International Journal of Pharmaceutics 379 (2009) 100–108

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

The effects of common solubilizing agents on the intestinal membrane barrier functions and membrane toxicity in rats

Khuriah Abdul Hamid[a,b], Hidemasa Katsumi[a], Toshiyasu Sakane[a], Akira Yamamoto[a,*]

[a] Department of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan
[b] Faculty of Pharmacy, MARA University of Technology, Puncak Alam Campus, Selangor 42300, Malaysia

ARTICLE INFO

Article history:
Received 31 January 2009
Received in revised form 5 June 2009
Accepted 13 June 2009
Available online 23 June 2009

Keywords:
Intestinal absorption
Intestinal transport
Solubilizing agent
Pharmaceutical excipient
Low water-soluble drug
Membrane toxicity

ABSTRACT

The use of solubilizing agents to improve the solubility of poorly water-soluble drugs often results in an alteration of intestinal membrane barrier function and intestinal membrane damage. In this study, 5(6)-carboxyfluorescein (CF) and fluorescein isothiocyanate-labeled dextran (MW 4400, FD4) were used as model compounds to examine the effects of twelve common solubilizing agents, sodium taurocholate (NaTC), Labrasol, polyethylene glycol 400 (PEG 400), Transcutol P, propylene glycol, Gelucire 44/14, HCO-60, ethanol, Cremophor EL, Tween 80, 2 hydroxypropyl-β-cyclodextrin (2HP-β-CyD) and dimethylsulfoxide (DMSO), on intestinal membrane barrier function and membrane toxicity in rats. Intestinal transport and absorption of CF were examined using an *in vitro* diffusion chamber and an *in situ* closed-loop technique. The *in vitro* diffusion chamber study showed that only 5 and 10% (w/v) NaTC significantly increased the transport of CF across the intestinal membrane. The *in situ* closed-loop study showed a remarkable increase in the absorption of CF and a bioavailability of more than 30% in the presence of 5 and 10% (v/v) Labrasol, 5 and 10% (w/v) NaTC and 10% (v/v) Transcutol P. Furthermore, we evaluated the effect of NaTC and Labrasol on the intestinal absorption of FD4, a high molecular weight compound. The results indicated that the absorption of FD4 also increased in the presence of 5 and 10% (w/v) NaTC and 10% (v/v) Labrasol, suggesting that these concentrations of NaTC and Labrasol may alter the intestinal membrane barrier functions in rats. We measured the release of protein and lactate dehydrogenase (LDH) from the intestinal membrane to examine the safety of solubilizing agents in the intestine. 5 and 10% (w/v) NaTC and 5 and 10% (v/v) Gelucire 44/14 significantly increased the presence of these toxicity markers compared to the control. The LDH level was also increased in the presence of 10% (v/v) of Cremophor EL. These findings suggest that the solubilizing agents at these concentrations except for NaTC, Gelucire 44/14 and Cremophor EL are considered safe and do not cause intestinal membrane damage. In conclusion, this study provides a basic approach in screening and predicting the effects of solubilizing agents for intestinal absorption studies using drugs poorly soluble in water.

1. Introduction

Pharmaceutical companies initially identify new drug candidates from vast libraries of compounds utilizing high-throughput screening. However, these compounds sometimes have very lipophilic characteristics and show very poor solubility in water (Lipinski et al., 2001). Furthermore, the variable absorption rate and low bioavailability of these compounds in the gastrointestinal tract present serious complications in the subsequent drug development process. Therefore, the solubility of these drug candidates must be increased to achieve higher bioavailability after oral administration.

Many strategies have been examined to improve the solubility of these lipophilic compounds, including the selection of particle size and crystal polymorphism, the use of a salt formation, the use of a solid dispersion system and the application of a dosage form such as emulsion (microemulsion) or liposome (Strickley, 2004). Of these strategies, the application of solubilizing agents is one of the simplest and easiest methods to improve the water solubility and to achieve the higher bioavailability after oral administration.

Solubilizing agents can be classified according to their chemical properties and their main solubilizing mechanism (Himmel, 2007). The classifications include: (1) water-soluble complexation builders/carriers such as cyclodextrins, (2) water-soluble organic (co)solvents (e.g., DMSO, ethanol, PEG 400, and propylene glycol), (3) water-soluble solubilizers/surfactants (e.g., Cremophor EL, Tween 80, Labrasol, HCO-60, Transcutol P, Gelucire 44/14, and NaTC), (4) lipid-based complexation builders (e.g., liposomes), and (5) nanoparticles. However, some of these solubilizing agents alter the intestinal membrane barrier functions and cause damage to

* Corresponding author. Tel.: +81 75 595 4661; fax: +81 75 595 4761.
E-mail address: yamamoto@mb.kyoto-phu.ac.jp (A. Yamamoto).

0378-5173/$ – see front matter.
doi:10.1016/j.ijpharm.2009.06.018

the intestinal epithelium. Indeed, our previous study demonstrated that NaTC, a bile salt and natural surfactant, increases the intestinal absorption of polar drugs and enhances the release of proteins and phospholipids from intestinal membranes (Yamamoto et al., 1996; Uchiyama et al., 1996). Furthermore, it was reported that NaTC enhances nasal absorption of insulin (Johansson et al., 2002).

The effect of solubilizing agents on intestinal membrane integrity was previously evaluated by *in vitro* experimental models such as Caco-2 cell monolayers (Sakai et al., 1997; Totterman et al., 1997; Rege et al., 2001; Takahashi et al., 2002) and excised rat intestinal segments, which are useful for examining transporter-mediated absorption (Sugiyama et al., 1997; Johnson et al., 2002). The solubilizing agents used in the Caco-2 cell system study (20% propylene glycol, 5% Tween 80, 5% PEG 400, 5% HP-β-CyD, and 5% Tween 80 + 5% PEG 400) were reported to exhibit no effect on 21 days cultures of Caco-2 monolayers (Takahashi et al., 2002). However, few studies have examined the effects of solubilizing agents on the intestinal membrane barrier functions and membrane toxicity using *in situ* or *in vivo* studies with intact animal models (Oda et al., 2004).

In the present study, we examined the effects on intestinal membrane barrier function of the following 12 solubilizing agents at varied concentrations: 5 and 10% (v/v) Labrasol, 5 and 10% (v/v) PEG 400, 5 and 10% (v/v) Transcutol P, 10% (v/v) propylene glycol, 5 and 10% (v/v) Gelucire 44/14, 10% (v/v) HCO-60, 5 and 10% (v/v) ethanol, 10% (v/v) Cremophor EL, 10% (v/v) Tween 80, 10% (w/v) 2HP-β-CyD, 10% (v/v) DMSO and 5 and 10% (w/v) NaTC. The model compounds 5(6)-carboxyfluorescein (CF) and fluorescein isothiocyanate-labeled dextran (MW 4400, FD4) were used as they are mainly transported across the intestinal membranes via a paracellular pathway and allow for easy estimation of intestinal epithelium barrier function. In addition to *in vitro* diffusion chamber method and *in situ* closed-loop techniques, we also measured the release of protein and lactate dehydrogenase (LDH) from the intestinal membranes in rats to evaluate membrane toxicity of the solubilizing agents.

2. Materials and methods

2.1. Materials

CF was obtained from Eastman Kodak Company (Rochester, NY, USA). FD4, NaTC, Cremophor EL, and Tween 80 were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Propylene glycol, 2HP-β-CyD and DMSO were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). PEG 400 was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Labrasol, Gelucire 44/14 and Transcutol P were kindly supplied from Gattefossé Corp. (Saint-Priest, France). HCO-60 was provided by Nikko Chemicals (Tokyo, Japan). Ethanol was purchased from Kanto Chemicals (Tokyo, Japan). All other chemicals and solvents were of analytical grade.

2.2. Preparation of drug solution

For the *in vitro* diffusion chamber studies, CF was dissolved in Hepes–Tris buffer solution at pH 7.4 to yield a final concentration of 10 μM. For intravenous administration, the drug dose was 1/10 as compared with the dose to the intestine. CF and FD4 solution were dissolved separately in a Hepes–Tris buffer solution adjusted to pH 7.4 to yield a final concentration of 0.05 and 0.8 mg/kg, respectively. For the intestinal absorption studies, CF was dissolved in a Hepes–Tris buffer solution of pH 7.4 to yield a final concentration of 0.125 mg/rat. FD4 was dissolved in the same buffer to a final concentration of 2 mg/rat. Solubilizing agents tested were NaTC, Labrasol, PEG 400, Transcutol P, propylene glycol, Gelucire 44/14, HCO-60, ethanol, Cremophor EL, Tween 80, 2HP-β-CyD and DMSO. CF was dissolved in Hepes–Tris buffer solution containing these solubilizing agents to yield a final concentration of 5 and 10% (v/v or w/v).

