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Modulation of intestinal P-glycoprotein function by polyethylene glycols and their derivatives by in vitro transport and in situ absorption studies

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Abstract

We examined the effect of polyethylene glycols (PEGs) with different molecular weights and their derivatives on the intestinal absorption of rhodamine123, a P-glycoprotein (P-gp) substrate, across the isolated rat intestinal membranes by an in vitro diffusion chamber system. The serosal to mucosal (secretory) transport of rhodamine123 was greater than its mucosal to serosal (absorptive) transport, indicating that the net movement of rhodamine123 across the intestinal membranes was preferentially secretory direction. The secretory transport of rhodamine123 was inhibited by the addition of PEGs with average molecular weights of 400, 2000 and 20,000, irrespective of its molecular weight. The inhibitory effects of these PEGs for the intestinal P-gp function were concentration dependent over the range 0.1–20% (v/v or w/v). Similar inhibitory effect for the intestinal P-gp function was observed when PEG derivatives including PEG monolaurate, PEG monooleate and PEG monostearate were added to the mucosal site of the chambers. Furthermore, we also examined effect of PEG20,000 on the intestinal absorption of rhodamine123 by an in situ closed loop method. The intestinal absorption of rhodamine123 was enhanced in the presence of PEG20,000. These findings suggest that PEGs and their derivatives are useful excipients to inhibit the function of intestinal P-gp, thereby improving the intestinal absorption of P-gp substrates, which are secreted by a P-gp-mediated efflux system.
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1. Introduction

Oral drug administration is generally preferred to treat chronic diseases. However, many drug candidates fail to fulfill their therapeutic potential owing to poor bioavailability as a result of low solubility, low permeability, and/or high metabolism. Among numerous factors that affect bioavailability, it is now generally recognized that active efflux of drugs by transporters should be considered to optimize oral bioavailability and to decrease variability at the absorption site. (Wacher et al., 1998; Toyobuku et al., 2003).

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 can act as an energy-dependent drug efflux pump that lowers intracellular drug concentrations. Expressed in tumor cells, P-gp causes the MDR phenotype by the active extrusion of a wide range of cancer chemotherapeutic agents. In addition to being expressed in tumor cells, P-gp is also expressed in various normal tissues including liver, kidney, adrenal glands, brain, testis and the intestinal brush border membranes. P-gp can transport a very broad range of substrates, including vinca alkaloids, anthracyclines, digoxin, and β-adrenergic agonists.

It has been demonstrated that the intestinal P-gp, an ATP-dependent multidrug efflux pump, can be an active secretion system or an absorption barrier by transporting some drugs from the intestinal cells into the lumen. Therefore, intestinal absorption of drugs that are secreted by a P-gp-mediated efflux system can be improved by inhibiting the function of P-gp in the intesti-

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nal membrane and the oral bioavailability of a wide range of drugs can be increased.

It is known that several excipients can reduce the function of P-gp in the intestine, thereby increasing the intestinal absorption of P-gp substrates. Among these excipients, PEGs are a class of polymer that is widely used in variety of pharmaceutical formulations. These polymers exist in a variety of molecular weight grades, ranking from 200 to 35,000 (Basit et al., 2002). Johnson et al. (2002) demonstrated that PEG400 and vitamin ED-α-tocopheryl polyethylene glycol 1000 succinate could inhibit the P-gp transporter in rat jejunal membrane. More recently, Hugger et al. (2002a) reported that PEG300 enhanced the absorptive transport of model drug taxol by increasing the absorptive directed transport and inhibiting the secretory directed transport of taxol in Caco-2 cells. In these previous studies, Caco-2 cell line, a human adenocarcinoma cell line, has been generally used to estimate drug permeability and substrate activity for efflux transport proteins such as P-gp. However, the expression levels of transporters in Caco-2 cells were usually variable and were dependent on the culture condition (Anderle et al., 1998), and it was suggested that P-gp was overexpressed in Caco-2 cells (Collett et al., 1999), which is one of the major disadvantages to estimate the function of P-gp in the presence or absence of some modulators and excipients using Caco-2 cells. Moreover, few studies have been examined the effect of PEGs with different molecular weights and PEGs derivatives on the function of P-gp in the intestine using intact intestines, especially in an in situ system.

