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Research paper

In vitro and in situ evidence for the contribution of Labrasol® and Gelucire 44/14 on transport of cephalexin and cefoperazone by rat intestine

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Abstract

In vitro and in situ intestinal transport of β-lactam antibiotics in the presence of two novel pharmaceutical excipients, caprylocaproyl and lauroyl macrogolglycerides (Labrasol® and Gelucire 44/14), is described. The objective was to compare the effects of both macrogolglycerides on the intestinal transport of cephalexin, a substrate of oligopeptide transporters, and cefoperazone, a non-substrate of them. The in vitro transport studies were performed using a sheet of rat jejunum mounted in Ussing-type diffusion chambers. The in situ studies used an isolated internal loop model in the rat. Labrasol® and Gelucire 44/14 were used as the excipients at low concentrations (0.01–0.5%, w/v). The membrane permeability of both drugs was compared by apparent permeability coefficients (P_{app}) determined from changes in the amount of permeation *vs*. time in in vitro studies and by apparent absorptive clearance (CL_{app}) determined from changes in the steady state drug concentration of perfusate in in situ studies. The P_{app} value of cephalexin increased with an increase in the concentration of Labrasol® (0.05–0.5%) compared to the value without Labrasol®. The enhancing effect of Labrasol® on cephalexin transport was similarly observed in in situ studies, and when 0.5% Labrasol® was used in the presence of glycyl-L-leucine or L-alanyl-L-alanine, 60 or 46% enhancement of the active transport of cephalexin by Labrasol® was obtained. On the other hand, Gelucire 44/14 did not affect the P_{app} and CL_{app} of either drug. The different effects of the excipients on cephalexin transport were thought to be due to the influences of size parameters such as a polydispersity index and particle size, and the change in the short-circuit current of jejunum by the addition of the excipient.

Keywords: β-Lactam antibiotic; Caprylocaproyl macrogolglyceride; Lauroyl macrogolglyceride; Polydispersity

1. Introduction

Caprylocaproyl macrogolglyceride (Labrasol®) and lauroyl macrogolglyceride (Gelucire 44/14) are pharmaceutical excipients which published to European Pharmacopoeial standards in 1998. Both excipients consist of mono-, di- and triglycerides and of mono-, and di-fatty acid esters of polyethylene glycol. Currently these excipients are used as bioavailability enhancers in, for example, proxyphylline release from matrix hard gelatin capsules [1], oral formulation of nicotine [2], dermal application of cyclosporin A [3] and lymphatic transport of an LTB4 inhibitor [4]. These excipients are a mixture of nonionic surfactants and form micelles in aqueous solution. We previously reported that nonionic surfactants with low polydispersity affected the membrane transport of ceftibuten, a β-lactam antibiotic, via oligopeptide transporters in rats [5]. Further, it has been reported that nonionic surfactants such as octyl-β-D-glucopyranoside and Tween-80 inhibit the transport of Na^+-dependent D-glucose by rat intestinal brush-border membrane vesicles [6] and the efflux of P-glycoprotein in Caco-2 cells [7], respectively.

The aim of this study was to compare the effects of the two excipients with different fatty acid distributions on cephalexin, a substrate of oligopeptide transporters, and cefoperazone, a non-substrate of the transporters.

2. Materials and methods

2.1. Materials

Labrasol® and Gelucire 44/14 (Gattefosse, Saint-Priest Cedex, France) were gifts from CBC Co. (Tokyo, Japan). The chemical definitions of both excipients are shown in

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Table 1
Physicochemical properties of Labrasol and Gelucire 44/14

	Labrasol	Gelucire 44/14
Caproic acid (C6)	0.9%	–
Caprylic acid (C8)	57.5%	8.2%
Capric acid (C10)	40.5%	6.3%
Lauric acid (C12)	0.7%	45.6%
Myristic acid (C14)	0.1%	16.7%
Palmitic acid (C16)	–	8.7%
Stearic acid (C18)	–	9.7%
HLB number	14	14

Table 1. Cephalexin was kindly donated by Shionogi Co. (Osaka, Japan). Cefoperazone sodium, glycyl-L-leucine (Gly–Leu) and L-alanyl-L-alanine (Ala–Ala) were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Animals

Wistar male rats weighing 220–250 g were fasted overnight prior to the in situ studies, but had free access to food in in vitro studies. Water was allowed ad libitum in in vitro and in situ studies. All animals received human care and were treated in accordance with the Guidelines for Animal Experimentation at Hokuriku University.

2.3. In vitro transport study

Rats were killed by removing blood from the main abdominal artery after intraperitoneal administration of pentobarbital sodium. Approximately 4 cm length of the jejunum was immediately excised with scissors. After the jejunum was spread to a flat sheet with scissors, the longitudinal muscle was removed with forceps, and then the jejunum sheet was set between Ussing-type diffusion chambers (No. 3440-s, Navicyte Inc., NV, USA), and the mucus was removed with Dulbecco's phosphate-buffered saline (Nissui Pharmaceutical Co., Tokyo, Japan). To deplete ATP, the intestinal segments were incubated for 20 min in phosphate buffered saline containing 10 mM NaF and 10 mM NaN_3 at room temperature according to the method of Terao et al. [8].

Isotonic phosphate buffer solution adjusted to pH 7.4 or 6.0 with HCl was used as the buffer on the serosal and mucosal sides of the chambers. Each β-lactam antibiotic was included in the buffer solution of one side chamber, and Labrasol® or Gelucire 44/14 was added to the mucosal-side chamber. The total volume of each chamber was 3 ml. During the experimental studies, both chambers were incubated at 37 °C and bubbled with O_2/CO_2 (95:5, v/v). A 100-μl sample was taken from the receptor side at 30, 45 and 60 min of incubation, and then samples were filtered with a Millipore filter (LCR13-LH, Millipore Japan, Tokyo, Japan) and a 30-μl volume was injected into an HPLC system.