2.3. Transport of CF across the intestinal membranes using in vitro diffusion chamber method

All studies were carried out in accordance with the guidelines of the animal ethics committee at Kyoto Pharmaceutical University. The *in vitro* transport of CF across the rat intestinal membrane in the presence or absence of solubilizing agents was evaluated by diffusion chamber method (Corning Coster Corp.) (Grass and Sweetana, 1988). Male Wistar albino rats, weighing 250–280 g, were fasted overnight and anesthetized with sodium pentobarbital (32 mg/kg body weight i.p.). The intestine was exposed through a midline abdominal incision, removed and washed with PBS. Intestinal segments were then isolated, cut open, and stripped off the muscle layer. Intestinal sheets were mounted to diffusion chamber pins and the half-chambers were clamped together. Drug solution (7 ml) was added to the donor side, and equal volume of drug free buffer was added to the receiver side. To mix each solution and to maintain the intestinal membrane viability, each side of the diffusion chamber was aerated with 95% O_2 and 5% CO_2 gas and maintained at 37 °C throughout the experiment. At predetermined times over 120 min, 0.1 ml aliquots were taken from the receiver side, and immediately replaced with an equal volume of buffer solution. Drugs were then assayed and the apparent permeability coefficient (P_{app}) was calculated using following equation:

$$P_{app} = \text{Flux} \times \frac{1}{\text{Area}} \times \frac{1}{C_0} \times \frac{1}{60} \quad (1)$$

where P_{app} is the apparent parameter of permeability (cm/s) and flux, F, is the slope of linear portion of cumulative transport curve (pmol/min), Area is the surface area of the diffusion chamber used for transport (1.78 cm^2), and C_0 is the initial drug concentration (pmol/ml).

2.4. Absorption experiments

Absorption experiments were performed using an *in situ* closed-loop technique, as reported previously (Yamamoto et al., 1994; Gotoh et al., 1996; Fetih et al., 2005). The concentrations of solubilizing agents used in these experiments were 5–10% (v/v or w/v) based on results from the *in vitro* transport study. Male Wistar rats (body weight, 250–280 g) were fasted overnight, 16 h prior to the start of the experiment, and anesthetized with sodium pentobarbital (32 mg/kg body weight, i.p.). The intestine was exposed through a midline abdominal incision, flushed with Hepes–Tris buffer solution (pH 7.4), and any remaining buffer was expelled with air. A closed jejunal loop was prepared with the distal portion cannulated with polyethylene tubing and clamped with forceps. Drug solution (1 ml), maintained at 37 °C, was introduced into the loop through a cannulated opening in the proximal portion and clamped with forceps. The jugular vein was exposed and blood samples (0.25 ml) were collected using heparinized syringes at predetermined time intervals over 240 min. Blood plasma (100 μl) was immediately obtained by centrifuging blood samples at 12,000 rpm for 5 min and then placed on ice until determination.

The concentrations of drugs in plasma were determined and the plasma concentrations–time profiles of drugs with or without various solubilizing agents were plotted. Peak concentration (C_{max}) and time to reach peak concentration (T_{max}) were determined directly from plasma concentration–time curves. The area under the curve (AUC) was calculated using the trapezoidal method from zero to the final sampling time (240 min). The extent of bioavailability was

calculated as follows:

$$F = \mathrm{AUC}_{(\mathrm{intestine})} \cdot \frac{D_{(\mathrm{i.v})}}{\mathrm{AUC}_{(\mathrm{i.v})}} \cdot D_{(\mathrm{intestine})} \times 100 \quad (2)$$

where F is bioavailability (%) and D is the administered dose. The $D_{(\mathrm{intestine})}$ is the drug dose administered to the intestine while $D_{(\mathrm{i.v})}$ is the dose for intravenous administration.

For intravenous administration, male Wistar rats (250–280 g) were fasted overnight and anesthetized with sodium pentobarbital (32 mg/kg body weight i.p.). 0.2 ml of CF (0.0625 mg/ml, 0.05 mg/kg) solution or 0.2 ml of FD4 (1 mg/ml, 0.8 mg/kg) solution was introduced directly by bolus injection into femoral vein, respectively. Then, the jugular vein was exposed and blood samples (0.25 ml) were collected using heparinized syringes at predetermined time intervals at 0–240 min. Plasma (100 μl) was immediately obtained by centrifuging blood samples at 12,000 rpm for 5 min and then placed on ice until determination with fluorescence spectrophotometer. The AUC value of i.v. administration was calculated using the trapezoidal method from zero to the final sampling time (240 min) and we also calculated the AUC from zero to infinity by the extrapolation method. The extent of bioavailability was calculated as shown in Eq. (2).

2.5. Assessment of intestinal membrane damage

The release of the toxicity markers, protein and LDH, from the jejunal membrane was measured by an *in situ* closed-loop technique to assess membrane damage, as reported previously (Gao et al., 2008a,b). Following completion of the absorption experiments (4 h after starting the experiment) intestinal perfusate was withdrawn and measured for the presence of protein and LDH. LDH activity was determined using an LDH CII assay kit (Wako Pure Chemical Industries, Osaka, Japan), and protein concentration was determined using a BCA™ Protein Assay kit (Pierce, WI, USA) with bovine serum albumin as a standard.

2.6. Analytical methods

Fluorescence intensities of CF and FD4 were measured with a fluorescence spectrophotometer (Spectrafluor Plus, TECAN, Switzerland) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm, respectively, as reported previously (Yamamoto et al., 2001a,b).

2.7. Statistical analyses

Results were expressed as the mean ± S.E. and statistical significance was performed with analysis of variance (ANOVA) for multiple comparisons with the minimum level of significance ($P < 0.05$).

3. Results

3.1. Effects of solubilizing agents on the transport of CF across the intestinal membranes using in vitro diffusion chamber method

The effect of various solubilizing agents on the transport of CF was examined using the *in vitro* diffusion chamber method, and Table 1 shows the P_{app} values of CF in the presence or absence of various solubilizing agents in the jejunum. Most of the solubilizing agents did not significantly alter the transport of CF across the jejunal region of the intestinal membrane. In contrast, the transport of CF remarkably increased in the presence of 5–10% (w/v) NaTC compared to the control, and slightly increased in the presence of 5% (v/v) Gelucire 44/14. Values of P_{app} for CF in the presence of 5 and 10% NaTC were about 2.3 and 3.7 times higher, respectively, than control values.

Table 1
Papp of CF with or without various solubilizing agents in the jejunum.

	Concentration	Papp ($\times 10^{-6}$ cm/s)	Enhancement ratio
Control	–	6.0 ± 0.8	–
NaTC	5%	13.7 ± 0.7**	2.3
NaTC	10%	22.1 ± 0.8**	3.7
Labrasol	5%	5.2 ± 0.5N.S.	0.9
Labrasol	10%	4.9 ± 0.3N.S.	0.8
PEG 400	5%	4.1 ± 0.6N.S.	0.7
PEG 400	10%	2.1 ± 0.5N.S.	0.4
Transcutol P	5%	6.2 ± 0.7N.S.	1.0
Transcutol P	10%	5.7 ± 0.5N.S.	0.9
Propylene glycol	10%	6.2 ± 1.2N.S.	1.0
Gelucire 44/14	5%	8.2 ± 0.4*	1.4
Gelucire 44/14	10%	5.7 ± 0.4N.S.	0.9
HCO-60	10%	4.1 ± 0.4N.S.	0.7
Ethanol	5%	6.1 ± 0.1N.S.	1.0
Ethanol	10%	7.2 ± 0.8N.S.	1.2
Cremophor EL	10%	3.5 ± 0.3N.S.	0.6
Tween 80	10%	2.8 ± 0.3N.S.	0.5
2HP-β-CyD	10%	4.4 ± 0.4N.S.	0.7
DMSO	10%	4.9 ± 0.7N.S.	0.8

Results are expressed as the mean ± S.E. of at least 3 experiments. ** $P < 0.01$, * $P < 0.05$, N.S. not significant difference, compared with the control.