In this study, therefore, we examined the effects of PEGs and their derivatives on the intestinal transport and absorption of rhodamine123, a P-gp substrate by both an in vitro diffusion chamber system using the isolated rat intestinal membranes and an in situ closed loop method.

2. Materials and methods

2.1. Materials

Rhodamine123 (MW 380.8), polyethylene glycol (PEG) monolaurate, polyethylene glycol (PEG) monooleate, polyethylene glycol (PEG) monostearate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polyethylene glycol (PEG) 400, 2000, 20,000 and verapamil were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Lucifer yellow CH dilithium salt (MW 457.3) was supplied by Sigma–Aldrich Chemical Co. Ltd. (St. Louis, MO). Cyclosporin A was obtained from Sandoz Pharmaceutical Company. All other reagents were of analytical grade.

2.2. Preparation of drug solution

Rhodamine123 and Lucifer yellow were dissolved in Tris–HEPES buffer solution at pH 7.4 to yield a final concentration of 10 and 100 μM. In some experiments, 0.1–20% of PEG400 (%, v/v), PEG2000 (%, w/v), PEG20,000 (%, w/v) and PEG derivatives (%, w/v), 20 μM cyclosporin A or 0.3 mM verapamil was added to the mucosal solution.

2.3. Transport of rhodamine123 across the intestinal membrane by an in vitro diffusion chamber system

The transport of P-gp substrate across the rat intestinal membrane was studied with the diffusion chamber (Corning Coster Corp.) (Grass and Sweetana, 1988; Saitoh and Aungst, 1995; Shono et al., 2004). Male Wistar rats, weighing 250–300 g, were fasted overnight and were anesthezided with Nembutal® (Dainippon Pharmaceutics, Osaka, Japan) (pentobarbital sodium, 50 mg/kg). The studies examined in this article have been 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 in ice-cold saline. Intestinal segments, excluding Peyer's patches, were isolated and immersed in ice-cold Tris–HEPES buffer solution. Segments were cut open and the intestinal sheets were mounted onto the pins of the cell, and the half-cells were clamped together. Drug solution (7 mL) was added to the donor site, whereas the same volume of drug-free buffer was added to the opposite site. The temperature of intestinal membranes was maintained at 37 °C, and both fluids were circulated by gas lift with 95% O_2/5%CO_2. During the transport studies, aliquots were taken from the receptor chamber at predetermined time up to ∼2 h. The receptor chamber samples were replaced with an equal volume of appropriate buffer. The permeated drugs were assayed. The apparent permeability coefficients (P_{app}) of drug were calculated from the slope of linear portion of permeability-time profiles of drug by the relationship $P_{app} = (dX_R/dt) \times (1/A\cdot C_0)$, where P_{app} is the apparent permeability coefficient, X_R is the amount of drug in the receptor side, A is the diffusion area, and C_0 is the initial concentration of drug in the donor side.

Efflux ratio was used to evaluate the extent of efflux (Liang et al., 2000; Eagling et al., 1999; Faassen et al., 2003; Zhang et al., 2004). The calculation was performed in the following equation:

$$\text{efflux ratio} = P_{app}\text{sm}/P_{app}\text{ms}$$

where P_{app}sm is the average of the permeability coefficient from serosal to mucosal side and P_{app}ms is the average of the 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.

2.4. Intestinal absorption of rhodamine123 by an in situ closed loop method

Absorption experiments were performed by the in-situ closed-loop methods (Hashizume et al., 1992; Yamada et al., 1992; Tozaki et al., 1998). Male Wistar rats, 250–300 g, were anesthetized with Nembutal® (Dainippon Pharmaceutics, Osaka, Japan) (pentobarbital sodium, 50 mg/kg). Animals were fasted for approximately 16 h before the experiments but water

