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Improvement of intestinal absorption of water-soluble macromolecules by various polyamines: Intestinal mucosal toxicity and absorption-enhancing mechanism of spermine

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Abstract

The absorption-enhancing effects of three different polyamines, spermine (SPM), spermidine (SPD) and putrescine (PUT) on the intestinal absorption of water-soluble macromolecules were examined in rats. Fluorescein isothiocyanate-labeled dextrans (FDs) with different average molecular weights were chosen as models of water-soluble macromolecules and intestinal absorption of FDs with or without these polyamines was examined by an *in situ* closed loop method. The intestinal absorption of fluorescein isothiocyanate-labeled dextran with an average molecular weight of 4400 (FD4) was relatively low in the absence of these polyamines. However, its absorption was improved in the presence of 5–10 mM SPM and 10 mM SPD in the jejunum and 10 mM SPM in the colon, while 10 mM PUT had almost no absorption-enhancing effect on the intestinal absorption of FD4. Overall, the enhancing effects of these polyamines were greater in the jejunal membranes than in the colonic membranes. The absorption-enhancing effect of SPM decreased as the molecular weights of FDs increased. The intestinal membrane toxicity of 10 mM SPM was evaluated by measuring the amount of protein and activity of lactate dehydrogenase (LDH) released from the intestinal epithelial cells. We also observed the morphological changes of intestinal mucosa in the presence or absence of SPM. The results indicated that the amount of protein and LDH was not changed in the presence of 10 mM SPM, although we observed a significant increase in these biological markers in the presence of 3% Triton X-100, as a positive control. Furthermore, we found no significant change in the intestinal membrane with 10 mM SPM by the morphological observation. These findings suggested that 10 mM SPM did not cause any significant membrane damage to the intestinal epithelium. To investigate the absorption-enhancing mechanism of SPM, the transepithelial electrical resistance (TEER) of the rat jejunal membranes was studied by using a diffusion chamber method. SPM decreased the TEER values in a concentration dependent manner and 10 mM SPM had almost the same effect to decrease the TEER value compared with 10 mM EDTA as a positive control. These findings suggest that SPM may loosen the tight junction of the epithelium, thereby increasing the intestinal absorption of drugs via a paracellular route. In summary, polyamines, especially SPM would be one of the suitable absorption enhancers with high effectiveness and low intestinal membrane toxicity.

Keywords: Intestinal absorption; Macromolecule; Absorption enhancer; Polyamine; Spermine; Membrane toxicity

1. Introduction

The intestinal absorption of water-soluble drugs is usually limited by their poor membrane permeability characteristics across the intestinal epithelium. Therefore, absorption enhancers have been often adopted to improve the absorption of these poorly absorbable drugs including hydrophilic antibiotics and peptide and protein drugs. These absorption enhancers include surfactants, bile salts, chelating agents, and fatty acids, etc. As for surfactants and bile salts, our previous study indicated that rectal permeability of insulin was enhanced by the co-administration of various bile salts such as sodium glycocholate (NaGC), sodium taurocholate and sodium deoxycholate (NaDC) (Yamamoto et al., 1992). We also demonstrated that *n*-dodecyl-