Table 2 shows the values of P_{app} for CF in the presence or absence of various solubilizing agents within the ileum. The transport of CF significantly increased in the presence of 5 and 10% (w/v) NaTC and 10% (v/v) ethanol. The other solubilizing agents did not affect the transport of CF across the ileum. The values of P_{app} for CF co-administered with 5 and 10% (w/v) NaTC and 10% (v/v) ethanol in the ileum were 5.3, 4.1 and 1.8 times higher, respectively, than control values.

Table 3 also shows that 5 and 10% (w/v) NaTC and 10% (v/v) ethanol significantly increased the transport of CF within the colon. The values of P_{app} for CF co-administered with of 5 and 10% (w/v) NaTC and 10% (v/v) ethanol were 5.3, 4.2 and 1.7 times higher, respectively, than control values. These cumulative results suggest that NaTC significantly enhances the transport of CF across all intestinal regions. In the presence of 10% (v/v) ethanol, the transport of CF across the ileal and colonic regions was significantly increased and only slightly increased in the jejunum. In contrast, we found almost no significant effect on the transport of CF across all

Table 2
Papp of CF with or without various solubilizing agents in the ileum.

	Concentration	Papp ($\times 10^{-6}$ cm/s)	Enhancement ratio
Control	–	5.9 ± 0.5	–
NaTC	5%	31.5 ± 3.5**	5.3
NaTC	10%	24.4 ± 0.5**	4.1
Labrasol	5%	6.2 ± 0.7N.S.	1.1
Labrasol	10%	5.5 ± 0.4N.S.	0.9
PEG 400	5%	4.6 ± 0.9N.S.	0.8
PEG 400	10%	2.1 ± 0.3N.S.	0.4
Transcutol P	5%	6.4 ± 0.7N.S.	1.1
Transcutol P	10%	5.5 ± 0.4N.S.	0.9
Propylene glycol	10%	7.9 ± 0.3N.S.	1.3
Gelucire 44/14	5%	7.7 ± 0.4N.S.	1.3
Gelucire 44/14	10%	7.5 ± 1.0N.S.	1.3
HCO-60	10%	3.9 ± 0.9N.S.	0.7
Ethanol	5%	7.2 ± 0.4N.S.	1.2
Ethanol	10%	10.4 ± 0.1**	1.8
Cremophor EL	10%	4.3 ± 0.8N.S.	0.7
Tween 80	10%	4.3 ± 0.5N.S.	0.7
2HP-β-CyD	10%	4.9 ± 0.5N.S.	0.8
DMSO	10%	6.6 ± 0.8N.S.	1.1

Results are expressed as the mean ± S.E. of at least 3 experiments. ** $P < 0.01$, N.S. not significant difference, compared with the control.

Table 3
Papp of CF with or without various solubilizing agents in the colon.

	Concentration	Papp ($\times 10^{-6}$ cm/s)	Enhancement ratio
Control	–	4.9 ± 0.5	–
NaTC	5%	25.9 ± 2.3**	5.3
NaTC	10%	20.8 ± 0.3**	4.2
Labrasol	5%	4.1 ± 0.4N.S.	0.8
Labrasol	10%	5.0 ± 0.6N.S.	1.0
PEG 400	5%	6.4 ± 0.1N.S.	1.3
PEG 400	10%	3.3 ± 0.7N.S.	0.7
Transcutol P	5%	5.5 ± 0.4N.S.	1.1
Transcutol P	10%	7.0 ± 0.5N.S.	1.4
Propylene glycol	10%	6.2 ± 0.5N.S.	1.3
Gelucire 44/14	5%	5.4 ± 0.7N.S.	1.1
Gelucire 44/14	10%	4.5 ± 0.3N.S.	0.9
HCO-60	10%	5.4 ± 0.9N.S.	1.1
Ethanol	5%	6.3 ± 0.5N.S.	1.3
Ethanol	10%	8.1 ± 0.4**	1.7
Cremophor EL	10%	6.9 ± 0.5N.S.	1.4
Tween 80	10%	6.0 ± 0.2N.S.	1.2
2HP-β-CyD	10%	6.0 ± 0.4N.S.	1.2
DMSO	10%	4.9 ± 0.7N.S.	1.0

Results are expressed as the mean ± S.E. of at least 3 experiments. $^{**}P < 0.01$, N.S. not significant difference, compared with the control.

intestinal regions when co-administered with the other solubilizing agents.

3.2. Effect of solubilizing agents on the intestinal absorption of CF and FD4 using in situ closed-loop technique

We examined the effects of these solubilizing agents on intestinal absorption of CF and FD4 based on results obtained using the *in vitro* diffusion chamber.

At first, we examined the plasma concentration profiles of CF and FD4 after their intravenous administration to rats. Fig. 1(a) and (b) shows the plasma concentration–time profiles of CF and FD4 after their intravenous administration to the rat femoral vein, respectively. As shown in Fig. 1(a) and (b), the plasma concentrations of both compounds rapidly decreased and the elimination of these compounds was quite fast. Table 4 shows the pharmacokinetic parameters of CF and FD4 after their administration to the rat femoral vein. The $AUC_{0-240\,min}$, $AUC_{0-\infty}$, MRT, $T_{1/2}$, CL_{tot}, and Vd_{ss} of CF were 5968.8 ± 905.3 ng min/ml, 6526.3 ± 1041.5 ng min/ml, 78.9 ± 13.5 min, 84.5 ± 12.5 min, 0.01 ± 0.0 L/(min kg) and 0.6 ± 0.1 L/kg, respectively. On the other hand, the $AUC_{0-240\,min}$, $AUC_{0-\infty}$, MRT, $T_{1/2}$, CL_{tot}, and Vd_{ss} of FD4 were 226.5 ± 23.8 μg min/ml, 321.7 ± 53.2 μg min/ml, 194.0 ± 24.7 min, 167.5 ± 2.4 min, 0.03 ± 0.0 L/(min kg) and 0.5 ± 0.0 L/kg, respectively.

Plasma concentration–time profiles following the administration of CF in the presence or absence of various solubilizing agents into the rat jejunum are shown in Fig. 2. The co-administration of 5 and 10% (v/v) PEG 400, 5 and 10% (v/v) Gelucire 44/14 and 10% (v/v) propylene glycol with CF showed little effect on intestinal absorption of CF with levels nearly comparable to the administration of CF alone (Fig. 2a). Similarly, results comparable to the control were observed for 10% (v/v) Tween 80, 10% (v/v) DMSO and 5 and 10% (v/v) of ethanol (Fig. 2b). Co-administration of CF with 10% (v/v) of HCO-60 and 10% (w/v) 2HP-β-CyD did not produce any significant effect on the intestinal absorption, but intestinal absorption of CF was remarkably increased in the presence of 5 and 10% (w/v) NaTC compared to the control (Fig. 2c). No significant effect on intestinal absorption of CF in the presence of 5% (v/v) Transcutol P or 10% (v/v) Cremophor EL was seen, but intestinal absorption of CF was significantly increased using 5 and 10% (v/v) Labrasol and 10% (v/v) Transcutol P compared to the control (Fig. 2d).

Table 4
Pharmacokinetic parameters of CF and FD4 after their intravenous administration to the femoral vein.

	CF (0.05 mg/kg)	FD4 (0.8 mg/kg)
$AUC_{0-240\,min}$	5968.8 ± 905.3 ng min/ml	226.5 ± 23.8 μg min/ml
$AUC_{0-\infty}$	6526.3 ± 1041.5 ng min/ml	321.7 ± 53.2 μg min/ml
MRT	78.9 ± 13.5 min	194.0 ± 24.7 min
$T_{1/2}$	84.5 ± 12.5 min	167.5 ± 2.4 min
CL_{tot}	0.01 ± 0.0 L/(min kg)	0.03 ± 0.0 L/(min kg)
Vd_{ss}	0.6 ± 0.1 L/kg	0.5 ± 0.0 L/kg

Results are expressed as the mean ± S.E. of at least 3 experiments.