was freely available. The intestine was exposed through a midline abdominal incision and 20 cm jejunum loop was prepared by cannulation with silicone tubing (i.d. 3 mm; o.d. 5 mm) at the proximal and distal ends of the loop. The luminal surface of the loop was washed with isotonic phosphate buffer. Rhodamine123 was dissolved in Tris–HEPES buffer at pH 7.4 to yield a final dose of 5 mg/kg. PEG20,000 was added to the dosing solutions. The drug solution was warmed to 37 °C and 1.5 mL was injected into the jejunum loop. The blood sample (0.25 mL) was collected from a jugular vein at a designated time. The intravenous administration of rhodamine123 was carried out separately via the femoral vein. The peak concentration (C_{max}) and the time to reach the peak concentration (T_{max}) were determined directly from the plasma concentration–time curves. The area under the curve (AUC) was calculated by the trapezoidal method from zero to the final sampling time (240 min). The extent of bioavailability was calculated as follows.

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

2.5. Determination (assay) of drugs

The fluorescein intensity of rhodamine123 was measured with microplate reader (Spectrafluor, TECAN Japan Co. Ltd., Tokyo, Japan) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm, respectively. Similarly, Lucifer yellow was determined spectrofluorometrically at an excitation wavelength of 430 nm and an emission wavelength of 535 nm, respectively.

Samples obtained from in situ closed loop studies were assayed on a reversed-phase HPLC system containing 5 μm Cosmosil (4.6 mm × 15 cm) particles in an analytical column from Nacalai Tesque, a Shimadzu LC-10 Pump System, a Shimadzu LC-10 Autoinjector, a Shimadzu LC-10 Detector and a Shimadzu CR-6A Integrator. The mobile phase was acetonitrile: 1% triethylamine (pH 3.0) = 28:72. The flow rate was 1.0 mL/min. The fluorescence detector (Shimadzu) for the assay of rhodamine123 was set at 485 and 546 nm of excitation and emission wavelengths, respectively.

Phosphate buffer (0.1 mL, pH 7.4), water (0.5 mL) and ethyl acetate (6 mL) were added to 0.1 mL of the plasma samples. It was then mixed for 10 min and centrifuged at 3000 rpm for 5 min. The organic layer (6 mL) was transferred to a clean test tube and evaporated in a centrifugal evaporator at 40 °C, the residue was then dissolved in a solution (acetonitrile: 1% triethylamine = 1:9), centrifuged at 10,000 rpm for 5 min, and the solution (50 μL) was injected into the HPLC system.

2.6. Statistics

Results are expressed as the mean ± S.E. of at least three experiments. Statistical significance was assessed using the Student's *t*-test or Dunnett's test for multiple comparison with $p < 0.05$ as the minimal level of significance.

3. Results

3.1. Transport characteristics of rhodamine123 across the intestinal membranes

Fig. 1 shows the time course of absorptive (mucosal to serosal) and secretory (serosal to mucosal) transport of rhodamine123 across the rat jejunal membranes. As shown in this figure, rhodamine123 was much more permeable in the secretory direction than in the absorptive direction, and the secretory transport (P_{app}) of rhodamine123 was more than seven-fold greater than its absorptive transport (P_{app}), indicating that the net movement of rhodamine123 across the rat jejunum was preferentially in the secretory direction.

When 5% PEG20,000 was added to mucosal side, the secretory transport of rhodamine123 significantly decreased. On the other hand, a little increase was observed in the absorptive transport of rhodamine123 in the presence of 5% PEG20,000.