The permeation rate of each β-lactam antibiotic was expressed as an apparent permeability coefficient (P_{app}) according to the following equation:

$$P_{app} = \frac{dC}{dt} \cdot \frac{V}{C_0 A}$$

where dC/dt is the change in concentration per unit time (nmol/ml per second); V is the solution volume of the receptor side (3 ml); C_0 is the initial drug concentration of the donor side (nmol/ml); and A is the apparent surface area of the jejunum sheet (1.2 cm^2).

2.4. Dynamic light scattering study

The average particle size and size distribution of micelle colloids of the excipients were determined using a laser particle analyzer (DLS-700, Otsuka Electronics Co., Osaka, Japan). The average diameters of the excipients were evaluated as a Z-average using a monomodal method, a cumulative analysis and particle size distribution as a ratio of average weight-weighed diameter (d_w) to average number-weighed diameter (d_n) using a multimodal method and an exponential sampling algorithm [9,10]. The analysis was performed at the excipient concentration of 0.5% (w/v) dissolved in phosphate buffer (pH 7.4), containing 0.5 mM cephalexin or 0.5 mM cefoperazone in the condition of 100 accumulation times at 37 °C.

2.5. Electrophysiological study in rat jejunum

Two electric parameters, membrane potential difference (PD) and short-circuit current (I_{sc}), were measured using a multi-channel voltage current clamp (EVC-4000, World Precision Instruments Inc., Sarasota, FL, USA) at 5-min intervals for 80 min. After rat jejunum sheet was prepared according to the method of Section 2.3, the jejunum sheet was mounted between Ussing-type diffusion chambers. Four glass-barrel micro-reference electrodes (Navicyte Inc., NV, USA) and two electrode cap holders (No. 3443, Navicyte Inc.) were set in a pair of chambers. Isotonic phosphate buffer solution adjusted to pH 7.4 with HCl was added to the serosal side of chambers and 0.5 mM cephalexin dissolved in phosphate buffer solution, pH 7.4, was added to the mucosal side. Labrasol® or Gelucire 44/14 at the final concentration of 0.5% was added to the mucosal side chamber after 20 min of incubation. The total volume of each chamber was 3 ml. During the experimental studies, both the chambers were incubated at 37 °C and bubbled with O_2/CO_2 (95:5, v/v).

The electrical resistance of membrane (R_m) is according to the following equation:

$$R_m = \frac{PD}{I_{sc}}$$

where R_m is the electrical resistance of membrane (Ω cm^2); PD is the membrane potential difference (mV); I_{sc} is the short-circuit current per unit of jejunum sheet area (μA/cm^2).

