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Modulating effect of polyethylene glycol on the intestinal transport and absorption of prednisolone, methylprednisolone and quinidine in rats by in-vitro and in-situ absorption studies

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Abstract

The effects of polyethylene glycol 20000 (PEG 20000) on the intestinal absorption of prednisolone, methylprednisolone and quinidine, three P-glycoprotein (P-gp) substrates, across the isolated rat intestinal membranes were examined by an in-vitro diffusion chamber system. The serosal-to-mucosal (secretory) transport of these P-gp substrates was greater than their mucosal-to-serosal (absorptive) transport, indicating that their net movement across the intestinal membranes was preferentially in the secretory direction. The polarized secretory transport of these drugs was remarkably diminished and their efflux ratios decreased in the presence of PEG 20000. In addition, PEG 20000 did not affect the transport of Lucifer yellow, a non-P-gp substrate. The intestinal membrane toxicity of PEG 20000 was evaluated by measuring the release of alkaline phosphatase (ALP) and protein from the intestinal membranes. The release of ALP and protein was enhanced in the presence of 20 mM sodium deoxycholate (NaDC), a positive control, while these biological parameters did not change in the presence of 0.1–5% (w/v) PEG 20000. These findings indicated that the intestinal membrane damage caused by PEG 20000 was not a main reason for the enhanced absorptive transport of these P-gp substrates in the presence of PEG 20000. Furthermore, the transepithelial electrical resistance (TEER) of rat jejunal membranes in the presence or absence of PEG 20000 was measured by a diffusion chamber method. PEG 20000 (0.1–5.0 % w/v) did not change the TEER values of the rat jejunal membranes, indicating that the increase in the absorptive transport of these P-gp substrates might not be due to the increased transport of these P-gp substrates via a paracellular pathway caused by PEG 20000. Finally, the effect of PEG 20000 on the intestinal absorption of quinidine was examined by an in-situ closed-loop method. The intestinal absorption of quinidine was significantly enhanced in the presence of 0.1–1.0% (w/v) PEG 20000. These findings suggest that PEG 20000 might be a useful excipient to improve the intestinal absorption of quinidine, which is mainly secreted by a P-gp-mediated efflux system in the intestine.

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Introduction

In general, the intestinal absorption of drugs after oral administration can be influenced by many factors, including drug solubility, drug stability in the gastrointestinal tract, intestinal membrane permeability and intestinal first pass metabolism. Among numerous factors affecting the intestinal absorption of drugs, it is now generally recognized that active efflux of drugs by transporters is one of the most important factors that can regulate the oral bioavailability of drugs (Wacher et al 1998; Toyobuku et al 2003). In these efflux transport systems, P-glycoprotein (P-gp) is the most widely studied and probably the most important efflux transporter in controlling the disposition of many P-gp substrates (Hunter & Hirst 1997). 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 intestinal membrane and the oral bioavailability of a wide range of drugs can be increased. Moreover, in addition to P-gp, it has been recently recognized that there are other important

efflux transporters, including multidrug resistant protein 2 and breast cancer resistance protein, that can influence the intestinal absorption of drugs (Lennernas 2003). Furthermore, recent studies indicate that CYP3A and P-gp share a remarkable number of substrates and inhibitors, suggesting that CYP3A and P-gp may form a concerted barrier to intestinal absorption of drugs (Wacher et al 2001).

It is known that several pharmaceutical excipients can reduce the function of P-gp in the intestine, thereby increasing the intestinal absorption of P-gp substrates (Nerurkar et al 1996, 1997; Lo & Huang 2000; Arima et al 2001; Hugger et al 2002a, 2002b, 2003; Johnson et al 2002; Rege et al 2002; Cornaire et al 2004; Shono et al 2004; Bogman et al 2005; Shen et al 2006; Lin et al 2007). Among these excipients, polyethylene glycols (PEGs) are a class of polymer that is widely used in a 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 the P-gp-mediated transport of drugs was inhibited by PEG 400 and vitamin E D-α-tocopheryl PEG 1000 succinate in the rat jejunal membrane. In addition, it was reported that PEG 300 increased the absorptive-directed transport of taxol, a model P-gp substrate, and inhibited its secretory-directed transport, thereby enhancing its net absorptive transport in Caco-2 cells (Hugger et al 2002a).