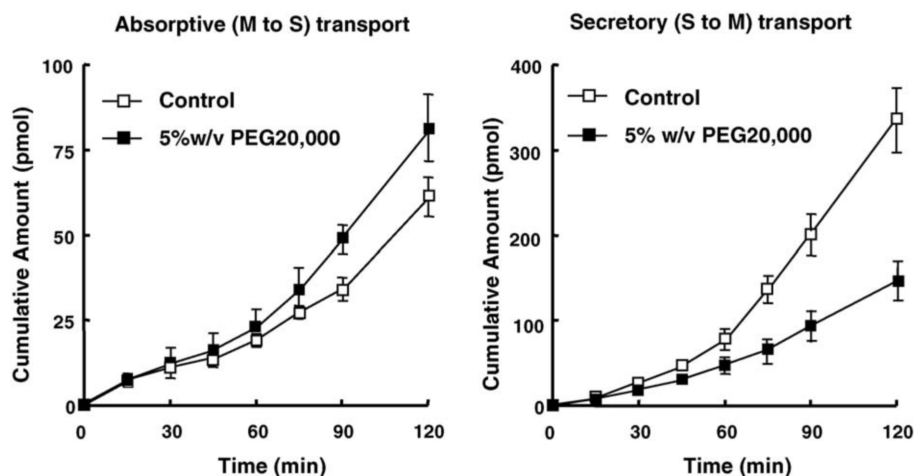

Fig. 1. Time course of absorptive (M to S) and secretory (S to M) transport of rhodamine123 and effect of 5% (w/v) PEG20,000 on the transport of rhodamine123 across the rat jejunal membrane. Keys: (□) control, (■) 5% (w/v) PEG20,000. Results are expressed as the mean ± S.E. of at least three experiments.

Table 1
Effects of various PEGs on the permeability of rhodamine123 across the intestinal jejunal membranes

Drug	Excipient	P_{app} ($\times10^{-6}$ cm/s)		Ratio: P_{app}sm/P_{app}ms
		M to S	S to M	
Rhodaminel23	Control	0.45 ± 0.08	3.48 ± 0.29	7.73
	5% (v/v) PEG400	0.44 ± 0.13 N.S.	1.22 ± 0.12**	2.77
	5% (w/v) PEG2000	0.48 ± 0.19 N.S.	2.22 ± 0.30 N.S.	4.63
	5% (w/v) PEG20,000	0.58 ± 0.10 N.S.	1.25 ± 0.02**	2.16
	0.3 mM verapamil	0.54 ± 0.08 N.S.	1.55 ± 0.21**	2.87
	20 μM cyclosporin A	0.78 ± 0.15*	1.40 ± 0.31**	1.79
Lucifer yellow	Control	5.67 ± 0.41	6.28 ± 0.25	1.10
	5% (v/v) PEG400	4.47 ± 0.65 N.S.	5.43 ± 0.67 N.S.	1.21
	5% (w/v) PEG2000	3.86 ± 0.76 N.S.	5.68 ± 0.51 N.S.	1.47
	5% (w/v) PEG20,000	4.53 ± 0.71 N.S.	5.97 ± 0.41 N.S.	1.32

Each value represents the mean ± S.E. of at least three experiments. N.S.: not significantly different.
* $p < 0.05$, compared with the control.
** $p < 0.01$, compared with the control.

3.2. *Effects of various PEGs with different molecular weights on the permeability of rhodamine123 across the intestinal membranes*

Table 1 shows the effect of various PEGs with different average molecular weights on the transport of rhodamine123 across the rat jejunal membranes. In the positive control studies, the absorptive or secretory transport of rhodamine123 was slightly enhanced or significantly reduced in the presence of 0.3 mM verapamil and 20 μM cyclosporin A, typical P-gp substrates, suggesting that the effect of some additives on the function of P-gp can be evaluated using this transport system. The absorptive transport of rhodamine123 was not significantly affected in the presence of PEG400, PEG2000 and PEG20,000, while the secretory transport of rhodamine123 was significantly reduced with these PEGs, suggesting that PEGs might inhibit the function of P-gp in the intestine.

Table 1 also indicates the efflux ratios of rhodamine123 in the presence of PEGs. In this study, we also evaluated the transport direction of rhodamine123 and function of P-gp by calculating the efflux ratio (P_{app}sm/P_{app}ms ratio) of rhodamine123. As is evident from the figure, the efflux ratio of rhodamine123 significantly decreased in the presence of 0.3 mM verapamil and 20 μM cyclosporin A. PEG400, PEG2000 and PEG20,000 also reduced the efflux ratio of rhodamine123, suggesting that PEGs might inhibit the function of P-gp, irrespective of the molecular weight of PEGs.