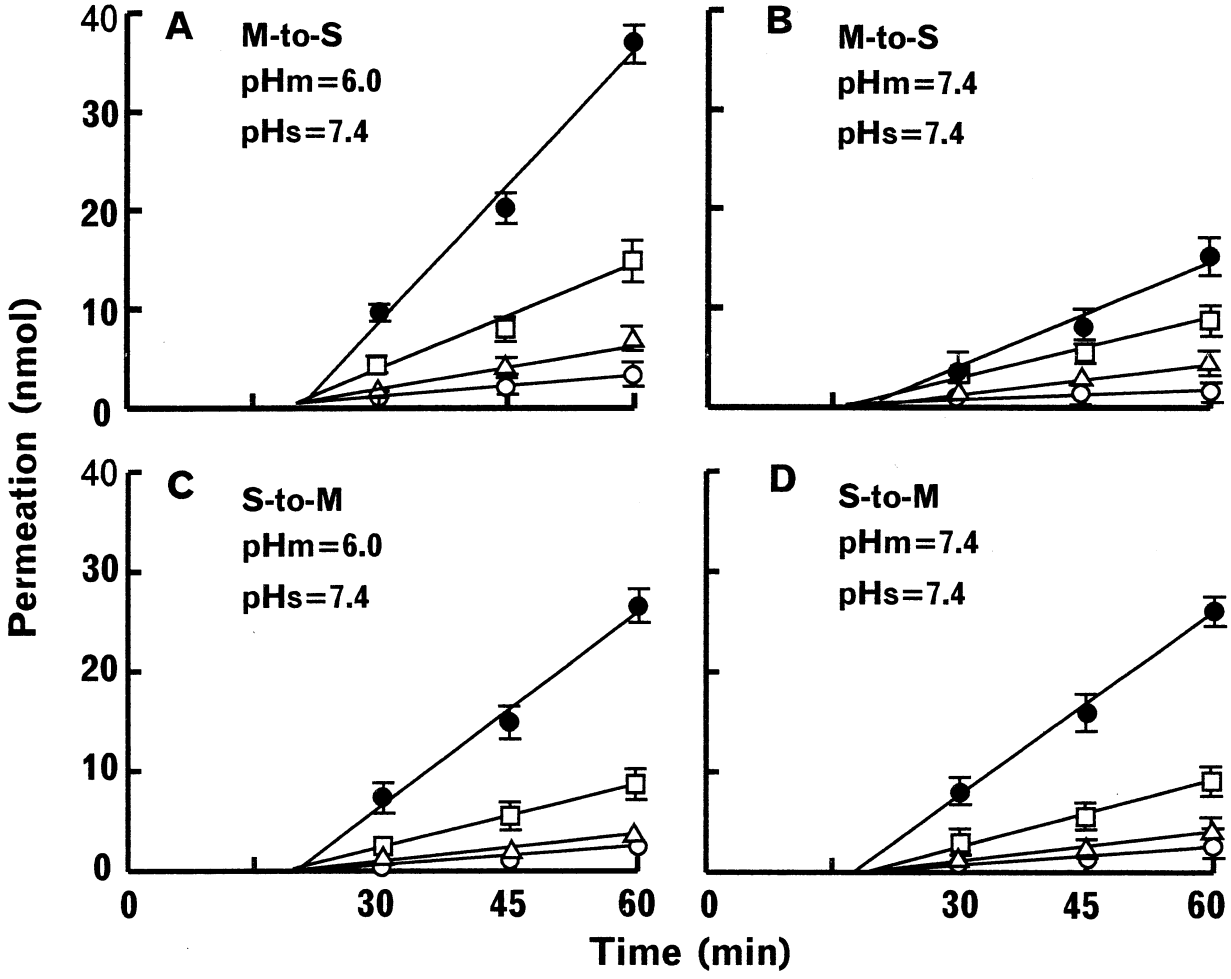

Fig. 1. Mucosal-to-serosal (M-to-S) and serosal-to-mucosal (S-to-M) cephalexin permeation by rat jejunum in the presence (A,C) and absence (B,D) of a proton-gradient. The pH of mucosal side (pHm) was 6.0 or 7.4. The pH of serosal side (pHs) was 7.4. Symbols indicate the concentrations of cephalexin: ○, 0.5 mM; △, 1.0 mM; □, 2.0 mM; ●, 5.0 mM. Data represent the mean ± SE of four experiments.

2.6. Surgical technique for isolated internal loop model

The establishment of the internal loop model was carried out by modification of the method of Poelma and Tukker [11]. Rats were anesthetized by intraperitoneal administration of pentobarbital sodium, and the abdomen was incised. Eight to 10 cm of the jejunum with an intact blood supply was severed from 10 cm under the stomach. The head–tail connection of the remaining intestine was restored by end-to-end anastomosis with sutures. The terminal ends of the 8–10 cm piece of jejunum were bound to two silicon tubes (2.4 mm inside diameter, 3.0 mm outside diameter, 2 cm length) with a strand of suture. Both silicon tubes were forced through the ventral muscle to the skin with a pointed forceps. Two silicon tubes (2.8 mm inside diameter, 4.5 mm outside diameter, 2 mm length) were inserted to 1 cm from the terminal of both penetrating tubes as stoppers. The abdominal opening was closed after a prophylactic injection of 100 000 IU penicillin. The isolated internal loop of jejunum was washed with saline. After the internal loop model rats were awake, total parenteral nutrition (9–15 ml/day Ensure Liquid, Dainabot Co., Osaka, Japan) was administered orally for 3 days, and then the rats were able to eat normal food and drink water ad libitum. The rats were considered fully recovered from the operation and ready for use in absorption experiments. The internal loop was washed out twice per day with saline.

2.7. In situ absorption study

The isolated internal loop model rats were placed in restraining cages. After the loop was washed with 10 ml saline, Tyrode solution adjusted to pH 6.5 with HCl and containing drug and excipient was perfused at the rate of 0.2 ml/min using a microsyringe pump (IC-3100, Kd Scientific Co., PA, USA). After the start of perfusion, the perfusate solution was collected from the outside tube of the loop at 10-min intervals for 90 min. The samples collected were filtered using a Millipore filter (LCR13-LH), and then 30 μl of the sample was injected into an HPLC system.

The absorption data of cephalexin were expressed as a steady-state concentration of perfusate (C_{ss}) and apparent