We have already examined the effect of PEGs with different molecular weights and their derivatives on the intestinal absorption of rhodamine 123, a P-gp substrate, across the isolated rat intestinal membranes by an in-vitro diffusion chamber system (Shen et al 2006). PEGs with average molecular weights of 400, 2000 and 20 000, inhibited the secretory transport of rhodamine 123 in the intestine (Shen et al 2006). In our previous report, although the secretory transport of rhodamine 123 was remarkably inhibited by PEGs, its absorptive transport was not clearly enhanced by the coadministration of PEGs. This may be due to the low contribution of the P-gp-mediated pathway of rhodamine 123 in the absorptive direction. Indeed, Troutman et al (2003a, b) reported that in the absorptive direction, rhodamine 123 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. Due to this drawback of the characteristics of rhodamine 123, new P-gp substrates that are mainly transported by a P-gp-mediated transport system in the absorptive direction were selected for this study.

Prednisolone, methylprednisolone and quinidine were chosen as model P-gp substrates in this study. We examined the effect of PEG 20000 on the intestinal transport and absorption of these P-gp substrates by an in-vitro diffusion chamber system using isolated rat intestinal membranes. PEG 20000 was selected in this study because in our previous study it showed the greatest absorption enhancing effect for the intestinal absorption of rhodamine 123 among various PEGs with different molecular weights (Shen et al 2006). We also examined the effect of PEG 20000 on membrane damage and membrane resistance of the rat intestines, and determined if PEGs cause intestinal membrane damage and increase the absorptive transport of these P-gp substrates by a non-P-gp-mediated mechanism. Finally, the effect of PEG 20000 on the intestinal absorption of quinidine was examined by an in-situ closed-loop method. Quinidine was chosen as a model P-gp substrate in the in-situ absorption study, since PEG 20000 could increase the absorptive transport of quinidine and decrease its secretory transport in the in-vitro transport study, and quinidine in plasma was easily assayed by high-performance liquid chromatography (HPLC).

Materials and Methods

Materials

Prednisolone, methylprednisolone and quinidine were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). PEG 20000 and verapamil were obtained from Nacalai Tesque, Inc., (Kyoto, Japan). Ciclosporin A was obtained from Novartis International AG, Basel, Switzerland. All other reagents were of analytical grade.

Preparation of drug solution

Prednisolone, methylprednisolone, quinidine and verapamil were dissolved in Tris-HEPES buffer solution at pH 7.4 to yield a final concentration. In some experiments, 0.1–5% (w/v) of PEG 20000 or 20 μM ciclosporin A was added to the mucosal side. For an in-vitro transport study, 0.1 mM of quinidine was used, while the concentration of prenisolone and methylprednisolone was 0.2 mM. In addition, 5 mg kg^{-1} of quinidine was used for the in-situ absorption study.

Transport of P-gp substrates across the intestinal membranes by an in-vitro diffusion chamber system

The transport of P-gp substrates across the rat intestinal membrane was studied using a diffusion chamber (Corning Coster Corp., Cambridge, MA, USA) (Grass & Sweetana 1988; Saitoh & Aungst 1995). Male Wistar rats, 250–300 g, were fasted overnight and anaesthetized with Nembutal (Dainippon Sumitomo Pharmaceutics, Osaka, Japan) (50 mg kg^{-1} pentobarbital sodium). The experiments were carried out in accordance with the guidelines of the Animal Ethics Committee at Kyoto Pharmaceutical University. The intestine was exposed through a midline abdominal incision, removed and washed in ice-cold saline. Intestinal segments, excluding Peyer's patches, were isolated and immersed in ice-cold Tris-HEPES buffer solution (pH 7.4, 1 M Tris solution) containing 25 mM HEPES, 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$ and 5 mM glucose. 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 side; the same volume of drug-free buffer was added to the opposite side. 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 times up to 2 h. The receptor chamber samples were replaced with an equal volume of appropriate buffer. The permeated drugs were assayed using HPLC. The apparent permeability coefficient (P_{app}) values of the drugs were calculated from the slope of linear portion of permeability–time profiles by the relationship:

$$P_{app} = (dX_R/dt) \times (1/(A \cdot C_0)) \times 1/60$$

where P_{app} is the apparent permeability coefficient (cm s^{-1}), X_R is the amount of drug in the receptor side (nmol), A is the diffusion area (cm^2), and C_0 is the initial concentration of drug in the donor side (nmol mL^{-1}).

The efflux ratio was used to evaluate the extent of efflux (Eagling et al 1999; Liang et al 2000; Faassen et al 2003; Zhang et al 2004). We examined both the absorptive and secretory transport of drugs and P_{app} values of absorptive and secretory transport of drugs were calculated as described above. The efflux ratios of drugs were obtained by the following equation:

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

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

In order to confirm that the viability of the intestinal membrane was maintained during the transport studies, the transport of trypan blue dye was examined. Trypan blue solution (1%) was added to the mucosal side and the transport of this dye across the rat intestinal membrane was monitored for 2 h by an in-vitro diffusion chamber method. There was no transport of the dye, confirming that the viability of the intestinal membranes was maintained during the transport experiments.