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β-D-maltopyranoside (LM), a nonionic surfactant, and bile salts such as NaGC and NaDC enhanced the permeability of insulin across the intestinal membrane (Uchiyama et al., 1999). With regard to chelating agents, it was found that sodium salicylate and 5-methoxysalicylate remarkably enhanced the rectal absorption of insulin in rats (Nishihata et al., 1983; Aungst and Rogers, 1988). Furthermore, it was reported that linoleic acid (fatty acid)-surfactant-mixed micelles improved the intestinal absorption of streptomycin and gentamicin in rats (Muranishi, 1985, 1990). More recently, we found that nitric oxide donors including *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP), etc., could improve the intestinal transport and absorption of insulin and other poorly absorbable drugs without severe intestinal membrane damage (Yamamoto et al., 2001; Fetih et al., 2005). These different types of enhancers have been known to increase the intestinal absorption of poorly absorbable drugs by various mechanisms. These mechanisms involve increase in membrane fluidity, interaction with the ability of calcium ion to maintain the dimension of intracellular space, solubilization of mucous membrane, change in non-protein and protein sulfhydryl levels in mucosal tissues, increase in water flux, and reduction of the viscosity of mucus layer adhering to all mucosal surfaces (Lee and Yamamoto, 1989; Lee et al., 1991). However, the absorption enhancers with high effectiveness often cause damage and irritate the intestinal mucosal membrane (Swenson and Curatolo, 1992). Indeed, our previous studies indicated that there exists an almost linear relationship between the absorption-enhancing effects of various absorption enhancers in the small and large intestine and their membrane toxicity (Uchiyama et al., 1996; Yamamoto et al., 1996). Therefore, novel effective and less toxic absorption enhancers should be developed and used in clinical practice.

Polyamines including spermine (SPM), spermidine (SPD), and putrescine (PUT) are indispensable components of living cells and have been known to be essential for cellular growth and proliferation. It is also well known that polyamines including SPM, SPD, and PUT, are contained in many foods coming from vegetables and animals (Landete et al., 2005; Haba et al., 2004; Til et al., 1997). These polyamines are also produced by microflora in the digestive tract of mammals. They exist naturally in the millimolar range in gut luminal contents and may thus affect cells in the intestinal mucosa (Seidel and Scemama, 1997). They are ubiquitous structural components of all eukaryotic cells (Zhang et al., 2000), and have been implicated in a wide variety of biological functions. They can stabilize conformations of DNA and prevent DNA fragmentation (Zhang et al., 2000), and maintain the integrity of normal intestinal mucosa (Guo et al., 2003). In addition, exogenous polyamines have been shown to effectively substitute for endogenously synthesized polyamines in duodenal mucosal repair processes and to increase the normal healing rate (Wang and Johnson, 1992). Furthermore, it was also reported that SPM or SPD given in the diet to young rats induced precocious intestinal maturation (Dufour et al., 1988).

As for the absorption-enhancing effect of polyamines, it was reported that the effects of SPM on the intestinal permeability to different-sized molecules generally depended on the intestinal region and polyamine concentrations (Osman et al., 1998). More recently, Miyake et al. (2006a) reported that SPM and SPD could improve the absorption of rebamipide, a poorly absorbable drug in the gastrointestinal tract after oral administration in rats without any significant membrane damage. They also reported that a synergistic absorption-enhancing effect was observed when they used SPM and SPD with bile salts to improve the intestinal absorption of rebamipide (Miyake et al., 2006a,b). However, few studies have been carried out the effects of these polyamines on the intestinal absorption of water-soluble macromolecular drugs in rats. Furthermore, the absorption-enhancing mechanisms of the polyamines were not clearly understood in the previous studies.

In the present study, therefore, fluorescein isothiocyanate-labeled dextrans (FDs) with different average molecular weights (FD4, FD10 and FD70) were chosen as models of water-soluble macromolecules and we examined the effect of exogenous polyamines including SPM, SPD and PUT on the intestinal absorption of FDs by an *in situ* closed loop method. We also examined the membrane toxicity caused by SPM by measuring the release amount of protein and activity of lactate dehydrogenase (LDH). In addition, the morphological changes of the intestinal epithelium were also examined by light microscopy. Furthermore, we examined to elucidate the absorption-enhancing mechanism of SPM by measuring the TEER of rat jejunal membranes in the presence of various concentrations of SPM by an *in vitro* diffusion chamber method.