The summary of AUC values and bioavailability (*F*%) of CF after co-administration with solubilizing agents to the jejunum using the *in situ* closed-loop technique shows that 5 and 10% (w/v) NaTC, 10% (v/v) Labrasol, and 10% (v/v) Transcutol P increased the AUC values and *F*% of CF in the jejunum (Table 5). The *F*% of CF in the presence of 5 and 10% (w/v) NaTC and 5 and 10% (v/v) Labrasol was more than 45%, and 10% (v/v) Labrasol displayed the largest AUC value and *F*% of CF in the absorption studies. The *F*% of CF in the presence of Transcutol P was about 35%, and the remaining solubilizing agents showed no significant effect on the intestinal absorption of CF using the *in situ* closed-loop technique.

To confirm whether the presence of NaTC and Labrasol enhances drug concentration in blood plasma of a high molecular weight substance, we examined the effect of 5 and 10% (w/v) NaTC and 5 and 10% (v/v) Labrasol on the intestinal absorption of FD4 (MW 4400) which has a higher molecular weight than CF (MW 376.32).

Both 5 and 10% (w/v) NaTC significantly increased the absorption of FD4 in the jejunum (Fig. 3). In addition, 10% (v/v) Labrasol remarkably increased the intestinal absorption of FD4, while 5% (v/v) Labrasol only slightly increased absorption.

The AUC values and *F*% of FD4 after administration of FD4 with 5 and 10% (w/v) NaTC and 5 and 10% (v/v) Labrasol to the jejunum

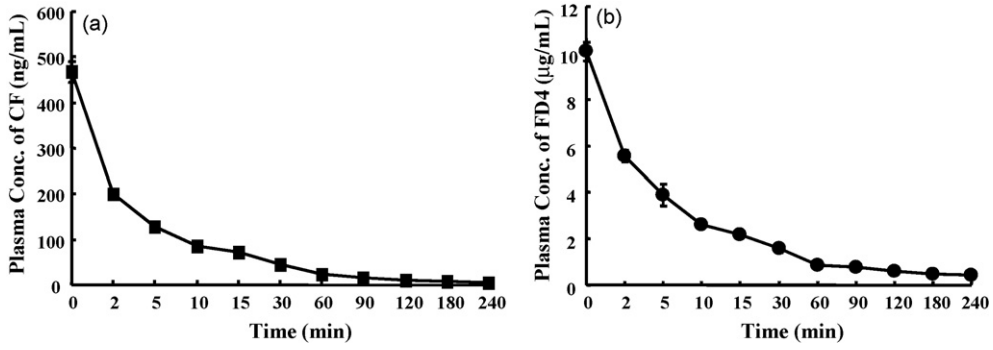

Fig. 1. Plasma concentration–time profiles of CF (0.0625 mg/ml) (a) and FD4 (1 mg/ml) (b) after their intravenous administration to the rat femoral vein. Results are expressed as the mean ± S.E. of at least 3 experiments.

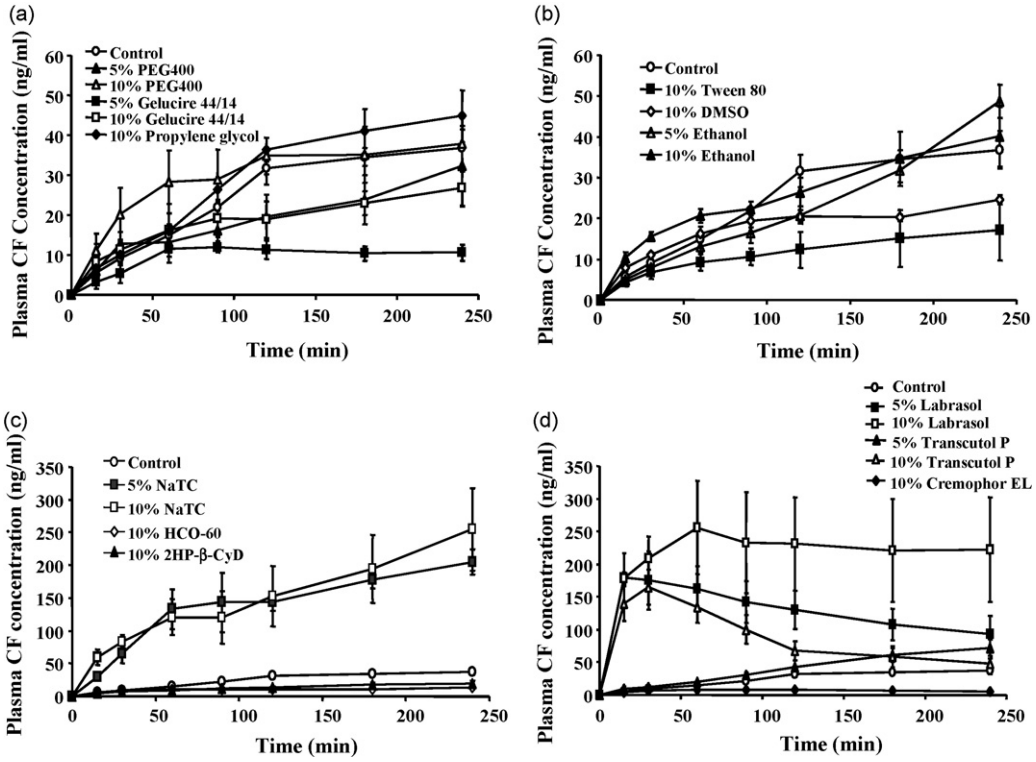

Fig. 2. Plasma concentration–time profiles of CF (0.125 mg/rat) after jejunal administration in the presence or absence of solubilizing agents of varying concentrations. Results are expressed as the mean ± S.E. of 3–4 experiments.

are summarized in Table 6. The AUC values and *F*% of FD4 significantly increased in the presence of 5 and 10% (w/v) NaTC and 10% (v/v) Labrasol, respectively, resulting in *F*% values of more than 19%. However, we found no significant effect on the intestinal absorption of FD4 in the presence of 5% (v/v) Labrasol. These results confirmed that higher concentrations of NaTC and Labrasol significantly increased the intestinal absorption of drugs regardless of molecular weight.

Table 5
Pharmacokinetic parameters of CF (0.5 mg/kg) with or without solubilizing agents in the jejunum.

	C_{max} (ng/ml)	T_{max} (min)	$AUC_{0-240\,min}$ (ng min/ml)	*F* (%)
Control	–	–	5993.5 ± 385.1	10.0 ± 0.7
NaTC (5%)	176.8 ± 13.0	220.0 ± 20.0	29229.1 ± 1958.5	49.0 ± 3.3
NaTC (10%)	254.5 ± 20.4	240.0 ± 0.0	36129.1 ± 5492.2**	60.5 ± 9.2
Labrasol (5%)	193.8 ± 39.0	30.0 ± 10.6	30927.3 ± 6692.6*	51.8 ± 11.2
Labrasol (10%)	263.5 ± 66.0	45.0 ± 15.0	52364.0 ± 15494.7**	87.7 ± 26.0
PEG 400 (5%)	32.3 ± 9.9	240.0 ± 0.0	4533.2 ± 1161.5$^{N.S.}$	7.6 ± 2.0
PEG 400 (10%)	41.7 ± 6.3	180.0 ± 60.0	7148.0 ± 1124.4$^{N.S.}$	12.0 ± 1.9
Transcutol P (5%)	72.4 ± 12.9	240.0 ± 0.0	9675.2 ± 1491.6$^{N.S.}$	16.2 ± 2.5
Transcutol P (10%)	164.7 ± 26.6	30.0 ± 0.0	20802.9 ± 3836.0*	34.9 ± 6.4
Propylene glycol (10%)	45.2 ± 6.5	225.0 ± 15.0	7034.4 ± 763.1$^{N.S.}$	11.8 ± 1.3
Gelucire 44/14 (5%)	14.5 ± 2.5	90.0 ± 12.3	2313.7 ± 447.0$^{N.S.}$	3.9 ± 0.8
Gelucire 44/14 (10%)	30.3 ± 4.6	195.0 ± 45.0	4476.3 ± 596.6$^{N.S.}$	7.5 ± 1.0
HCO-60 (10%)	14.5 ± 2.5	190.0 ± 50.0	2358.6 ± 307.0$^{N.S.}$	4.0 ± 0.5
Ethanol (5%)	48.7 ± 4.0	240.0 ± 0.0	5571.0 ± 338.8$^{N.S.}$	9.3 ± 0.6
Ethanol (10%)	40.1 ± 7.4	240.0 ± 0.0	6158.1 ± 901.8$^{N.S.}$	10.3 ± 1.5
CremophorEL (10%)	8.9 ± 1.6	90.0 ± 12.3	1699.5 ± 337.5$^{N.S.}$	2.9 ± 0.6
Tween 80 (10%)	17.7 ± 7.3	165.0 ± 43.3	2788.2 ± 1002.8$^{N.S.}$	4.7 ± 1.7
2HP-β-CyD (10%)	20.4 ± 3.7	210.0 ± 17.3	3070.2 ± 459.1$^{N.S.}$	5.1 ± 0.8
DMSO (10%)	24.6 ± 1.1	240.0 ± 0.0	4384.7 ± 160.5$^{N.S.}$	7.4 ± 0.3

Results are expressed as the mean ± S.E. of at least 3 experiments. $^{**}P < 0.01$, $^{*}P < 0.05$, N.S. not significant difference, compared with the control.