Evaluation of intestinal membrane toxicity caused by PEG 20000

When the intestinal absorption of P-gp substrates is enhanced in the presence of PEG 20000, one possible reason for this finding is that PEG 20000 has some toxic effect on the intestinal membrane and may cause intestinal membrane damage, although PEGs are known to be relatively safe excipients. To evaluate intestinal membrane damage caused by PEG 20000, the activity of alkaline phosphatase (ALP) and the amount of protein released from the intestinal membranes were measured in the mucosal solution. The concentrations of PEG 20000 used in this study were 0.1%, 1% and 5% (w/v). Sample solution (50 μL) was collected from the donor side. The amount of ALP from the intestinal membrane was determined using a Wako alkaline phosphatase kit (Wako Pure Chemical Industries., Co., Japan). In addition, the protein concentration released from the intestinal membranes in the perfusate was determined by a BCA protein assay kit (Pierce Tech., Holmdel, NJ, USA).

Measurement of transepithelial electrical resistance (TEER) in the presence of PEG 20000

When the intestinal absorption of P-gp substrates was enhanced in the presence of PEG 20000, another possible mechanism is that PEG 20000 might loosen the tight junction of the intestinal epithelium, thereby increasing the intestinal absorption of P-gp substrates via a paracellular pathway. To confirm this possibility, the TEER values of the intestinal membranes were measured by an in-vitro diffusion chamber method using stripped rat jejunal membranes, as reported previously (Uchiyama et al 1999; Yamamoto et al 2001; Gao et al 2008a, b). After surgical operation, the small intestine was isolated, the first 10 cm of the top of small intestine was cut away and the next 10 cm was used as the jejunum. The underlying muscularis of the jejunal membranes was removed and the jejunal segments were mounted in a diffusion chamber in which a surface area of 1.78 cm^2 was exposed. Two pairs of electrodes connected to a short-circuit amplifier (CEZ-9100; Nihon Kohden, Tokyo, Japan) were inserted into each side of the diffusion chamber. Phosphate-buffered saline (7 mL, pH 7.4) was added to the serosal side, and an equal volume of PEG solution (0.1–5.0% w/v) was added to the mucosal side. Each side of the chamber was bubbled with a mixture of 95% O_2 and 5% CO_2 in order to maintain the viability of the intestinal membranes. The temperature was maintained at 37°C during the experiment by a circulating water bath. At different time intervals up to 2 h, the potential difference and the short-circuit current were measured, and then TEER values were calculated by Ohm's law. The initial TEER value in the rat jejunal membranes was 46.4 ± 7.9 (ohm·cm^2). In this study, the decrease in TEER values was an index of loosening the tight junction of the intestinal epithelium.

Intestinal absorption of quinidine by an in-situ closed-loop method

Absorption experiments were performed by an in-situ closed-loop method, as reported previously (Yamamoto et al 1994; Tozaki et al 1998; Fetih et al 2005; Gao et al 2008a, b). Male Wistar rats, 250–300 g, were anaesthetized with Nembutal (Dainippon Sumitomo Pharmaceutics, Osaka, Japan) (50 mg kg^{-1} pentobarbital sodium). 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 a 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. Quinidine was dissolved in Tris-HEPES buffer (pH 7.4, 1 M Tris solution) containing 25 mM HEPES, 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$ and 5 mM glucose to a final concentration of 5 mg kg^{-1}. Then, 0.1% or 1% (w/v) of PEG 20000 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 (0, 15, 30, 60, 90, 120, 180, 240 min). 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 time 0 to the final sampling time (240 min).

Determination of drugs

Samples obtained from in-vitro transport studies and in-situ absorption 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 RF-10F or LC-10 detector and a Shimadzu CR-6A integrator.

For the in-vitro diffusion chamber, the mobile phase of quinidine was acetonitrile/0.4% triethylamine (pH 2.5) 10:90. The flow rate was 1.0 mL min^{-1}. The concentration of quinidine

was determined using a fluorescence detector (Shimadzu RF-10F) at excitation and emission wavelengths of 350 and 444 nm, respectively. The mobile phase of prednisolone and methylprednisolone was acetic acid buffer (pH 4.0)/acetonitrile 72:28. The flow rate was 1.0 mL min^{-1}. The concentrations of prednisolone and methylprednisolone were determined using a detector (Shimadzu LC-10) at 242 nm wavelength.