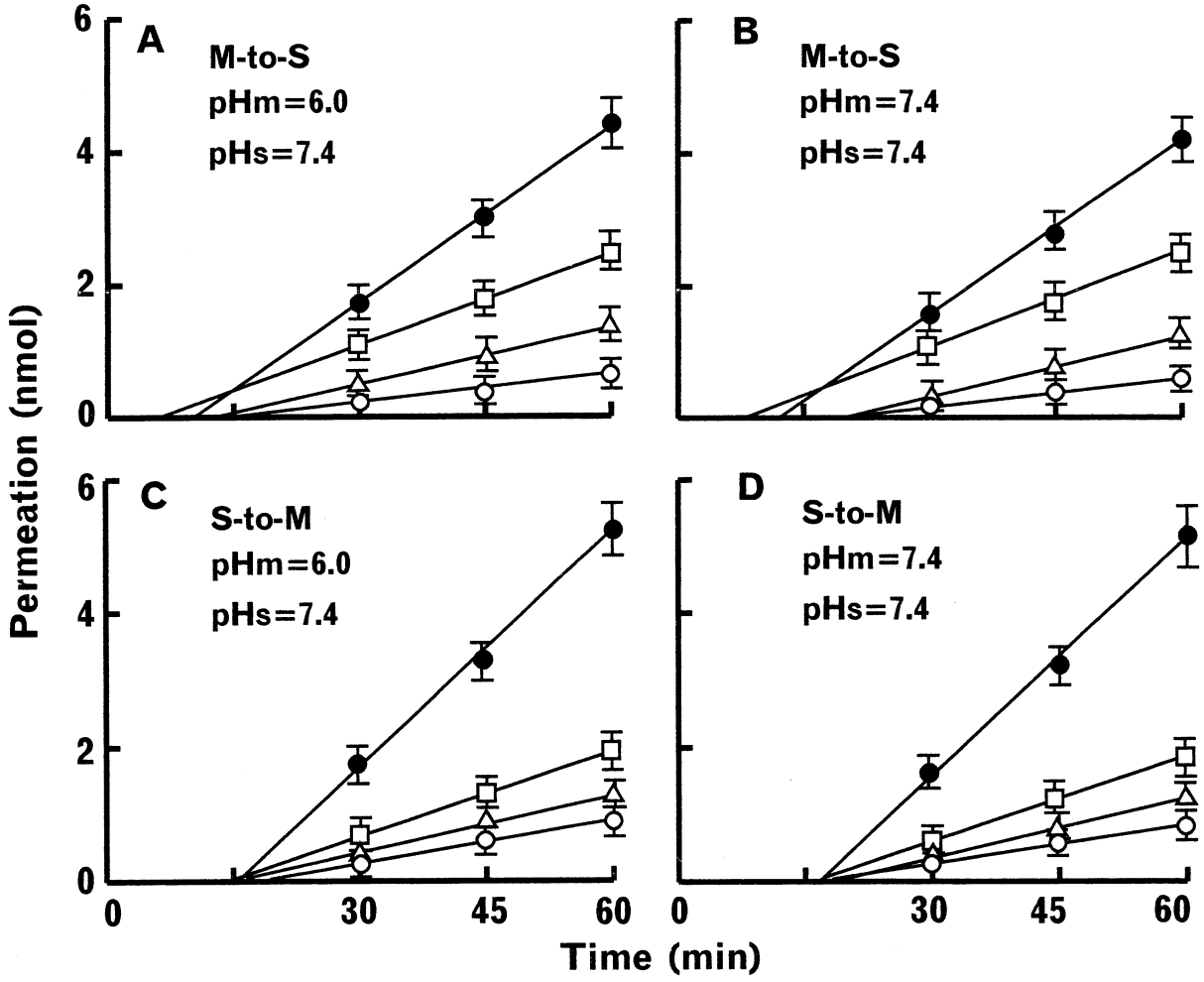

Fig. 2. Mucosal-to-serosal (M-to-S) and serosal-to-mucosal (S-to-M) cefoperazone permeation by rat jejunum in the presence (A,C) and absence (B,D) of a proton-gradient. The pH of mucosal side (pHm) was 6.0 or 7.4. The pH of serosal side (pHs) was 7.4. Symbols indicate the concentrations of cefoperazone: ○, 0.5 mM; △, 1.0 mM; □, 2.0 mM; ●, 5.0 mM. Data represent the mean ± SE of four experiments.

absorption clearance (CL_{app}). The CL_{app} values were calculated according to the following equation:

$$CL_{app} = \frac{dC}{dt} \cdot \frac{1}{C_0 tsl}$$

where dC/dt is the change in concentration per unit of time (μmol/ml per min), C_0 is the initial drug concentration, t is the perfusion time in steady state (80 min), s is the flow rate (0.2 ml/min), and l is the length of isolated intestinal loop (cm).

2.8. HPLC analysis

The concentrations of cephalexin and cefoperazone were measured with a high-performance liquid chromatograph (HPLC) LC-9A (Shimadzu, Kyoto, Japan). Both conditions used a Nucleosil 5C18 column (25 cm length, 4.6 mm internal diameter); a mobile phase of methanol/100 mM acetate buffer, pH 6.0 (35:65, v/v); a 0.6 ml/min flow rate; a 262 nm wavelength and a column oven temperature of 40 °C.

3. Results and discussion

3.1. Membrane permeability of cephalexin and cefoperazone

We focused on the different mechanisms of transport of cephalexin and cefoperazone. As the reason, it has been known that cephalexin is cotransported from the mucosal side to the serosal side of small intestine by an inward proton-gradient via oligopeptide transporters [12]. In particular, cephalexin among oral β-lactam antibiotics has an almost complete availability after oral dosing [13]. On the other hand, cefoperazone is not recognized by the oligopeptide transporters and is transported from the serosal side to the mucosal side by an efflux pump [14]. In order to clarify the transport kinetics of cephalexin and cefoperazone in the small intestine, the permeability of both drugs was compared using Ussing-type diffusion chambers in mucosal-to-serosal and serosal-to-mucosal directions.

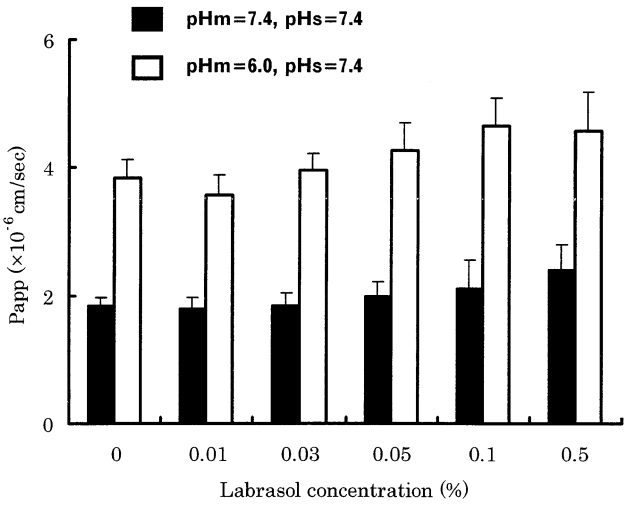

Fig. 3. Effects of concentration of Labrasol on the apparent permeability coefficient (P_{app}) of cephalexin by rat jejunum in the absence and presence of a proton-gradient. The pH of the mucosal side (pHm) was 6.0 or 7.4. The pH of the serosal side (pHs) was 7.4. The cephalexin concentration was 0.5 mM. Labrasol was added to the mucosal side. Data represent the mean ± SE of three experiments.