Table 6
Pharmacokinetic parameters of FD4 (8 mg/kg) with or without some solubilizing agents in the jejunum.

	Concentration	C_{max} (μg/ml)	T_{max} (min)	$AUC_{0-240\,min}$ (ng min/ml)	*F* (%)
Control (FD4)	–	0.6 ± 0.1	200 ± 40.0	100.1 ± 18.4	4.4 ± 0.8
NaTC	5% (v/v)	3.1 ± 0.6	240 ± 0.0	431.6 ± 81.7*	19.1 ± 3.6*
NaTC	10% (v/v)	5.4 ± 0.8	240 ± 0.0	737.8 ± 175.3**	32.6 ± 7.7**
Labrasol	5% (v/v)	1.8 ± 0.3	26 ± 3.8	281.7 ± 41.9$^{N.S.}$	12.4 ± 1.8$^{N.S.}$
Labrasol	10% (v/v)	4.4 ± 0.8	30 ± 0.0	688.5 ± 183.6**	30.4 ± 8.1**

Results are expressed as the mean ± S.E. of at least 3 experiments. $^{**}P < 0.01$, $^{*}P < 0.05$, N.S. not significant difference, compared with the control.

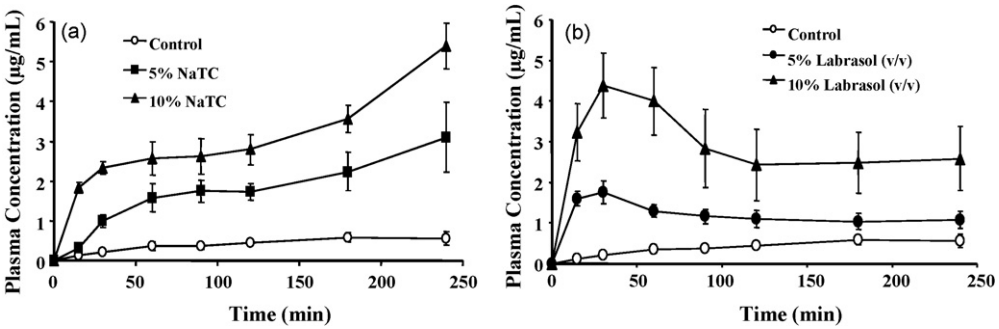

Fig. 3. Plasma concentration–time profiles of FD4 (2 mg/rat) after jejunal administration in the presence or absence of Labrasol and NaTC of varying concentrations. Results are expressed as the mean ± S.E. of at least 3 experiments. Key: (a) (○) control, (■) 5% (w/v) NaTC, (▲), 10% (w/v) NaTC; (b) (○) control, (■) 5% (v/v) Labrasol, (▲) 10% (v/v) Labrasol.

3.3. Effect of solubilizing agents on intestinal membrane toxicity

Finally, we examined the effects of various solubilizing agents on intestinal membrane toxicity by evaluating the total amount of protein and LDH released from intestinal epithelial cells in the presence or absence of solubilizing agents. As shown in Fig. 4, the total protein level at 4 h after administration of the solubilizing agents significantly increased in the presence of 5 and 10% (w/v) NaTC and 5% (v/v) Gelucire 44/14. The remaining solubilizing agents showed no significant effect on protein levels, suggesting that they may not cause intestinal membrane damage.

The release of LDH at 4 h after administration of solubilizing agents significantly increased for 5 and 10% (w/v) NaTC, 5 and 10% (v/v) Gelucire 44/14, 5 and 10% (v/v) Labrasol and 10% (v/v) Cremophor EL compared to the control (Fig. 5). FD4 in the presence of all other solubilizing agents produced nearly identical LDH levels to control values.

4. Discussion

In the present study, we first examined the effects of several solubilizing agents on the transport of CF in various intestinal regions (Tables 1–3). Only NaTC significantly increased the transport of CF in every region of the intestine, but toxicity studies indicate that this may be due to intestinal membrane damage. On the other hand, there was a regional difference in the absorption enhancing effects of Gelucire 44/14 and ethanol. Using 5% (v/v) Gelucire 44/14 significantly increased the transport of CF only within the jejunum, and ethanol increased the transport of CF across the lower intestinal regions (ileum and colon). All other solubilizing agents did not sig-

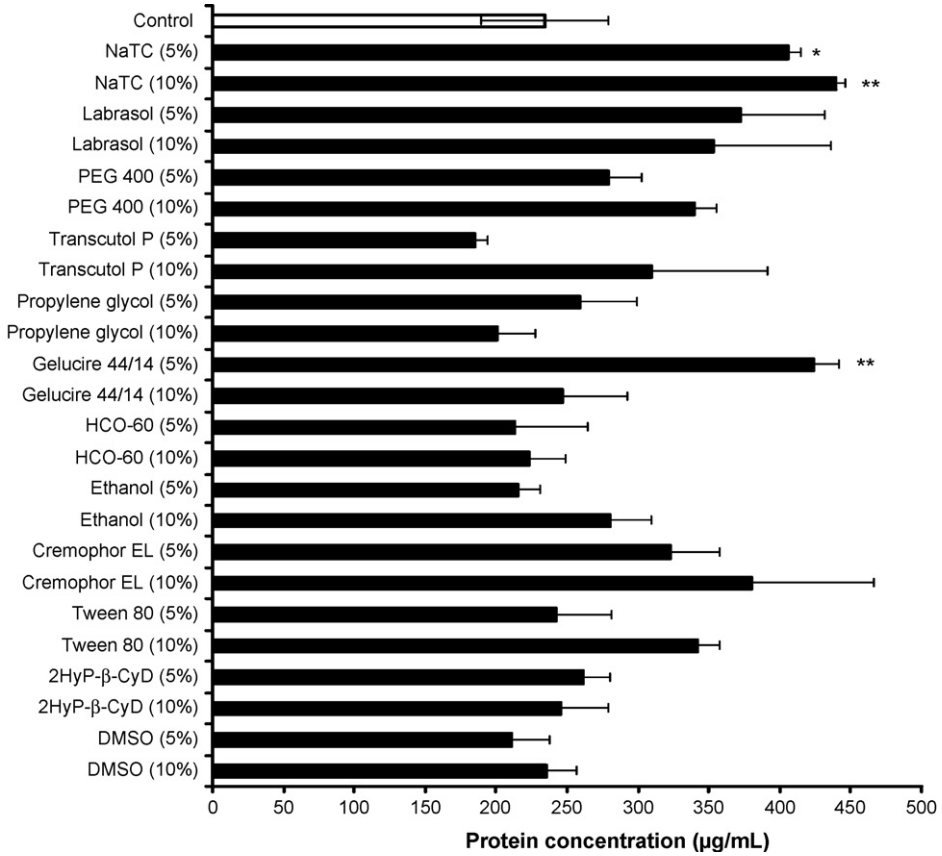

Fig. 4. The total protein level at 4 h after jejunal administration of various solubilizing agents. Results are expressed as the mean ± S.E. of at least 3–4 experiments. $^{**}P < 0.01$, $^{*}P < 0.05$, compared with the control.