Furthermore, Table 1 demonstrated that the effect of PEGs on the transport of Lucifer yellow, a non-P-gp substrate and a marker compound of paracellular transport, across the rat jejunal membranes. The absorptive and secretory transport of Lucifer yellow was not affected by the addition of 5% PEG400 (%, v/v), PEG2000 (%, w/v) and PEG20,000 (%, w/v). In addition, these PEGs did not change the efflux ratios of Lucifer yellow across

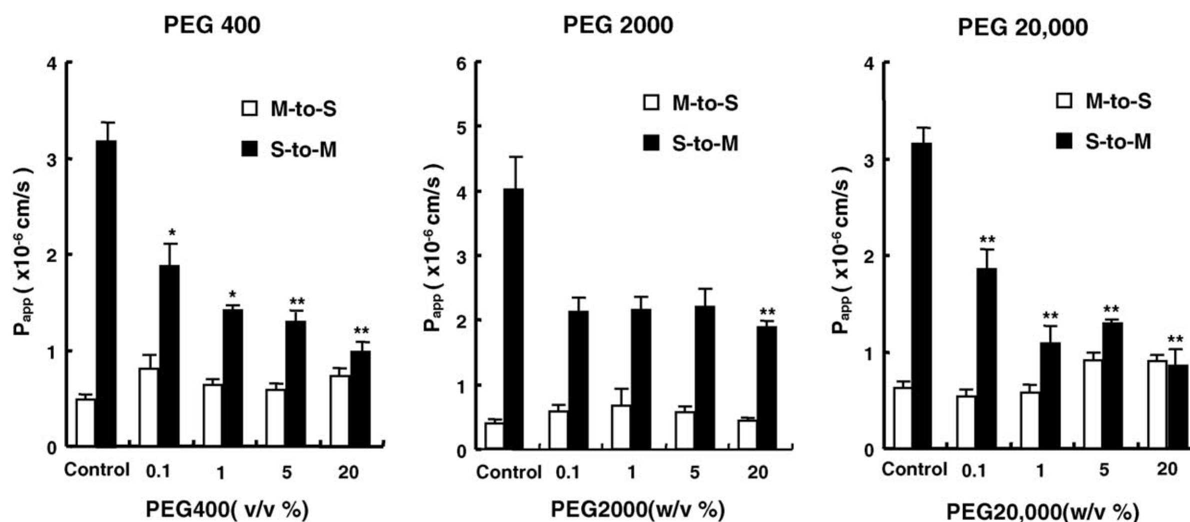

Fig. 2. Concentration-dependent effects of PEG400, PEG2000 and PEG20,000 on absorptive (M to S) and secretory (S to M) transport of rhodamine123 in the rat jejunum. Open bar indicates the absorptive transport, while the closed bar shows the secretory transport. The concentration of PEGs were 0.1–20% (v/v or w/v). Results are expressed as the mean ± S.E. of at least three experiments.
*$p < 0.05$, **$p < 0.01$ compared with the control.

Table 2
Effect of PEGs derivatives on the transport of rhodamine123 across the intestinal membranes

	P_{app} ($\times 10^{-6}$ cm/s)		Ratio: P_{app}sm/P_{app}ms
	M to S	S to M	
Control	0.63 ± 0.09	3.12 ± 0.47	4.95
1% (w/v) monolaurate	0.58 ± 0.26 N.S.	1.28 ± 0.11*	2.21
5% (w/v) monolaurate	0.43 ± 0.09 N.S.	1.16 ± 0.23*	2.70
1% (w/v) monooleate	0.49 ± 0.12 N.S.	1.34 ± 0.14*	2.73
5% (w/v) monooleate	0.33 ± 0.05 N.S.	2.60 ± 0.88 N.S.	7.88
1% (w/v) monostearate	0.39 ± 0.12 N.S.	1.44 ± 0.25*	3.69

Each value represents the mean ± S.E. of at least three experiments. N.S.: not significantly different.
* $p < 0.05$, compared with the control.

the jejunal membranes. Therefore, these findings indicated that the PEGs only reduced the efflux ratio of rhodamine123, a P-gp substrate and PEGs might possibly reduce the action of P-gp in the intestine.

3.3. Effects of various concentrations of PEGs on the permeability of rhodamine123 across the intestinal membranes

Fig. 2 shows the effect of various concentrations of PEG400 on the absorptive and secretory transport of rhodamine123 in the rat jejunal membranes. The secretory transport of rhodamine123 gradually decreased as the concentration of PEG400 increased, and the inhibitory effect of PEG400 for the secretory transport of rhodamine123 was concentration dependent over the range 0.1–20% (v/v). However, the absorptive transport of rhodamine123 was not enhanced by the addition of PEG400 at the concentrations over the range 0.1–20% (v/v).