When the concentrations of quinidine in plasma were determined in the case of the in-situ closed-loop method, 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 sample. It was then mixed using a vortex mixer and centrifuged at 3000 rev min^{-1} (150 *g*) 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 acetonitrile/1% triethylamine (1:9), centrifuged at 10 000 rev min^{-1} (1120 *g*) for 5 min, and the solution (50 μL) was injected into the HPLC system.

The calibration curve for the determination of quinidine in plasma was linear over the range 15–500 ng mL^{-1}. The linearity of this method was statistically confirmed. The following regression equation was obtained: A = 3554C (ng mL^{-1}) + 5077, r = 0.9998, for quinidine. The limit of quantification was 15 ng mL^{-1} for quinidine. We assessed the precision of the method by repeated analysis of plasma specimens containing known concentrations of quinidine: the within-day precision values of quinidine concentrations were less than 3.5%; between-day precision values were less than 5.1%.

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.

Results

Effects of PEG 20000 on the permeability of various P-gp substrates across intestinal membranes

The effect of PEG 20000 on the intestinal absorption of prednisolone, methylprednisolone and quinidine, three P-gp substrates, across isolated rat intestinal membranes was examined by an in-vitro diffusion chamber system. Figure 1 shows the time course of absorptive (mucosal to serosal) and secretory (serosal to mucosal) transport of prednisolone, methylprednisolone and quinidine across rat jejunal membranes in the presence or absence of PEG 20000. In the control studies without PEG 20000, the secretory transport of these P-gp substrates was greater than their absorptive transport, indicating that their net movement across the intestinal membranes was preferentially in the secretory direction. The polarized secretory transport of these drugs was significantly diminished in the presence of PEG 20000.

Table 1 summarizes the P_{app} values of prednisolone, methylprednisolone and quinidine across rat jejunal membranes in the absorptive and secretory directions, and their efflux ratios. In the control studies, the efflux ratios of prednisolone, methylprednisolone and quinidine were 1.90, 2.93 and 2.50, respectively. In order to confirm whether this transport system was suitable to evaluate the effect of various pharmaceutical excipients on the intestinal absorption of P-gp substrates, we first examined the effect of ciclosporin A, a typical P-gp inhibitor used as a positive control, on the intestinal transport of these P-gp substrates. The absorptive or secretory transport of these drugs was significantly enhanced or significantly reduced in the presence of 20 μM ciclosporin A, suggesting that the effect of some additives on the function of P-gp can be evaluated using this transport system. When 0.1% (w/v) PEG 20000 was administered to the mucosal side,

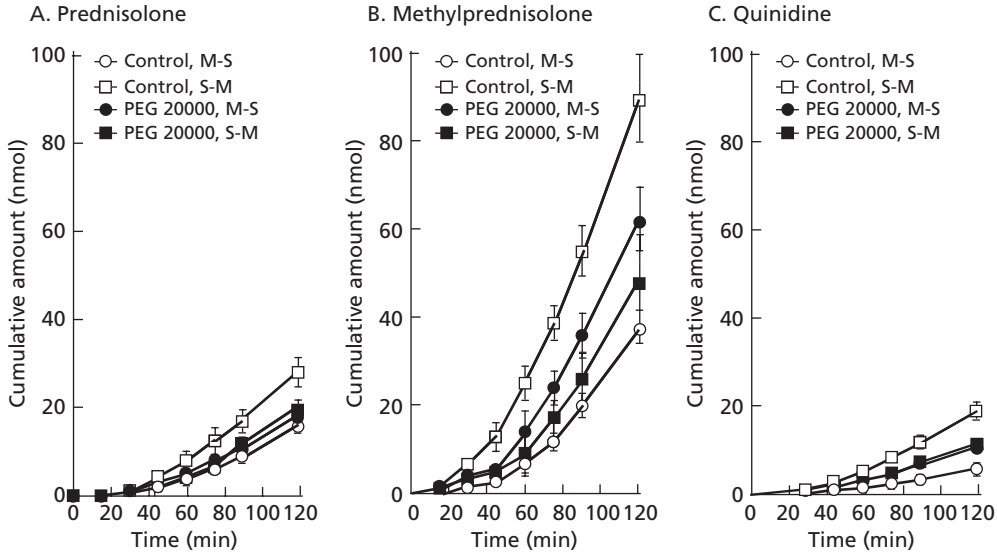

Figure 1 Time course of absorptive (mucosal to serosal: M-S) and secretory (serosal to mucosal: S-M) transport of prednisolone, methylprednisolone and quinidine across the rat jejunal membrane in the presence or absence of 0.1% (w/v) PEG 20000. Results are expressed as the mean ± s.e. of at least three experiments.