The kinetics in membrane permeability of cephalexin are shown in Fig. 1.

The membrane permeability of cephalexin in the mucosal-to-serosal direction under an inward proton-gradient condition was increased compared to that in the absence of the proton-gradient. In particular, when 5.0 mM cephalexin was used, the extent of cephalexin permeability with the proton-gradient increased 2.5-fold compared to that in the absence of the proton-gradient (Fig. 1A,B). These results are consistent with the already-known mechanism by which cephalexin is transported via oligopeptide transporters.

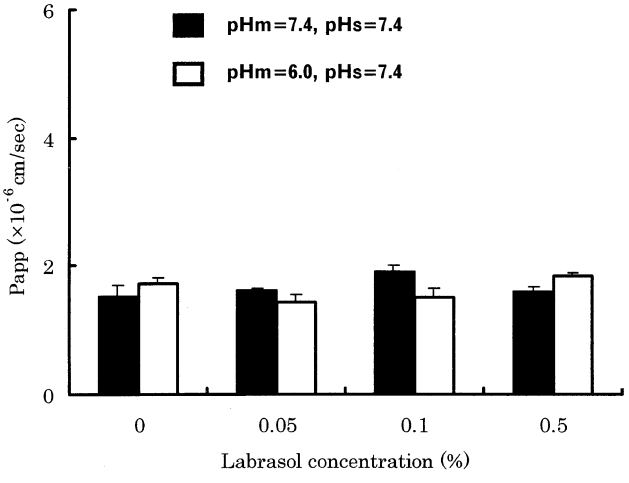

Fig. 4. Effects of concentration of Labrasol on the apparent permeability coefficient (P_{app}) of cefoperazone by rat jejunum in the absence and presence of a proton-gradient. The pH of the mucosal side (pHm) was 6.0 or 7.4, and the pH of the serosal side (pHs) was 7.4. The cefoperazone concentration was 0.5 mM. Labrasol was added to the mucosal side. Data represent the mean ± SE of three experiments.

From the comparison of the permeation kinetics in Fig. 1C,D, the proton-gradient did not affect the membrane permeability of cephalexin in the serosal-to-mucosal direction.

The kinetics of the membrane permeability of cefoperazone are shown in Fig. 2. Cefoperazone showed almost identical kinetic patterns in the mucosal-to-serosal (Fig. 2A,B) or serosal-to-mucosal (Fig. 2C,D) directions in the presence and absence of the proton-gradient conditions. The identical kinetic patterns in Fig. 2A,B support the evidence that cefoperazone is not cotransported by a proton-gradient, that is, cefoperazone is not a substrate of oligopeptide transporters. Furthermore, cefoperazone permeability in the mucosal-to-serosal direction was almost identical compared to that in the serosal-to-mucosal direction except for 5.0 mM cefoperazone. Namely, it was suggested that the effect of efflux pump was small at the low concentration of cefoperazone.

From the results in Figs. 1 and 2, it was considered that the action of the efflux pump does not reflect the membrane permeability in the mucosal-to-serosal direction when a drug at low concentration is used in the permeability studies. Therefore, it was decided to carry out the permeability studies at the concentration of 0.5 mM for both drugs based on the hypothesis that the effect of the efflux pump on membrane permeability of both drugs is very small.

3.2. Effects of Labrasol® and Gelucire 44/14 on apparent permeability coefficient

The effect of Labrasol® on the P_{app} value of cephalexin is shown in Fig. 3. The P_{app} value of cephalexin was gently increased depending on the concentration of Labrasol® (0.01–0.1%) under the proton-gradient condition. From the results, it seemed that Labrasol® enhanced the active transport of cephalexin. The proportion of active transport was considered a difference in the P_{app} value in the presence of proton-gradient and that in the absence of proton-gradient. The P_{app} value of cephalexin at the concentration of 0.5% Labrasol® was increased compared to that without Labrasol® in the absence of the proton-gradient. Therefore, it is suggested that Labrasol® enhanced not only the active transport but also the passive diffusion of cephalexin.

On the other hand, the effect of Labrasol® on the P_{app} value of cefoperazone is shown in Fig. 4. No difference was observed between the P_{app} value of cefoperazone either with or without Labrasol® in the presence or absence of the proton-gradient, suggesting that Labrasol® did not affect the transport of cefoperazone.

The effect of Gelucire 44/14 on P_{app} value of cephalexin is shown in Fig. 5. Gelucire 44/14 did not affect the P_{app} value of cephalexin in the presence of the proton-gradient. Gelucire 44/14 gradually decreased the P_{app} value of cephalexin in the absence of the proton-gradient. These results suggested that Labrasol® and Gelucire 44/14 have different effects on the membrane permeability of cephalexin. Based