2. Materials and methods

2.1. Materials

SPM, SPD and PUT were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). The chemical structure of these polyamines is shown in Fig. 1. FD4, FD10 and FD70 with average molecular weights of 4400, 9300 and 69,000 were also obtained from Sigma–Aldrich Chemical Co. Ltd. (St. Louis, MO, USA). Ethylenediaminetetraacetic acid disodium salt (EDTA) was obtained from Nakalai tesque Inc. (Kyoto, Japan). All other reagents used were of analytical grade.

2.2. Preparation of drug solution

During the *in situ* absorption experiment, FDs (FD4, FD10 and FD70) were dissolved in the PBS to yield a final concentration of 2 mg/ml. Polyamines including SPM, SPD and PUT with different concentrations were added to the dosing solutions of FDs. In addition, the solution containing only SPM was prepared in PBS at a concentration of 10 mM for evaluating the intestinal membrane toxicity caused by SPM. Furthermore, the PBS solution containing 1–10 mM SPM was used for the measurement of TEER values of the rat jejunal membranes.

2.3. Absorption experiments

In our preliminary studies at 1, 10 and 50 mM SPM, 1 mM SPM did not enhance the intestinal absorption of FD4 so much.

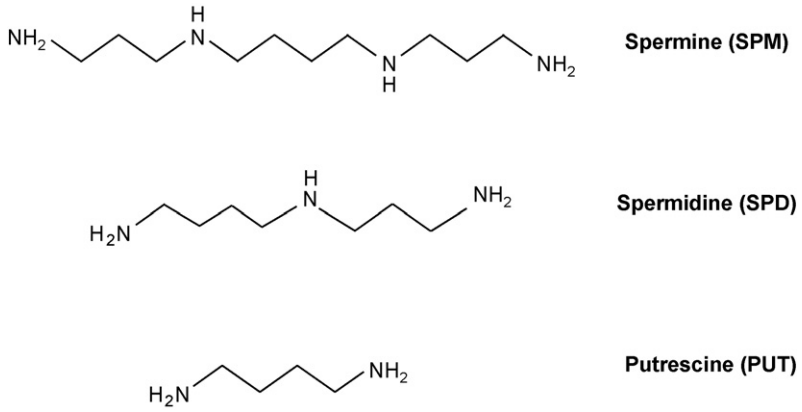

Fig. 1. Chemical structure of spermine (SPM), spermidine (SPD) and putrescine (PUT).

On the other hand, 50 mM SPM caused intestinal mucosal damage including a significant amount of protein released from the intestinal mucosa compared with the control and exfoliation and shrinkage of epithelial cells. Therefore, the concentrations of polyamines were fixed between 1 and 10 mM from the point of efficacy and toxicity in the present study.

Absorption experiments were performed by an *in situ* closed loop method, as reported previously (Yamamoto et al., 1994; Gotoh et al., 1996). Male Wistar rats (210–250 g) were anesthetized with sodium pentobarbital (32 mg/kg body weight i.p.). Animals were fasted for about 16 h before experiment. The studies were performed in accordance with the guidelines of animal ethics committee at Kyoto Pharmaceutical University. The intestine was exposed through a midline abdominal incision and flushed by PBS (pH 7.4). The remaining buffer was expelled with air. A jejunal or colonic closed loop was prepared. The distal part of the loop was cannulated with polyethylene tubing, and then closed by clipping with forceps. One milliliter drug solution, kept at 37 °C, was introduced into the loop through a cannulated opening in the proximal part of the loop, which was then closed with forceps. The jugular vein was exposed and blood samples (0.25 ml) were collected into heparinized syringes at predetermined time intervals up to 240 min. Samples were immediately centrifuged at 12,000 rpm for 5 min to obtain the plasma fraction (100 μl), which was then kept in ice until determination.