Fig. 2 also indicated the effect of various concentrations of PEG2000 and PEG20,000 on the absorptive and secretory transport of rhodamine123 across the rat jejunal membrane. We observed a similar result, as was the case of PEG400. As the concentrations of PEG2000 and PEG20,000 increased, the secretory transport of rhodamine123 was significantly decreased. However, we found no significant increase in the absorptive transport of rhodamine123 with PEG2000 and PEG20,000.

3.4. Effects of PEGs derivatives on the transport of rhodamine123 across the intestinal membranes

We next examined the effect of PEG derivatives on the transport of rhodamine123 across the intestinal membranes whether the PEG derivatives also inhibit the function of P-gp in the intestine. Table 2 shows the effect of PEG derivatives on the absorptive and secretory transport of rhodamine123 in the rat jejunal membrane. The absorptive transport of rhodamine123 was not affected by the addition of PEG derivatives (1% (w/v) PEG monolaurate, 5% (w/v) PEG monolaurate, 1% (w/v) PEG monooleate and 1% (w/v) PEG monostearate, 5% (w/v) PEG monooleate). However, the secretory transport of rhodamine123 was inhibited by the addition of 1% (w/v) PEG monolaurate, 5% (w/v) PEG monolaurate, 1% (w/v) PEG monooleate and 1% (w/v) PEG monostearate, while 5% w/v PEG monooleate has no effect on the secretory transport of rhodamine123 due to the low solubility in the buffer solution. These findings suggested that PEGs derivatives as well as PEG itself might inhibit the function of P-gp in the intestine.

3.5. Effect of PEG20,000 on the intestinal absorption of rhodamine123 by an in situ closed loop method

Finally, we examined the effect of PEG20,000 on the intestinal absorption of rhodamine123 whether PEG20,000 might also affect the intestinal absorption of rhodamine123 in in situ absorption studies as well as in vitro transport studies. Therefore, a solution containing rhodamine123 was administered into the jejunal loop, and the jejunal absorption of rhodamine123 was examined by the in situ closed loop method.

Fig. 3 shows plasma concentration–time profiles of rhodamine123 after the jejunal administration of rhodamine123 in the presence or absence of different concentrations of PEG20,000 (1, 5 and 20% (w/v)). A remarkable increase in the plasma concentrations of rhodamine123 was observed

Table 3
Pharmacokinetic parameters of jejunal absorption of rhodamine123 in the presence or absence of various concentrations of PEG20,000 by an in situ closed loop method

	C_{max} (ng/mL)	T_{max} (min)	$AUC_{0\rightarrow 240}$ (ng/mL·min)	F%
Control	20.4 ± 3.0	57.0 ± 12.0	3937 ± 392	6.47 ± 0.64
1% (w/v) PEG20,000	31.6 ± 5.2 N.S.	48.8 ± 14.4 N.S.	5560 ± 1009*	9.14 ± 1.66
5% (w/v) PEG20,000	51.3 ± 7.8**	48.8 ± 12.8 N.S.	8405 ± 666*	13.82 ± 1.10
20% (w/v) PEG20,000	42.1 ± 7.3*	27.0 ± 3.0*	5880 ± 983*	9.67 ± 1.62

Each value represents the mean ± S.E. of at least three experiments. N.S.: not significantly different.
* $p < 0.05$, compared with the control.
** $p < 0.01$, compared with the control.