Table 1 Effects of PEG 20000 on the permeability of various P-glycoprotein substrates across intestinal jejunal membranes

Drug	Excipient	P_{app}($\times 10^{-6}$ cm s^{-1}) mucosal to serosal	P_{app}($\times 10^{-6}$ cm s^{-1}) serosal to mucosal	Ratio: $P_{app}sm/P_{app}ms$
Prednisolone	Control	8.01 ± 0.83	15.22 ± 1.07	1.90
	0.1% (w/v) PEG 20000	10.21 ± 0.7	11.93 ± 0.68*	1.17
	20 μM ciclosporin A	11.67 ± 0.68*	7.11 ± 1.37*	0.61
Methyl-prednisolone	Control	6.29 ± 1.28	18.45 ± 1.14	2.93
	0.1% (w/v) PEG 20000	12.47 ± 1.41*	14.98 ± 0.46*	1.20
	20 μM ciclosporin A	13.71 ± 1.78*	13.05 ± 0.95**	0.95
Quinidine	Control	6.71 ± 0.56	16.64 ± 1.37	2.50
	0.1% (w/v) PEG 20000	10.68 ± 0.62*	12.18 ± 0.36*	1.14
	20 μM ciclosporin A	9.19 ± 0.85*	9.31 ± 2.63*	1.01
Lucifer yellow	Control	5.67 ± 0.41	6.28 ± 0.25	1.11
	0.1% (w/v) PEG 20000	6.76 ± 0.50	7.25 ± 0.52	1.07

P_{app}, apparent permeability coefficient; $P_{app}sm$, average of the permeability coefficient from the serosal to the mucosal side; $P_{app}ms$, average of the permeability coefficient from the mucosal to the serosal side. Each value represents the mean ± s.e. of at least three experiments. *$P < 0.05$, **$P < 0.01$, significantly different compared with the control.

the absorptive transport of methylprednisolone and quinidine was significantly increased, but the transport of prednisolone was not affected in the presence of PEG 20000. On the other hand, the secretory transport of methylprednisolone, quinidine and prednisolone was significantly reduced with PEG 20000. These findings indicate that PEG 20000 reduced the efflux ratio of methylprednisolone, quinidine and prednisolone. Table 1 also shows the effect of PEG 20000 on the transport of Lucifer yellow, a non-P-gp substrate used as a negative control and a paracellular marker compound, across the rat jejunal membranes. The absorptive and secretory transport of Lucifer yellow was not affected by the addition of 0.1% (w/v) PEG 20000. In addition, PEG 20000 did not change the efflux ratios of Lucifer yellow across the jejunal membranes. Therefore, the inhibitory action of PEG 20000 for the efflux ratios of drugs in the intestine was specific for P-gp substrates. These findings suggest that PEG 20000 might inhibit the function of P-gp in the intestine, thereby reducing the efflux ratios of these P-gp substrates in the transport studies.

Effects of PEG 20000 on intestinal membrane toxicity

The intestinal membrane toxicity of PEG 20000 was evaluated by measuring the release of ALP and protein from the intestinal membranes (Figure 2). The release of ALP and protein was enhanced in the presence of 20 mM sodium deoxycholate (NaDC), a positive control, while these biological parameters did not change in the presence of 0.1–5% (w/v) of PEG 20000. These findings indicate that PEG 20000 did not cause any membrane damage to the intestinal epithelium.

Effects of PEG 20000 on TEER of the rat jejunal membranes

A possible mechanism for the enhanced intestinal absorption of P-gp substrates in the presence of PEG 20000 might be that PEG 20000 loosens the tight junction of the intestinal epithelium, thereby increasing the intestinal absorption of P-gp substrates via the paracellular pathway. To confirm this