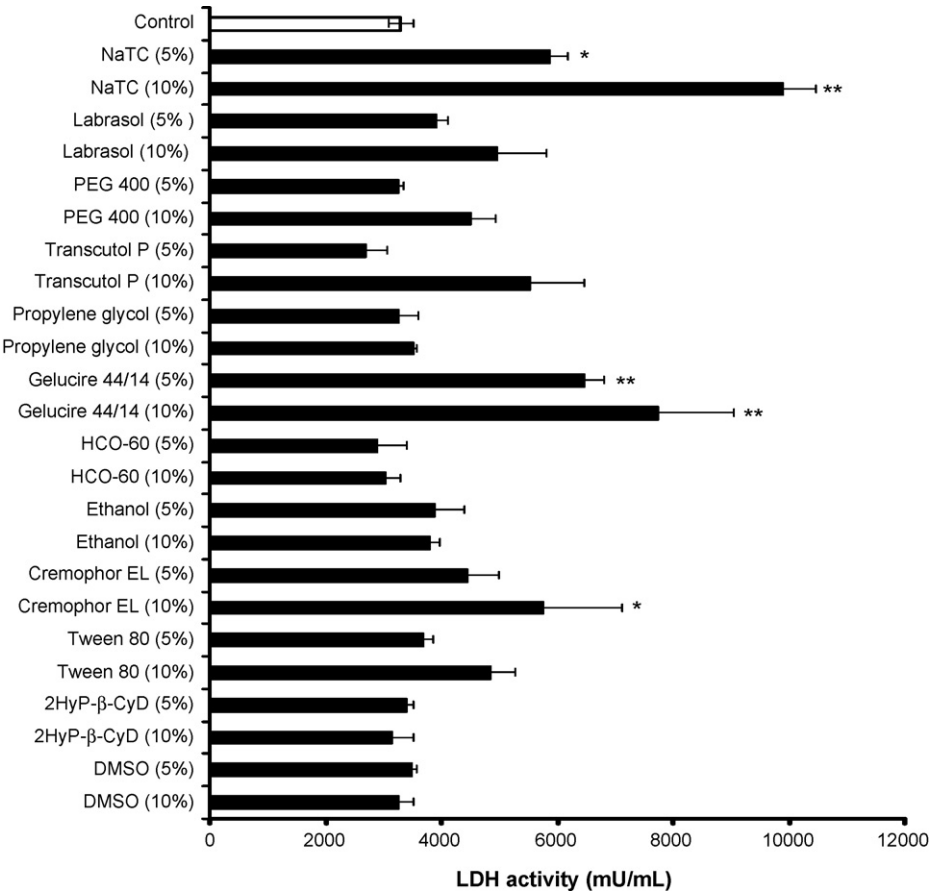

Fig. 5. The activity of lactate dehydrogenase (LDH) at 4 h after jejunal administration of various solubilizing agents. Results are expressed as the mean ± S.E. of at least 3–4 experiments. $^{**}P < 0.01$, $^{*}P < 0.05$, compared with the control.

nificantly affect the transport of CF across the intestinal membrane. These *in vitro* transport results offer a preliminary look at the effect of solubilizing agents on the transport of CF across the intestinal membrane.

The absorption enhancing effects of various adjuvants, including absorption enhancers and these solubilizing agents, were generally greater in lower intestinal regions than in upper intestinal regions. Previously, we reported that the effects of conventional absorption enhancers were greater in the large intestine than in the small intestine for improving the intestinal absorption of ebiratide (Yamamoto et al., 1997). Similarly, the rank order of the effectiveness of various absorption enhancers after their administration to sites was reported as rectum > colon > small intestine > stomach > skin (Muranishi, 1990). However, the absorption enhancing effects of nitric oxide (NO) donors, novel absorption enhancers in the small intestine, were nearly identical to effects seen in the large intestine for improving the intestinal absorption of insulin (Fetih et al., 2005). A more recent study shows that the absorption enhancing effect of spermine, one of the typical polyamines, was greater in the jejunum than in the colon (Gao et al., 2008a).

The reason for regional differences in absorption enhancing effects of adjuvants is not fully understood, but may be related to physiological differences, such as variations in mucous layer thickness, number and tightness of cell junctions, or dissimilarities in lipid composition.

Based on results obtained using the *in vitro* diffusion chamber, we evaluated the effect of the solubilizing agents on the intestinal absorption of CF using an *in situ* closed-loop technique. The jejunal region, having the largest surface area available for drug absorption, was chosen for the closed-loop study (Lin et al., 1994). Results of the present study indicate that most of the solubilizing agents examined using the *in situ* closed-loop technique had no significant effect on intestinal absorption, which includes the non-ionic solubilizers Cremophor EL, Tween 80, 2HP-β-CyD, Gelucire 44/14, HCO-60, (co)solvents of PEG 400, propylene glycol, DMSO and ethanol in concentrations of 5 and 10% (v/v or w/v). A previous study using Caco-2 cells reported that five different solubilizing agents (20% propylene glycol, 5% Tween 80, 5% PEG 400, 5% HP-β-CD, and 5% Tween 80 + 5% PEG 400) did not affect the viability of 21 days cultured Caco-2 monolayers (Takahashi et al., 2002). Furthermore, 0.2 mg/ml Cremophor EL had no influence on the permeability of amoxicillin (Legen et al., 2006) and on the transport of rhodamine123 (Shono et al., 2004). However, these non-ionic surfactants/solubilizers are often discussed in drug solubility (Kawakami et al., 2004; Zerrouk et al., 2006; Wang et al., 2007) and in P-gp transport studies (Shono et al., 2004; Shen et al., 2006; Lin et al., 2007).

Organic co-solvents are normally used to solubilize drugs that are poorly soluble in water to a desired concentration in oral solutions, and the maximum amount of solvent used is up to 55% for propylene glycol, 17% for PEG 400 and up to 42% for ethanol (Strickley, 2004). Ethanol is a common solubilizing excipient and is often used in conjunction with propylene glycol in mixed aqueous or organic co-solvents, or with Cremophor EL in formulations composed entirely of organic solvents (Strickley, 2004). DMSO was reported to be inert towards other chemicals (David, 1972), easily penetrates cell membranes without causing irreversible damage and has been widely used because of a large solubilization capacity and a low toxicity (Himmel, 2007). Lin et al. (2007) reported that 0.01–10% (v/v) Gelucire 44/14 did not change the transport of

rhodamine123 in the ileum and colon and was confirmed to form micelles by surface tension (Kawakami et al., 2004).

Labrasol and NaTC with 5 and 10% (v/v, w/v) displayed remarkable enhancing effects with bioavailability at more than 45%, and 10% (v/v) Transcutol P showed similar but less significant effects (Fig. 2c and d). Labrasol has been widely used in solubilizing hydrophobic drugs (Strickley, 2004), was reported to have a strong absorption enhancing effect and was shown to improve the intestinal absorption of poorly absorbed drugs, including gentamicin, insulin and vancomycin (Hu et al., 2001; Eaimtrakarn et al., 2002; Rama Prasad et al., 2003). Additionally, NaTC also plays a pivotal role in enhancing the rectal absorption of insulin (Yamamoto et al., 1992). Our current results correlate well with these previous studies.

The effects of NaTC and Labrasol on the intestinal absorption of FD4, a high molecular weight model drug were studied and the results are summarized in Fig. 3 and Table 6. A significant absorption enhancing effect, similar to that for CF, was observed for both concentrations of NaTC as well as 10% (v/v) Labrasol. No significant effect was observed using 5% (v/v) Labrasol and may be attributed to the high molecular weight of FD4. Overall, our results suggest that both NaTC and Labrasol have a notable effect on intestinal absorption, regardless of the drug molecular weight. NaTC, a natural bile salt and commonly used absorption enhancer, increases paracellular absorption by opening tight junctions (Gizurarson, 1990). Recent *in vitro* transport and *in vivo* absorption studies indicate that NaTC has a significant effect in each intestinal region and correlates well with the *in vivo* results.