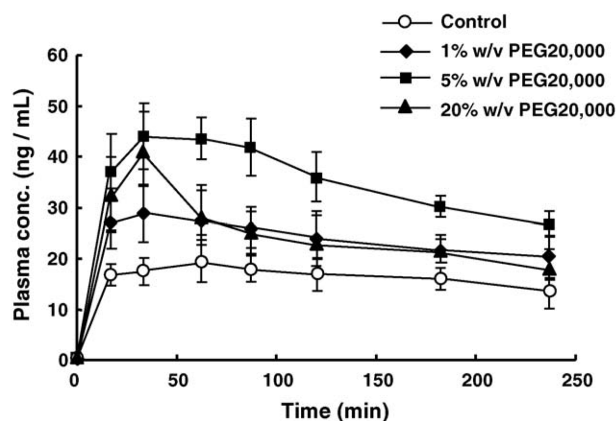

Fig. 3. Plasma concentration–time profiles of rhodamine123 after the jejunal administration of rhodamine123 in the presence or absence of different concentrations of PEG20,000 (1, 5 and 20% (w/v)) by an in situ closed loop method. Keys: (◯) control, (♦) 1% (w/v) PEG20,000, (■) 5% (w/v) PEG20,000, (▲) 20% (w/v) PEG20,000. Results are expressed as the mean ± S.E. of at least three experiments.

when rhodamine123 was co-administered with 1–20% (w/v) PEG20,000 to the jejunum.

Table 3 shows the pharmacokinetic parameters (C_{max}, T_{max}, $AUC_{0\rightarrow240}$ and *F%*) of rhodamine123 after its administration with PEG20,000 into the jejunum. In the presence of different concentrations of PEG20,000, we observed a significant increase in $AUC_{0\rightarrow240}$ values and *F%* after administration of rhodamine123 into the jejunum. We found a greatest enhancing effect of PEG20,000 for the intestinal absorption of rhodamine123, when 5% (w/v) PEG20,000 was administered to the jejunal loops of rats.

4. Discussion

The present study demonstrated that the secretory directed transport of rhodamine123, a well-known P-gp substrate, was inhibited by PEGs and their derivatives. In this study, rhodamine123 was used as a typical model of P-gp substrate, because this compound was easily assayed and was widely used for evaluating the function of P-gp in the field of cancer chemotherapy as well as biopharmaceutics (Fontaine et al., 1996).

As shown in Fig. 1, the secretory transport of rhodamine123 was much greater than its absorptive transport. In addition, the P_{app}sm/P_{app}ms ratio of rhodamine123 was clearly reduced in the presence of 0.3 mM verapamil and 20 μM cyclosporin A (Table 1). Verapamil and cyclosporin A are the most extensively characterized inhibitors of P-gp and were the first multidrug resistance reversal agents (Fisher and Sikic, 1995; Gottesman and Pastan, 1989). Therefore, we could confirm to estimate the function of P-gp using this in vitro diffusion chamber system.

Recently, it was known that many new chemical entities were poorly absorbed from the gastrointestinal tract which may be partly due to the P-gp efflux system, and the oral administration of P-gp inhibitors including verapamil or cyclosporin A, can enhance the oral bioavailability of P-gp substrate drugs, but such inhibitors themselves have pharmacological activities. Thus, it is necessary to develop a new generation of P-gp inhibitors without undesired toxicologic effects (van Asperen et al., 1997; Cornaire et al., 2004). Recently, it was reported that some excipients, which are commonly added to pharmaceutical formulations, could inhibit the function of P-gp in the intestine.

Of these pharmaceutical excipients, PEGs are polyethoxylated excipients commonly added in pharmaceutical formulations to increase the aqueous solubility of drugs and drug candidates (Price, 1994; Leyland, 1994; Yu, 1994). These excipients, which are added to food products, are considered to be non-toxic and inert. PEGs are amphiphilic excipients distributed in various molecular weight forms (Hugger et al., 2002b). To achieve an increase in drug transport by P-gp inhibition, a property that have polyoxyethylene groups should be required (Cornaire et al., 2004).

As shown in Fig. 2, we observed that when 20% (w/v or v/v) PEGs, especially PEG20,000 and PEG400 were added to the mucosal side, the absorptive and secretory transport of rhodamine123 became almost equal. Therefore, these findings suggested that PEGs might inhibit the function of P-gp in the intestine and reduced the secretory transport of rhodamine123 (Oka et al., 2002). Although the secretory transport of rhodamine123 was remarkably inhibited by PEGs, its absorptive transport was not so much affected by the co-administration with PEGs. The reason for the negative effect of PEGs for enhancing the absorptive transport of rhodamine123 is not fully understood. However, recently, Troutman and Thakker (2003a,b) reported that in the absorptive direction, rhodamine123 might be transported via a paracellular pathway rather than the P-gp-mediated transport system, although it could be mainly transported by a P-gp-mediated transport system in the secretory direction. Thus, such low contribution of the P-gp-mediated pathway of rhodamine123 in the absorptive direction may be attributed to the negative effect of PEGs in the absorptive transport of rhodamine123.