2.4. Assessment of membrane toxicity

2.4.1. Release amount of protein and activity of lactate dehydrogenase (LDH)

The amount of protein and LDH released from the intestinal epithelial cells was selected as two biological markers to assess intestinal membrane damage caused by SPM. Phosphate buffered saline (PBS), 10 mM SPM and 3% Triton X-100 were injected into the intestinal loop respectively in a similar manner to that used for the absorption experiment. Rats were left for 4 h after administration and at the end of the experiments, the perfusate in the intestine was withdrawn for the determination of the release of protein and LDH. The protein concentrations in the perfusate were determined by BCA™ Protein Assay Kit (Pierce Tech., USA) using bovine serum albumin as a standard. The activity of LDH was determined using an assay kit LDH CII (Wako Pure Chemical Industries, Osaka, Japan).

2.4.2. Morphological observation

After *in situ* closed loop experiments were carried out for 4 h, the intestine was washed with PBS (pH 7.4) and the loop segments were removed and immersed in the 4% neutral paraform aldehyde buffer and fixed. Vertical sections were prepared, stained with hematoxylin-eosin, and examined by light microscopy.

2.5. Measurement of transepithelial electrical resistance (TEER)

The TEER values of the intestinal membranes were measured by an *in vitro* diffusion chamber method using stripped rat jejunal membranes. After surgical operation, the small intestine was isolated and the underlying muscularis 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, respectively. PBS (7 ml) at pH 7.4 was added to the serosal side, and an equal volume of SPM solution with different concentrations (1–10 mM) 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 (PD) and the short-circuit current (Isc) were measured respectively, and then TEER values were calculated by Ohm's law.

2.6. Analytical methods

The fluorescence intensities of FDs were measured with a fluorescence spectrophotometer (Spectrafluor Plus, TECAN, Switzerland) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm, respectively.

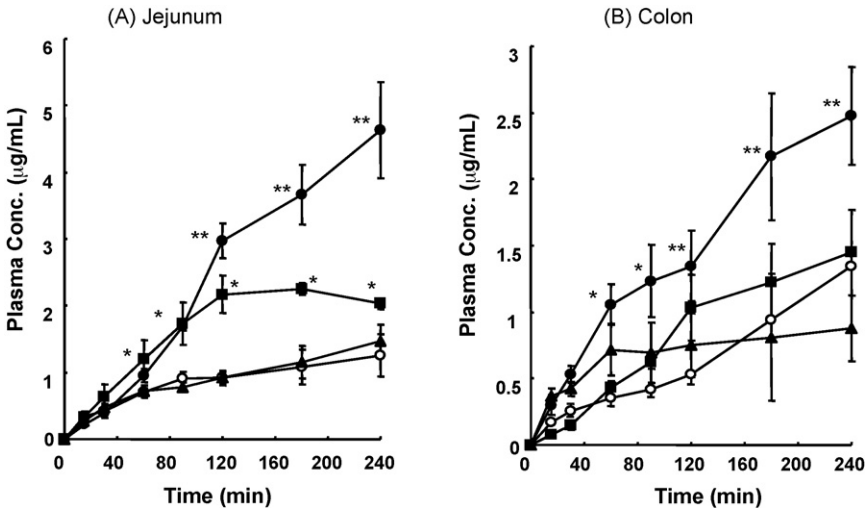

Fig. 2. Plasma concentration–time profiles of FD4 after administration to the jejunum (A) and the colon (B) in the presence or absence of various polyamines. Results are expressed as the mean ± S.E. of at least four experiments. *$P < 0.05$, **$P < 0.01$, compared with the control. Keys: (◯) control, (●) 10 mM spermine (SPM), (■) 10 mM spermidine (SPD), (▲) 10 mM putrescine (PUT).

2.7. Statistical analyses

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

3. Results

3.1. Effects of polyamines on the intestinal absorption of FD4 in different regions

The effects of SPM, SPD and PUT on the absorption of FD4 from the jejunum and the colon were examined by an *in situ* closed loop method. Fig. 2 shows the plasma concentration–time profiles of FD4 in the presence or absence of polyamines following administration of FD4 with various polyamines to the intestine of rats. The absorption of FD4 from the jejunum was enhanced by 10 mM SPM and 10 mM SPD, and the absorption-enhancing effect of SPM was greater than that of SPD. However, 10 mM PUT showed no significant effect in the intestinal absorption of FD4 (Fig. 2A). However, in the large intestine, only 10 mM SPM could increase the absorption of FD4 significantly (Fig. 2B).