All the other solubilizing agents showed similar results between *in vitro* and *in vivo* studies, and had no significant effect on the transport or absorption of CF in the rat intestine. Conversely, there existed almost no correlation of absorption enhancing effect of Labrasol between *in vitro* and *in vivo* studies with Labrasol indicated as more potent *in vivo* than *in vitro*. Labrasol is mainly composed of PEG esters and glyceride with medium acyl chains. Recent studies observed hydrolysis (lipolysis) of Labrasol by lipase, especially gastric lipase, pancreatic lipase-related protein 2 (PLRP2) and carboxylester hydrolase (CEH) after oral administration (Fernandez et al., 2007), and the digested components resulting from Labrasol lipolysis appeared to be more potent absorption enhancers compared to Labrasol (Dahan and Hoffman, 2008). These findings suggest that some components of Labrasol lipolysis have absorption enhancing effects and subsequently improve intestinal absorption of CF in the present *in situ* closed-loop study, where digestion may occur more easily than in the *in vitro* diffusion chamber.

Gelucire 44/14 and ethanol, on the other hand, showed greater absorption enhancing effects in the *in vitro* studies than the *in vivo* studies, although the mechanism is not fully understood. One possible explanation for the different results is the dilution of solubilizing agents in luminal fluid. During the course of the *in situ* closed-loop absorption studies, concentrations of solubilizing agents within the loop decreased as intestinal fluids were secreted into the lumen from epithelial cells. In contrast, the concentrations of solubilizing agents applied to the donor site in the *in vitro* transport studies could be relatively maintained, because intestinal fluids were not secreted nearly as much from the isolated intestinal membrane.

The present toxicity studies indicate that most of the solubilizing agents do not damage or irritate the rat intestinal membrane, and are corroborated by previous investigations showing non-ionic surfactants to be less toxic than ionic surfactants to biological membranes (Davis et al., 1970). Only NaTC and Gelucire 44/14 showed a significant increase in the release of protein and LDH, and 10% (v/v) Cremophor EL exhibited a significant increase ($P < 0.05$) only in LDH activity. Measuring leakage of cytoplasm components has been accepted as a valid method for estimating membrane damage, and the release of protein and lactate dehydrogenase (LDH) from the intestinal mucosa has been used as an index to assess tissue damage (Uchiyama et al., 1999; Yamamoto et al., 2001a). The results clearly indicate that 5 and 10% (w/v) NaTC significantly increased both the amount of protein released and the activity of LDH. NaTC has also been shown to have a concentration-dependent toxicity when administered to Caco-2 cell monolayers and these concentration dependent effects are seen using NaTC above the critical micelle concentration (CMC), or about 2 mM in HBSS at 37 °C (Johansson et al., 2002). Gelucire 44/14 also showed approximately ten times more cytotoxic compared to control in Caco-2 cells system (Sachs-Barrable et al., 2007). NaTC, a bile salt and natural surfactant, may increase permeability by altering or damaging tight junctions of the intestinal epithelium.

Membrane damage was not seen in the presence of Labrasol, and suggests that it increases intestinal absorption of CF and FD4 by alternate mechanisms, possibly by means of a paracellular pathway established through the loosening of tight junctions in the intestinal epithelium.

Previous studies have shown that 0.1% Labrasol does not damage the intestinal membrane (Lin et al., 2007) and shows high tolerance and low toxicity (Rama Prasad et al., 2003). Based on previous toxicity findings, we consider that 5 and 10% (v/v) Labrasol, 5 and 10% (v/v) PEG 400, 5 and 10% (v/v) Transcutol P, 10% (v/v) propylene glycol, 10% (v/v) HCO-60, 5 and 10% (v/v) ethanol, 10% (v/v) Tween 80, 10% (v/v) 2HP-β-CyD and 10% (v/v) DMSO do not cause significant membrane damage to the intestinal epithelium.

Solubility is an important requirement for absorption and solubilizing agents are normally used to increase the solubility of lipophilic drugs. Other important factors to consider for absorption include the drug's molecular weight, lipid solubility, the partition coefficient, the ionization constant, and pH of the administration site. Unfavorable factors may negatively affect the absorption process despite the presence of solubilizing agents or having achieved acceptable solubility rates *in vitro* (Shah et al., 1996).

The present results indicate that 5 and 10% (v/v) of PEG 400, 10% (v/v) propylene glycol, 10% (v/v) HCO-60, 5 and 10% (v/v) ethanol, 10% (v/v) Cremophor EL, 10% (v/v) Tween 80, 10% (w/v) 2HP-β-CyD and 10% (v/v) DMSO are suitable solubilizing agents for evaluating drugs that are poorly soluble in water using the *in vitro* diffusion method and the *in situ* closed-loop technique. Additionally, these solubilizing agents resulted in no significant damage to membrane integrity and can be safely used in both *in vitro* and *in situ* absorption studies with drugs having very limited water solubility. In conclusion, the present study increases the fundamental knowledge of screening and evaluating solubilizing agents, using *in vitro* and *in situ* absorption methods, for drugs that are poorly absorbed.

Acknowledgement

This work was supported by the 21st Century Center of Excellence Program, 'Development of Drug Discovery Frontier Integrated from Traditional to Proteome' from the Ministry of Education, Science, Sports and Culture in Japan.

References

Dahan, A., Hoffman, A., 2008. Rationalizing the selection of oral lipid based drug delivery systems by an *in vitro* dynamic lipolysis model for improved oral bioavailability of poorly water soluble drugs. J. Control Release 129, 1–10.

David, N.A., 1972. The pharmacology of dimethyl sulfoxide. Ann. Rev. Pharmacol. 12, 353–374.

Davis, W.W., Pfeiffer, R.R., Quay, J.F., 1970. Normal and promoted gastrointestinal absorption of water-soluble substances. I. Induced rapidly reversible hyperabsorptive state in the canine fundic stomach pouch. J. Pharm. Sci. 59, 960–963.

Eaimtrakarn, S., Rama Prasad, Y.V., Ohno, T., Konishi, T., Yoshikawa, Y., Shibata, N., Takada, K., 2002. Absorption enhancing effect of Labrasol on the intestinal absorption of insulin in rats. J. Drug Target. 10, 255–260.

Fernandez, S., Jannin, V., Rodier, J.D., Ritter, N., Mahler, B., Carriere, F., 2007. Comparative study on digestive lipase activities on the self emulsifying excipient