In this study, we found no significant effect of PEG20,000 on the transport of Lucifer yellow, a model drug of non-P-gp substrate transported by a passive diffusion (Cao et al., 1993) in both absorptive and secretory direction. This finding suggests that PEGs did not cause any severe membrane damage and toxicity to the intestinal mucosa at these concentrations used in this study and they only affected the secretory transport of P-gp substrates.

We next examined the effect of PEG derivatives on the transport of rhodamine123 whether they also inhibit the function of P-gp as well as PEG itself. We demonstrated that PEG monolaurate, PEG monooleate and PEG monostearate at a concentration of 1 and 5% (w/v), reduced the secretory transport of rhodamine123. The rank order for inhibiting the secretory transport of rhodamine123 was as follows; PEG monolaurate > PEG monooleate > PEG monostearate. This ranking indicates that alkyl and unsaturated C–C bond in the chemical structure of PEG derivatives may be essential and important for inhibiting the function of P-gp, and thereby reducing the secretory transport of rhodamine123 in the intestine.

The mechanisms by which PEGs could inhibit the function of P-gp in the intestine were not fully studied in this

paper. However, some researchers have already explored the inhibitory mechanisms of P-gp function by pharmaceutical excipients (Dudeja et al., 1995). It was suggested that PEGs may reduce P-gp activity by interfering with the structure of the apical membrane and thereby either directly or indirectly affecting the function of the transporter (Johnson et al., 2002). In addition, the results obtained from membrane fluidity studies suggested that increasing concentrations of PEG300 caused a significant decrease in the fluidity of the polar head group regions of membranes. In this case, the concentration-dependent increase in membrane fluidity paralleled the P-gp inhibitory activity of the excipient (Hugger et al., 2002b), which is very similar to our findings in this study. Moreover, the significance of cell membrane environment in relation to P-gp function has also been discussed by Ferte (2000). He reported that there is an intimate association between P-gp, the cell membrane as a whole, and its lipid components. In this paper, he demonstrated that P-gp was highly sensitive to the lipid environment, and P-gp may be involved in lipid trafficking and metabolism. Furthermore, PEGs, which contain many oxyethylene groups, may alter the lipid phase of the membrane or they may change the fluidity of the polar head group regions of cell membranes. Therefore, such mechanisms may be involved in the reduced function of P-gp in the intestine, although we do not have any direct evidence to support these mechanisms at present.

It is of great importance to investigate the significance and role of P-gp in the intestinal absorption of P-gp substrates by in situ absorption studies. Choi and Jo (2004) reported that PEGylation could significantly increase the absorption of paclitaxel, and the mean absolute bioavailability of this prodrug was 3.9-fold higher than paclitaxel. In the present study, we compared jejunal membrane absorption after the administration of rhodamine123 and coadministration of PEG20,000 by the in situ closed loop method to determine whether P-gp contributes the absorption barrier for rhodamine123 in the intestine. We showed that co-administration of PEG20,000 was associated with a significant 79, 122, 87% increase in rhodamine123 AUC and $F\%$ by 1, 5, 20% (w/v) PEG20,000, respectively. It can therefore be suspected that PEG20,000 inhibit the function of intestinal P-gp, and it increased the intestinal absorption of rhodamine123 by the in situ loop method (Bogman et al., 2005).

In conclusion, PEGs and their derivatives were effective to inhibit the P-gp-mediated efflux system, thereby improving the intestinal absorption of poorly absorbable drugs that are secreted by P-gp from the cells into the lumen. Thus, the use of PEGs and their derivatives in oral drug formulations might have an influence on the pharmacokinetics of the drugs, if they are P-gp substrates. Furthermore, they are very useful excipients to inhibit the function of intestinal P-gp, thereby improving the intestinal absorption of P-gp substrates.

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