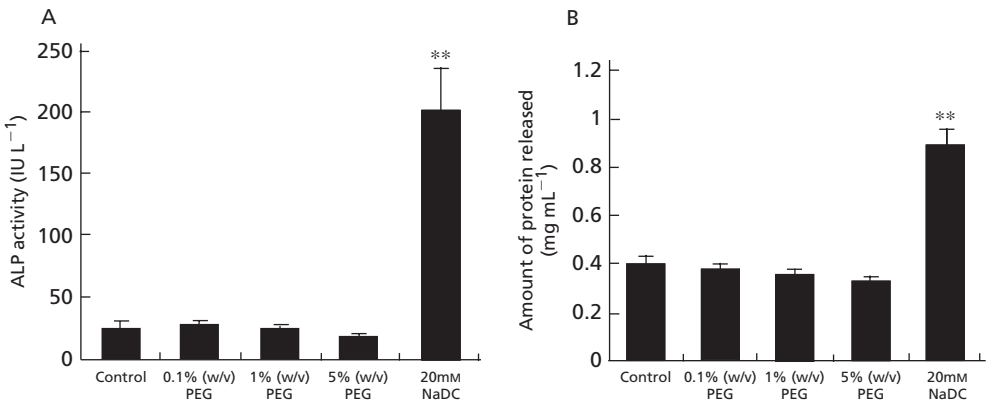

Figure 2 Effect of different concentrations of PEG 20000 on the toxicity of rat jejunal membranes. Intestinal membrane toxicity was evaluated by measuring the release of alkaline phosphatase (ALP) and protein from the intestinal membranes. Sodium deoxycholate (NaDC) was used as a positive control. Results are expressed as the mean ± s.e. of at least three experiments. **$P < 0.01$, significantly different compared with the control.

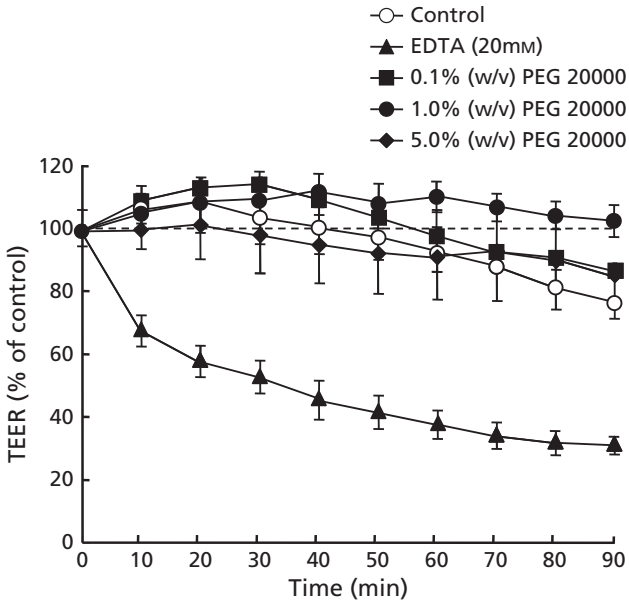

Figure 3 Effect of different concentrations of PEG 20000 on the transepithelial electrical resistance (TEER) of rat jejunal membranes by a diffusion chamber method. EDTA (20 mM) was used as a positive control. Results are expressed as the mean ± s.e. of at least three experiments.

possibility, the effect of various concentrations of PEG 20000 on the TEER values of the intestinal membranes were measured by an in-vitro diffusion chamber method (Figure 3). The TEER values of rat jejunal membranes were significantly reduced in the presence of EDTA, a positive control. However, PEG 20000 did not change the TEER values of the rat jejunal membranes over the concentration range of 0.1–5% (w/v).

Effect of PEG 20000 on the intestinal absorption of quinidine by an in-situ closed-loop method

We examined the effect of PEG 20000 on the intestinal absorption of quinidine to determine if PEG 20000 might affect the intestinal absorption of quinidine in in-situ absorption studies as well as in in-vitro transport studies. Therefore, a solution containing quinidine was administered into the jejunal loop, and the jejunal absorption of quinidine was examined by the in-situ closed-loop method. Figure 4 shows the plasma concentration–time profiles of quinidine after the jejunal administration of quinidine in the presence

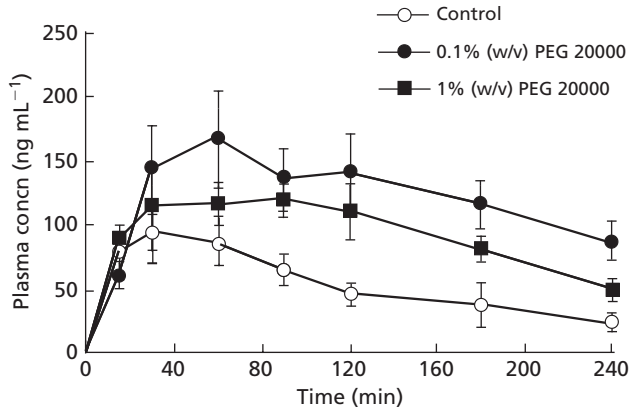

Figure 4 Plasma concentration–time profile of quinidine after jejunum administration with or without 0.1–1.0% (w/v) PEG 20000 by an in-situ closed-loop experiment. Results are expressed as the mean ± s.e. of at least three experiments.

