the brush border membrane, is also used as a biological marker for evaluating the membrane toxicity.[31] As shown in Fig. 2, Labrasol did not cause any serious membrane damage or toxicity to the intestinal mucosa at this concentration used in this study; however, it slightly increased the release of protein from the intestinal membrane. Therefore, Labrasol at higher concentrations (above 0.1% (v/v)) may cause intestinal membrane damage to some extent, thereby affecting the intestinal transport of some drugs.

In this study, we also evaluated the effect of other pharmaceutical excipients-Gelucire44/14 and Transcutol P on the transport of rhodamine123 to investigate whether they also inhibit the function of P-gp in the intestine as well as Labrasol. Gelucire44/14 is well-defined mixtures of mono-, di- and triglycerides and mono- and di-fatty esters of polyethylene glycol, which contain predominant fatty acids composed of caprylate caprate and laurate, respectively.[21] Transcutol P is purified diethylene glycol monoethyl ether, which is widely used as an effective absorption enhancer to apply in transdermal, oral and topical pharmaceutical preparations.[32] Our findings indicated that Transcutol P did not affect the transport of rhodamine123 in the ileum, although Gelucire44/14 significantly reduced the secretory transport of rhodamine123 in a concentration dependent manner. Therefore, these findings indicate that Transcutol P had almost no inhibitory effect on the function of P-gp, while Gelucire44/14 had some inhibitory action on this function, although we have not yet elucidated the inhibitory mechanism of these pharmaceutical excipients on this function. Furthermore, we demonstrated that the absorptive transport of rhodamine123 was significantly reduced in the presence of high concentrations 1.0—10.0% of Gelucire44/14. Since the critical micelle concentration of this excipient is known to be 0.001—0.01%,[33] the decrease in the absorptive transport of rhodamine123 with high concentrations (1.0—10.0%) of Gelucire44/14 may be due to the entrapment of rhodamine123 into its micelles.

We found that Labrasol significantly enhanced the intestinal absorption of rhodamine123, although this absorption enhancing effect was less than the effect of verapamil (Fig. 3, Table 4). These findings suggested that Labrasol might also inhibit the function of P-gp by the *in situ* closed loop method, thereby increasing the intestinal absorption of rhodamine123. However, Labrasol at a concentration of 0.1% (v/v) also enhanced the intestinal transport of Lucifer yellow, a typical

marker compound of passive transport *via* a paracellular pathway by the *in vitro* transport studies, as described previously. Therefore, we may consider the contribution to the enhanced intestinal absorption of rhodamine123 *via* a paracellular pathway in addition to the inhibitory action of Labrasol for the function of P-gp in the intestine. More recently, it was reported that Labrasol improved the permeability of gentamicin sulfate by modulating the membrane lipid fluidity.[34] Therefore, Labrasol might also increase the transport of rhodamine123 *via* a transcellular passive transport pathway.

On the other hand, there was no significant difference in the plasma concentration of rhodamine110 and its pharmacokinetic parameters after administration of rhodamine123 with or without Labrasol (Fig. 4, Table 4). Moreover, we also examined the plasma concentration of rhodamine123 after its intravenous administration with or without Labrasol and found no significant difference in the plasma concentration with or without this excipient. These findings suggested that Labrasol might not affect the distribution, metabolism or elimination process of rhodamine123 but might only influence its absorption process of rhodamine123 in the intestine.

In conclusion, low concentrations of Labrasol (0.075% (v/v), 0.1% (v/v)) might inhibit the function of P-gp in rat ileum and colon, thereby increasing intestinal absorption and bioavailability of P-gp substrates. Labrasol might be a useful excipient for improving the intestinal absorption of P-gp substrates by reducing the function of P-gp in the intestine, although the absorption enhancing mechanism of Labrasol may partially involve the passive transport *via* both a paracellular and a transcellular pathway.

Acknowledgments This work was supported by a Grant-in-Aid for Scientific Research (12672231), and the 21st Century Center of Excellence Program “Development of Drug Discovery Frontier Integrated from Traditional to Proteome” from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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