labrasol, medium chain glycerides and PEG esters. Biochim. Biophys. Acta 1771, 633–640.
Fetih, G., Habib, F., Okada, N., Fujita, T., Attia, M., Yamamoto, A., 2005. Nitric oxide donors can enhance the intestinal transport and absorption of insulin and [Asu[1,7]]-eel calcitonin in rats. J. Control Release 106, 287–297.
Gao, Y., He, L., Katsumi, H., Sakane, T., Fujita, T., Yamamoto, A., 2008a. Improvement of intestinal absorption of water-soluble macromolecules by various polyamines: intestinal mucosal toxicity and absorption-enhancing mechanism of spermine. Int. J. Pharm. 354, 126–134.
Gao, Y., He, L., Katsumi, H., Sakane, T., Fujita, T., Yamamoto, A., 2008b. Improvement of intestinal absorption of insulin and water-soluble macromolecular compounds by chitosan oligomers in rats. Int. J. Pharm. 359, 70–78.
Gizurarson, S., 1990. Intranasal application of insulin. Doctoral Dissertation, the Royal Danish School of Pharmacy, Copenhagen, Denmark.
Gotoh, S., Nakamura, R., Nishiyama, M., Quan, Y.S., Fujita, T., Yamamoto, A., Muranishi, S., 1996. Effects of protease inhibitors on the absorption of phenol red and fluorescein isothiocyanate dextrans from the rat intestine. J. Pharm. Sci. 85, 858–862.
Grass, G.M., Sweetana, S.A., 1988. *In vitro* measurement of gastrointestinal tissue permeability using a new diffusion cell. Pharm. Res. 5, 372–376.
Himmel, H.M., 2007. Suitability of commonly used excipients for electrophysiological *in-vitro* safety pharmacology assessment of effects on hERG potassium current and on rabbit Purkinje fiber action potential. J. Pharm. Toxicol. Method 56, 145–158.
Hu, Z., Tawa, R., Konishi, T., Shibata, N., Takada, K., 2001. A novel emulsifier, labrasol, enhances gastrointestinal absorption of gentamicin. Life Sci. 69, 2899–2910.
Johansson, F., Hjertberg, E., Eirefelt, S., Tronde, A., Bengtsson, U.H., 2002. Mechanisms for absorption enhancement of inhaled insulin by sodium taurocholate. Eur. J. Pharm. Sci. 17, 63–71.
Johnson, B.M., Charman, W.N., Porter, C.H., 2002. An *in vitro* examination of the impact of polyethylene glycol 400, pluronic P85, and Vitamin E D-α-tocopheryl polyethylene glycol 1000 succinate on P-glycoprotein efflux and enterocyte-based metabolism in excised rat intestine. AAPS Pharm. Sci. 4, E40.
Kawakami, K., Miyoshi, K., Ida, Y., 2004. Solubilization behaviour of poorly soluble drugs with combined use of gelucire 44/14 and cosolvent. J. Pharm. Sci. 93, 1471–1479.
Legen, I., Kracun, M., Salobir, M., Kerc, J., 2006. The evaluation of some pharmaceutically acceptable excipients as permeation enhancers for amoxicillin. Int. J. Pharm. 308, 84–89.
Lin, J.H., Chen, I.-W., deLuna, F.A., 1994. On the absorption of alendronate in rats. J. Pharm. Sci. 83, 1741–1746.
Lin, Y., Shen, Q., Katsumi, H., Okada, N., Fujita, T., Jiang, X., Yamamoto, A., 2007. Effects of Labrasol and other pharmaceutical excipients on the intestinal transport and absorption of rhodamine 123, a P-glycoprotein substrate, in rats. Biol. Pharm. Bull. 30, 1301–1307.
Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 46, 3–26.
Muranishi, S., 1990. Absorption enhancers. Crit. Rev. Ther. Drug Carrier Syst. 7, 1–33.
Oda, M., Saitoh, H., Kobayashi, M., Aungst, B.J., 2004. β-Cyclodextrin as a suitable solubilizing agent for *in situ* absorption study of poorly water-soluble drugs. Int. J. Pharm. 280, 95–102.
Rama Prasad, Y.V., Puthli, S.P., Eaimtrakarn, S., Ishida, M., Yoshikawa, Y., Shibata, N., Takada, K., 2003. Enhanced intestinal absorption of vancomycin with Labrasol and D-α-tocopheryl PEG 1000 succinate in rats. Int. J. Pharm. 250, 181–190.
Rege, B.D., Yu, L., Hussain, A.S., Polli, J.E., 2001. Effect of common excipients on Caco-2 transport of low-permeability drugs. J. Pharm. Sci. 90, 1776–1786.
Sachs-Barrable, K., Thamboo, A., Lee, S.D., Wasan, K.M., 2007. Lipid excipients peceol and gelucire 44/14 decrease P-glycoprotein mediated efflux of rhodamine 123 partially due to modifying P-glycoprotein protein expression within Caco-2 cells. J. Pharm. Pharm. Sci. 10, 319–331.
Sakai, M., Imai, T., Ohtake, H., Azuma, H., Otagiri, M., 1997. Effects of absorption enhancers on the transport of model compounds in Caco-2 cell monolayers: assessment by confocal laser scanning microscopy. J. Pharm. Sci. 86, 779–785.
Shah, V.P., Yacobi, A., Barr, W.H., Benet, L.Z., Breimer, D., Dobrinska, M.R., Endrenyi, L., Fairweather, W., Gillespie, W., Gonzalez, M.A., Hooper, J., Jackson, A., Lesko, L.J., Midha, K.K., Noonan, P.K., Petnaik, R., Williams, R.L., 1996. Workshop report: evaluation of orally administered highly variable drugs and drug formulations. Pharm. Res. 13, 1590–1594.
Shen, Q., Lin, Y., Handa, T., Doi, M., Sugie, M., Wakayama, K., Okada, N., Fujita, T., Yamamoto, A., 2006. Modulation of intestinal P-glycoprotein function by polyethylene glycols and their derivatives by *in vitro* transport and *in situ* absorption studies. Int. J. Pharm. 313, 49–56.
Shono, Y., Nishihara, H., Matsuda, Y., Furukawa, S., Okada, N., Fujita, T., Yamamoto, A., 2004. Modulation of intestinal P-glycoprotein function by Cremophor EL and other surfactants by an *in vitro* diffusion chamber method using the isolated rat intestinal membranes. J. Pharm. Sci. 93, 877–885.
Strickley, R.G., 2004. Solubilizing excipients in oral and injectable formulations. Pharm. Res. 21, 201–230.
Sugiyama, T., Yamamoto, A., Kawabe, Y., Uchiyama, T., Quan, Y.-S., Muranishi, S., 1997. Effects of various absorption enhancers on the intestinal absorption of water soluble drugs by *in vitro* Ussing chamber method: correlation with an *in situ* absorption experiment. Biol. Pharm. Bull. 20, 812–814.
Takahashi, Y., Kondo, H., Yasuda, T., Watanabe, T., Kobayashi, S., Yokohama, S., 2002. Common solubilizers to estimate the Caco-2 transport of poorly water-soluble drugs. Int. J. Pharm. 246, 85–94.
Totterman, A.M., Schipper, N.G., Thompson, D.O., Mannermaa, J.-P., 1997. Intestinal safety of water soluble β-cyclodextrins in paediatric oral solutions of spironolactone: effects on human intestinal epithelial Caco-2 cells. J. Pharm. Pharmacol. 49, 43–48.
Uchiyama, T., Yamamoto, A., Hatano, H., Fujita, T., Muranishi, S., 1996. Effectiveness and toxicity screening of various absorption enhancers in the large intestine: intestinal absorption of phenol red and protein and phospholipid release from the intestinal membrane. Biol. Pharm. Bull. 19, 1618–1621.
Uchiyama, T., Sugiyama, T., Quan, Y.-S., Kotani, A., Okada, N., Fujita, T., Muranishi, S., Yamamoto, A., 1999. Enhanced permeability of insulin across the rat intestinal membrane by various absorption enhancers: their intestinal mucosal toxicity and absorption enhancing mechanism of n-lauryl-β-D-maltopyranoside. J. Pharm. Pharmacol. 51, 1241–1250.
Wang, L., Jiang, X., Xu, W., Li, C., 2007. Complexation of tanshinone IIA with 2-hydroxypropyl-β-cyclodextrin: effect on aqueous solubility, dissolution rate, and intestinal absorption behavior in rats. Int. J. Pharm. 341, 58–67.
Yamamoto, A., Hayakawa, E., Kato, Y., Nishiura, A., Lee, V.H.L., 1992. A mechanistic study on enhancement of rectal permeability to insulin in the albino rabbit. J. Pharmacol. Exp. Ther. 263, 25–31.
Yamamoto, A., Taniguchi, T., Rikyuu, K., Tsuji, T., Fujita, T., Murakami, M., Muranishi, S., 1994. Effects of various protease inhibitors on the intestinal absorption and degradation of insulin in rats. Pharm. Res. 11, 1496–1500.
Yamamoto, A., Uchiyama, T., Nishikawa, R., Fujita, T., Muranishi, S., 1996. Effectiveness and toxicity screening of various absorption enhancers in the rat small intestine: effects of absorption enhancers on the intestinal absorption of phenol red and the release of protein and phospholipids from the intestinal membrane. J. Pharm. Pharmacol. 48, 1285–1289.
Yamamoto, A., Okagawa, T., Kotani, A., Uchiyama, T., Shimura, T., Tabata, S., Kondo, S., Muranishi, S., 1997. Effects of different absorption enhancers on the permeation of ebiratide, an ACTH analogue, across intestinal membranes. J. Pharm. Pharmacol. 49, 1057–1061.
Yamamoto, A., Tatsumi, H., Maruyama, M., Uchiyama, T., Okada, N., Fujita, T., 2001a. Modulation of intestinal permeability by nitric oxide donors: implications in intestinal delivery of poorly absorbable drugs. J. Pharmacol. Exp. Ther. 296, 84–90.
Yamamoto, A., Iseki, T., Ochi-Sugiyama, M., Okada, N., Fujita, T., Muranishi, S., 2001b. Absorption of water-soluble compounds with different molecular weights and [Asu[1,7]]-eel calcitonin from various mucosal administration sites. J. Control Release 76, 363–374.
Zerrouk, N., Corti, G., Ancillotti, S., Maestrelli, F., Cirri, M., Mura, P., 2006. Influence of cyclodextrins and chitosan, separately or in combination, on glyburide solubility and permeability. Eur. J. Pharm. Biopharm. 62, 241–246.