or absence of different concentrations of PEG 20000 (0.1 and 1% w/v). A significant increase in the plasma concentrations of quinidine was observed when quinidine was coadministered with 0.1 and 1% (w/v) PEG 20000 to the jejunum. Table 2 shows the pharmacokinetic parameters (C_{max}, T_{max} and $AUC_{0\rightarrow240}$) of quinidine after its administration with PEG 20000 into the jejunum. In the presence of different concentrations of PEG 20000, we observed a significant increase in C_{max} and $AUC_{0\rightarrow240}$ values after administration of quinidine with PEG 20000 into the jejunum. In addition, the AUC value of quinidine with 0.1% PEG 20000 was greater than that with 1% of PEG 20000. These findings suggest that PEG 20000 might be a useful excipient to improve the intestinal absorption of quinidine, which may be mainly secreted by a P-gp-mediated efflux system in the intestine.

Discussion

The present study demonstrated that PEG 20000 could reduce the efflux ratios of P-gp substrates in the intestine using the diffusion chamber method. In previous studies, the Caco-2 cell line, a human adenocarcinoma cell line, has generally been used to estimate drug permeability and substrate activity for efflux transport proteins including P-gp. However, the expression levels of transporters in Caco-2 cells are usually variable and are dependent on the culture conditions (Anderle

Table 2 Pharmacokinetic parameters of jejunal absorption of quinidine in the presence or absence of PEG 20000 by the in-situ closed-loop method

Excipient	C_{max} (ng mL^{-1})	T_{max} (min)	$AUC_{0\rightarrow240}$ (ng min mL^{-1})
Control	95.7 ± 3.75	25.0 ± 5.0	12800 ± 986
0.1% (w/v) PEG 20000	184.8 ± 21.6*	40.0 ± 10.0	28750 ± 2418*
1% (w/v) PEG 20000	135.0 ± 13.2*	67.0 ± 14.0	22015 ± 2928*

C_{max}, peak concentration; T_{max}, time to reach the peak concentration; $AUC_{0\rightarrow240}$, area under the curve from 0 to 240 min. Each value represents the mean ± s.e. of at least three experiments. *$P < 0.05$, significantly different compared with the control.

et al 1998), and it was suggested that P-gp is overexpressed in Caco-2 cells (Collect et al 1999), which is a major disadvantage for the estimation of P-gp function in the presence or absence of some modulators and excipients using Caco-2 cells. Therefore, in this study, the in-vitro diffusion chamber method was used for evaluating the effect of PEG 20000 on the intestinal function of P-gp in the intestine.

As shown in Figure 1 and Table 1, the secretory transport of P-gp substrates, including prednisolone, methylprednisolone and quinidine, was greater than its absorptive transport in the control studies. Oka et al (2002) reported that the secretory transport of prednisolone and methylprednisolone was about 1.9-times and 3.4-times greater than that of the absorptive transport in Caco-2 cells. In addition, it was reported that the secretory transport of quinidine was 3.5-times greater than the absorptive transport in rat jejunal membranes (Emi et al 1998). Therefore, our present finding was well correlated with previous studies.

Figure 1 and Table 1 also show that PEG 20000 could increase the absorptive transport of methyprednisolone and quinidine and it could decrease the secretory transport of prednisolone, methyprednisolone and quinidine in the intestine. The $P_{app}sm/P_{app}ms$ ratios of these P-gp substrates were clearly reduced in the presence of PEG 20000 compared with the control. Previous studies indicated that the secretory-directed transport of methyprednisolone and prednisolone was inhibited in the presence of verapamil (0.1 mM) in Caco-2 cells (Oka et al 2002). In addition, Emi et al (1998) reported that the secretion of quinidine was significantly inhibited and its absorption was significantly enhanced by several substrates of P-gp, including verapamil, diltiazem and digoxin, in rats. They also demonstrated that these effects were also observed in the presence of 2,4-dinitrophenol, a typical metabolic inhibitor (Emi et al 1998). Moreover, our previous study also indicated that PEG 20000 reduced the efflux ratio of rhodamine 123, a P-gp substrate, in the intestine (Shen et al 2006). Based on these findings, it was suggested that these drugs might be transported by a P-gp-mediated efflux system and their polarized secretory transport was inhibited in the presence of various P-gp substrates and inhibitors.

The present study also demonstrated that the absorptive transport of prednisolone was not significantly enhanced in the presence of PEG 20000, although it could increase the transport of methylprednisolone and quinidine. The reason for this difference was not clear. One possible reason for the negative effect of PEG 20000 on the absorptive transport of prednisolone is the concentration of prednisolone. We used 0.2 mM prednisolone for the transport studies because of its limit of quantification (detection limit of assay). However, the contribution of P-gp-mediated transport to the intestinal absorption decreased as the concentration of P-gp substrate increased. The P-gp-mediated absorptive transport of prednisolone was probably saturated at the concentration used in this study.