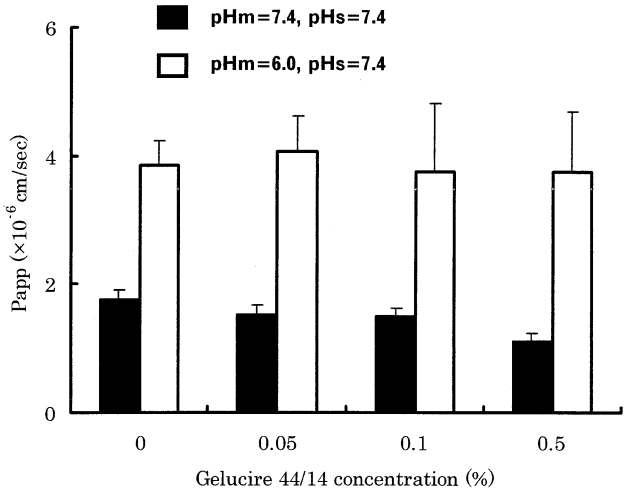

Fig. 5. Effects of concentration of Gelucire 44/14 on the apparent permeability coefficient (P_{app}) of cephalexin by rat jejunum in the absence and presence of a proton-gradient. The pH of the mucosal side (pHm) was 6.0 or 7.4, and the pH of the serosal side (pHs) was 7.4. The cephalexin concentration was 0.5 mM. Gelucire 44/14 was added to the mucosal side. Data represent the mean ± SE of three experiments.

on the difference in physicochemical properties of the excipients, it is predicted that the dispersity of the excipient will contribute to the membrane permeability of cephalexin because nonionic surfactants with low polydispersity (the ratio of weight-weighed average diameter (d_w) and number-weighed average diameter (d_n) is approximately 1.0) change the membrane lipid fluidity depending on an increase in the hydrophobic index, the hydrophile–lipophile balance (HLB) number, and then influence the membrane permeability of drugs; however, nonionic surfactants with high polydispersity ($d_w/d_n \gg 1.0$) do not affect them [15].

The HLB numbers of Labrasol® and Gelucire 44/14 were both 14. Therefore, the hydrophobicity of the excipients was the same. The average particle sizes of Labrasol® and Gelucire 44/14 containing 0.5 mM cephalexin or 0.5 mM cefoperazone were 189.2 or 188.8 nm and 827.7 or 824.5 nm, respectively. The d_w/d_n values of Labrasol® and Gelucire 44/14 were 1.2 and 2.4, respectively, in a dynamic light scattering study. The polydispersity of Labrasol® was approximately 1, i.e. low, and that of Gelucire 44/14, was clearly over 1, i.e. high. The results suggest that only Labrasol® would affect the membrane permeability of a drug and, in fact, Labrasol® affected the membrane permeability of cephalexin. Furthermore, the average particle size of Labrasol® was smaller than that of Gelucire 44/14. It was indicated that the difference of polydispersity index and/or average particle size of the excipients was deeply concerned in the membrane transport.

3.3. Effects of Labrasol® and Gelucire 44/14 on membrane functions

PD and I_{sc} of the membrane were measured using Ussing-type diffusion chambers in order to clarify the change in the electrical resistance of the membrane by the addition of Labrasol® and Gelucire 44/14. Fig. 6 shows the time-courses of R_m and I_{sc} when 0.5% Labrasol®, 0.5% Gelucire 44/14 or without excipient (control) was introduced to the mucosal side of the membrane after preincubation of phosphate buffer solution, pH 7.4, containing 0.5 mM cephalexin. The exclusion of Labrasol® or Gelucire 44/14 from the mucosal solution caused no change in R_m compared to the control. The R_m values with or without the excipients were in range of 63 to 50 Ω cm^2 during the experimental period. This result suggested that the membrane barrier function was maintained normally. Because, it is reported that the R_m value of small intestine is approximately 30 to 60 Ω cm^2 [16]. On the other hand, both excipients caused a decrease in I_{sc}. In particular, the addition of Labrasol®

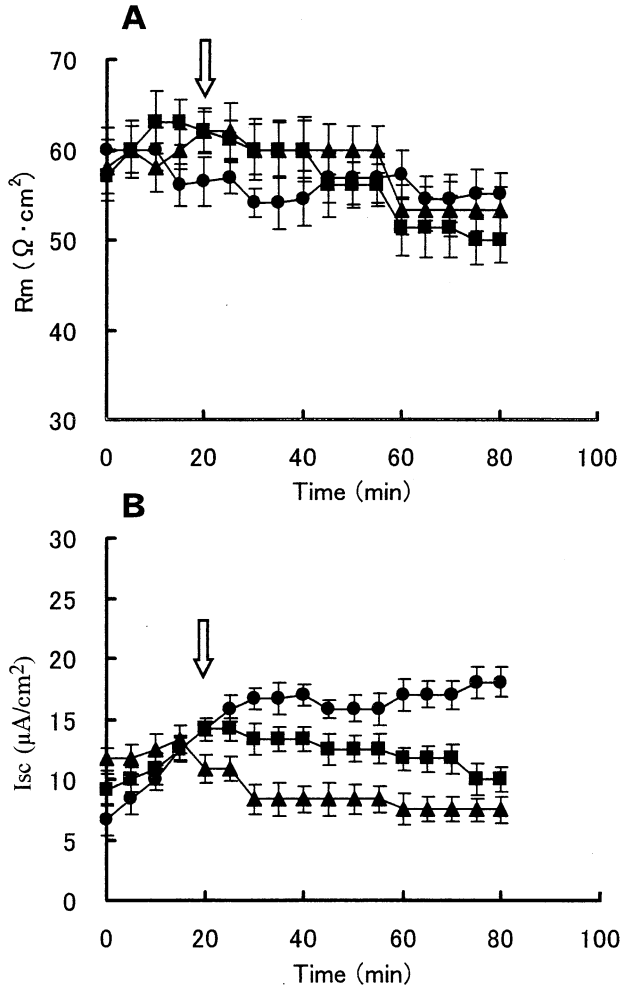

Fig. 6. Effect of Labrasol or Gelucire 44/14 on the membrane resistance (R_m) (A) and the short-circuit current (I_{sc}) (B) of rat jejunum. Isotonic phosphate buffer solution, pH 7.4, containing 0.5 mM cephalexin and 0.5% excipient was introduced to the mucosal side of the membrane after preincubation with isotonic phosphate buffer solution, pH7.4, containing 0.5 mM cephalexin for 20 min. Symbols indicate the concentrations of cefoperazone: ●, without excipient (control); ▲, 0.5% Labrasol; ■, 0.5% Gelucire 44/14. Data represent the mean ± SE of three experiments.