We found that PEG 20000 could increase the absorptive transport of methyprednisolone and quinidine. One possible mechanism for this increased absorptive transport could be that PEG 20000 causes membrane damage to the intestinal epithelium, thereby increasing the transport of drugs via a transcellular pathway. Therefore, we also evaluated the intestinal membrane toxicity with or without PEG 20000 by measuring the release of ALP and protein from the intestinal membranes in the mucosal site. ALP is a marker enzyme of apical membrane of the intestinal epithelium, and its presence in the apical compartment is generally regarded as evidence of cell membrane damage (Schasteen et al 1992). Protein is also an index of membrane toxicity (Uchiyama et al 1996; Yamamoto et al 1996), since protein is one of the major components of biological membranes including intestines. As shown in Figure 2, the release of ALP and protein was enhanced in the presence of 20 mM sodium deoxycholate (NaDC), a positive control, while these biological parameters did not change in the presence of 0.1–5% (w/v) PEG 20000. These findings indicate that PEG 20000 did not cause any membrane damage to the intestinal epithelium. Thus, the enhanced absorptive transport of P-gp substrates in the presence of PEG 20000 was not due to membrane damage caused by PEG 20000. Another possibility for the increase in the transport of P-gp substrates across the intestinal membrane in the presence of PEG 20000 is that this excipient might loosen the tight junctions of the epithelium, thereby increasing the transport of drugs via a paracellular pathway. In order to confirm this possibility, we measured the TEER of rat jejunal membranes with or without PEG 20000 by a diffusion chamber method (Figure 3). The TEER values of rat jejunal membranes were significantly reduced in the presence of EDTA, a positive control. However, PEG 20000 (0.1–5% w/v) did not change the TEER values of the rat jejunal membranes. These findings indicate that the increase in the absorptive transport of these P-gp substrates might not be due to the increased transport of these P-gp substrates via a paracellular pathway caused by PEG 20000. This was also supported by the fact that the transport of Lucifer yellow, a typical paracellular marker compound, was not changed in the presence of PEG 20000 as described above (Table 1).

It is important to investigate the contribution of P-gp in the intestine not only by in-vitro studies but also by in-situ or in-vivo studies, when these pharmaceutical excipients can be used for improving the intestinal absorption of P-gp substrates in clinical use. In the in-vivo absorption of P-gp substrates with some pharmaceutical excipients, Zhang et al (2003) reported that the commonly used surfactant, Tween 80, could improve the intestinal absorption of digoxin, a P-gp substrate, after oral administration in rats. In the present study quinidine was selected as a model P-gp substrate and we examined the intestinal absorption of quinidine in the presence or absence of PEG 20000 by an in-situ closed-loop method to determine if P-gp can act as a absorption barrier for quinidine in the intestine. Quinidine shares common characteristics (high lipophilicity, positive charge and planar structure) with a substrate of P-gp in tumour cells, brain capillary endothelial cells (Tsuji et al 1993) and in the rat small intestine. The transport of quinidine increased in the presence of P-gp substrates and/or inhibitors in jejunum (Emi et al 1998).

As shown in Figure 4, we found a significant increase in C_{max} and $AUC_{0\rightarrow240}$ values after administration of quinidine

with PEG 20000 into the jejunum. Using the in-situ closed-loop method, we previously demonstrated that PEG 20000 could increase the intestinal absorption of rhodamine 123, a P-gp substrate (Shen et al 2006). It can therefore be suggested that PEG 20000 might inhibit the function of intestinal P-gp, and it might increase the absorption of quinidine from the small intestine. In this study, the AUC of quinidine in the presence of 1% PEG 20000 was lower than that with 0.1% PEG 20000 and there was almost no concentration dependency in the absorption-enhancing effect of PEG 20000 for the intestinal absorption of quinidine. The reason for this finding is not clear. However, PEG at relatively high concentrations can become a viscous solution, and this would decrease the diffusion of quinidine in the solution. This characteristic of PEG 20000 solution would also decrease the T_{max} values of quinidine in the presence of 0.1% and 1% PEG 20000, although we do not have any evidence to support this hypothesis. Further studies are necessary to clarify these findings.

In conclusion, PEG 20000 effectively inhibited the P-gp-mediated efflux system, thereby improving the intestinal absorption of drugs that are secreted by P-gp from the cells into the lumen. Thus, PEG 20000 in oral drug formulations might be useful for improving the intestinal absorption of P-gp substrates including quinidine without any significant damage to the intestinal membranes.

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