Table 2
P_{app} of cefalexin in the mucosal-to-serosal direction in the presence and absence of Labrasol and/or dipeptide under an ATP-depleted condition[a]

	P_{app} ($\times 10^{-6}$ cm/s)	Rate of mean (%)
Control	3.86 ± 0.22	100
with Labrasol	4.23 ± 0.45	109.5
with Gly–Leu	3.26 ± 0.28	84.5
with Labrasol and Gly–Leu	3.41 ± 0.33	88.3
with Ala–Ala	3.16 ± 0.31	81.9
with Labrasol and Ala–Ala	3.36 ± 0.30	87.0

[a] The pHs of mucosal and serosal side were 6.0 and 7.4, respectively. The concentrations of cephalexin, Labrasol and dipeptide were 0.5 mM, 0.5% (w/v) and 10 mM, respectively. Labrasol and dipeptide were added to the mucosal side. Data represent the mean ± SE of three experiments.

resulted in a rapid decrease in I_{sc} to approximately 5 Ω cm^2. I_{sc} is identical with the total of the flow of electrogenic ion through the membrane [17]. Therefore, it may be considered that the effect of Labrasol® on active transport via ion flux is strong compared to that of Gelucire 44/14.

3.4. Effects of Labrasol® with the addition of dipeptides on the apparent permeability coefficient of cephalexin

In previous sections, it was impossible to clarify which degree Labrasol® affected the active transport of cephalexin. The aim of this section was to determine how much Labrasol® enhanced the active transport of cephalexin by in vitro membrane permeability studies. The P_{app} value of absorptive transport of cephalexin was examined under an ATP-depleted condition in order to suppress the effect of efflux pump. The results are shown in Table 2. When the P_{app} value of cephalexin without Labrasol® and dipeptide, i.e. the control value, was considered as 100%, the P_{app} value with Labrasol® increased to 109.5%. The P_{app} values with Gly–Leu and with Ala–Ala were decreased to 84.5 and 81.9%, respectively. Thus, cephalexin transport was suppressed 15.5% by the addition of Gly–Leu and 18.1% by the addition of Ala–Ala for in the presence of the proton-gradient. The P_{app} values for Labrasol® and Gly–Leu and for Labrasol® and Ala–Ala were 88.3 and 87.0%, respectively.

Table 3
Effects of Labrasol and Gelucire 44/14 on cephalexin absorption in situ[a]

Pharmaceutical excipient	C_{ss} (nmol/ml)	CL_{app} (μl/min/cm)
No addition	91.7 ± 3.3	8.1 ± 0.29
Labrasol		
0.01%	87.1 ± 2.4	12.6 ± 0.34
0.03%	89.9 ± 3.0	9.9 ± 0.33
0.05%	88.9 ± 8.5	10.8 ± 1.03
0.1%	88.9 ± 7.7	10.8 ± 0.93
0.5%	93.6 ± 5.3	6.3 ± 0.35
Gelucire 44/14		
0.05%	90.5 ± 3.8	9.8 ± 0.41
0.5%	90.3 ± 4.6	9.5 ± 0.48

[a] Data represent the mean ± SE of three experiments.

The results demonstrated that cephalexin transport was increased 3.8 and 5.1% by the addition of Labrasol® in the presence of the dipeptide. Therefore, the enhancement rates of Labrasol® on active cephalexin transport were calculated, by comparison of the rate of the P_{app} values with Labrasol® (109.5%), as

$(9.5 - 3.8)/9.5 \times 100 = 60.0\%$ when *Gly − Leu was used*,

$(9.5 - 5.1)/9.5 \times 100 = 46.3\%$ when *Ala − Ala was used*.

From the experiments using both dipeptides, the enhancement rate for the active transport among the membrane permeability of cephalexin enhanced by the addition of 0.5% Labrasol® was 60 and 46.3%, respectively, under the proton-gradient condition. These data are given based on the hypothesis that Gly–Leu and Ala–Ala sufficiently inhibited active transport of cephalexin. These results suggested that 60 or 46% of the enhancement of Labrasol® on cephalexin transport comes out for the active transport, and 40 or 54% of the remainder is for passive transport.

3.5. Effects of Labrasol® and Gelucire 44/14 on cephalexin absorption in situ

In order to clarify whether or not the enhancing effect of Labrasol® on cephalexin transport is reflected by intestinal absorption studies, the effects of Labrasol® and Gelucire 44/14 on the intestinal absorption of cephalexin were studied in situ. The results are shown in Table 3 as cephalexin concentration of steady state condition (C_{ss}) and apparent absorption clearance (CL_{app}) in perfusion studies. The CL_{app} values of cephalexin with 0.01–0.1% Labrasol® were higher than the CL_{app} value without an excipient. When 0.5% Labrasol® was used, the CL_{app} value was rather lower than the CL_{app} value without the excipient. This suppressive effect of 0.5% Labrasol® was different from that in vitro membrane permeability studies. As the reason, it is suggested that cephalexin adhering to the surface of the intestinal tract was washed out by the dispersion effect of Labrasol®. Although we did not perform experiments to resolve the problem of adhesive or adsorptive effects of drugs on the intestinal tract, it is reported that the adsorption of a drug is important for its drug absorption mechanism [18]. On the other hand, Gelucire 44/14 did not affect the CL_{app} of cephalexin at the concentrations of 0.05 and 0.5%, suggesting that these results were the same as that of in vitro transport studies. These results in in situ studies clearly showed that Labrasol® enhanced cephalexin absorption from the intestinal tract, and Glerucire 44/14 did not influence the cephalexin absorption.

3.6. Conclusion

In this study, we found that Labrasol® enhanced the membrane permeability and the intestinal absorption of cephalexin and that Gelucire 44/14 did not affect them. In particular, it was indicated that the effect of Labrasol® on

active transport of cephalexin affects the ion transport based on the result which the I_{sc} of the jejunum greatly changed by the addition of Labrasol®. From the membrane permeability experiments using the addition of dipeptide, the enhancement rate for the active transport of cephalexin in the presence of 0.5% Labrasol® was 60 and 46% for the experiments with Gly–Leu and Ala–Ala, respectively. Therefore, Labrasol® affected both active and passive transport of cephalexin. As the reason that Gelucire 44/14 did not affect them, it is suggested that the polydispersity and average particle size of excipients contribute to the interaction between membrane and drug.

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References

[1] V. Ratsimbazafy, E. Bourret, R. Duclos, C. Brossard, Rheological behavior of drug suspensions in Gelucire mixtures and proxyphylline release from matrix hard gelatin capsules, Eur. J. Pharm. Biopharm. 48 (1999) 247–252.

[2] J.T. Green, B.K. Evans, J. Rhodes, G.A. Thomas, C. Ranshaw, C. Feyerabend, M.A. Russell, An oral formulation of nicotine for release and absorption in the colon: its development and pharmacokinetics, Br. J. Clin. Pharmacol. 48 (1999) 485–493.

[3] H.S. Tran, D. Malli, F.A. Chrzanowski, M.M. Pu, Site-specific immunosuppression using a new formulation of topical cyclosporin A with polyethylene glycol-8 glyceryl caprylate/caprate, J. Surg. Res. 83 (1999) 136–140.

[4] D.J. Hauss, S.E. Fogal, J.V. Ficorilli, C.A. Price, T. Roy, A.A. Jayaraj, J.J. Keirns, Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor, J. Pharm. Sci. 87 (1998) 164–169.

[5] K. Koga, T. Ohyashiki, M. Murakami, S. Kawashima, Modification of ceftibuten transport by the addition of non-ionic surfactants, Eur. J. Pharm. Biopharm. 49 (2000) 17–25.

[6] M.T. Vincenzini, T. Iantomasi, M. Stio, C. Treves, F. Favilli, P. Vanni, 1-O-n-octyl-β-D-glucopyranoside as a competitive inhibitor of Na^+-dependent D-glucose cotransporter in the small intestine brush-border membrane, Biochim. Biophys. Acta 903 (1987) 273–276.

[7] M.M. Nerurkar, P.S. Burton, R.T. Borchardt, The use of surfactants to enhance the permeability of peptides through Caco-2 cells by inhibition of an apically polarized efflux system, Pharm. Res. 13 (1996) 528–534.

[8] T. Terao, E. Hisanaga, Y. Say, I. Tamai, A. Tsuji, Active secretion of drugs from the small intestinal epithelium in rats by P-glycoprotein functioning as an absorption barrier, J. Pharm. Pharmacol. 48 (1996) 1083–1089.

[9] H. Komatsu, S. Okada, Ethanol-induced aggregation and fusion of small phosphatidylcholine liposome: participation of interdigitated membrane formation in their processes, Biochim. Biophys. Acta 1235 (1995) 270–280.

[10] T. Imae, Light scattering investigation for growth and interaction of nonionic micelles in aqueous solution, J. Colloid Interface Sci. 127 (1989) 256–264.

[11] F.G.L. Poelma, J.J. Tukker, Evaluation of a chronically isolated internal loop in the rat for the study of drug absorption kinetics, J. Pharm. Sci. 76 (1987) 433–436.

[12] A.H. Dantzig, L. Bergin, Carrier-mediated uptake of cephalexin in human intestinal cells, Biochem. Biophys. Res. Commun. 155 (1988) 1082–1087.

[13] R. Kohda, Y.-H. Li, Y. Shitara, K. Ito, Y. Tsuda, H. Yamada, T. Itoh, Prediction of oral absorption of cephalosporins in humans, Xenobio. Metab. Dispos. 15 (2000) 196–200.

[14] H. Saitoh, H. Fujisaki, B.J. Aungst, K. Miyazaki, Restricted intestinal absorption of some β-lactam antibiotics by an energy-dependent efflux system in rat intestine, Pharm. Res. 14 (1997) 645–649.

[15] K. Koga, M. Murakami, S. Kawashima, Modulation of ceftibuten transport by changes in lipid fluidity caused by fatty acid glycerol esters, Biol. Pharm. Bull. 22 (1999) 103–106.

[16] D.W. Powell, Barrier function of epithelia, Am. J. Physiol. 241 (1981) G275–G288.

[17] S.G. Schultz, R. Zalusky, Ion transport in isolated rabbit ileum. I. Short-circuit current and Na fluxes, J. Gen. Physiol. 47 (1964) 567–584.

[18] T. Hashimoto, M. Nomoto, K. Komatsu, M. Haga, M. Hayashi, Improvement of intestinal absorption of peptides: adsorption of B1-Phe monoglucosylated insulin to rat intestinal brush-border membrane vesicles, Eur. J. Pharm. Biopharm. 50 (2000) 197–204.