Fig. 3 shows the effect of various concentrations (1–10 mM) of SPM on the intestinal absorption of FD4 after administration of FD4 with different concentrations of SPM. As shown in these figures, the absorption-enhancing effect of SPM was concentration-dependent over the concentration range of 1–10 mM, and 10 mM SPM showed the greatest absorption-enhancing effects both in jejunum (Fig. 3A) and in colon (Fig. 3B).

Table 1 summarizes the AUC values of FD4 after administration with various polyamines to the jejunum and colon. As

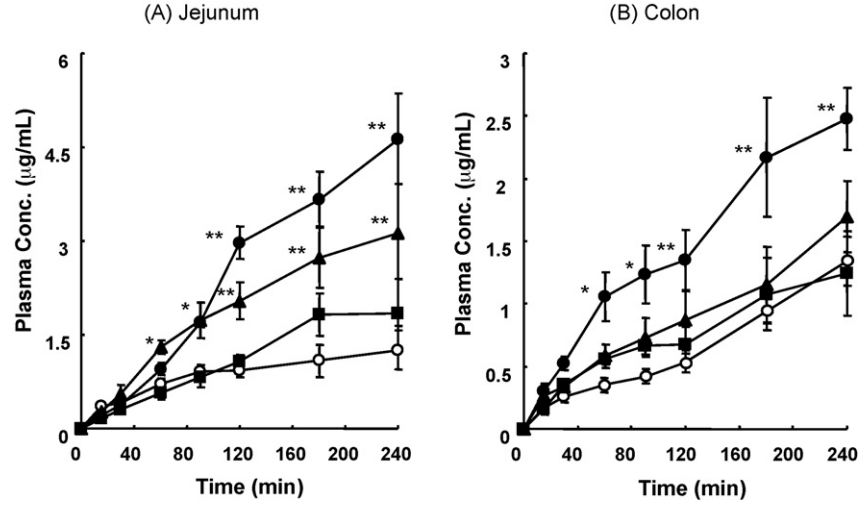

Fig. 3. Plasma concentration–time profiles of FD4 after administration to the jejunum (A) and the colon (B) in the presence or absence of various concentrations of spermine (SPM). Results are expressed as the mean ± S.E. of at least four experiments. *$P < 0.05$, **$P < 0.01$, compared with the control. Keys: (◯) control, (■) 1 mM spermine (SPM), (▲) 5 mM spermine (SPM), (●) 10 mM spermine (SPM).

Table 1
Pharmacokinetic parameters of FD-4 after intestinal administration with or without polyamines

	Jejunum		Colon	
	$AUC_{0-240\,min}$ (μg·min/ml)	Ratio	$AUC_{0-240\,min}$ (μg·min/ml)	Ratio
Control	207.5 ± 30.9	–	152.2 ± 8.2	–
Spermine (1 mM)	263.6 ± 26.9 ns	1.3	179.3 ± 35.6 ns	1.2
Spermine (5 mM)	457.2 ± 81.3**	2.2	210.0 ± 23.5 ns	1.4
Spermine (10 mM)	584.0 ± 60.7**	2.8	347.3 ± 49.2**	2.3
Spermidine (10 mM)	400.8 ± 40.6*	1.9	199.6 ± 38.2 ns	1.3
Putrescine (10 mM)	215.2 ± 23.5 ns	1.0	165.8 ± 28.2 ns	1.1

Results are expressed as the mean ± S.E. of four to five rats. $^{**}P < 0.01$; $^{*}P < 0.05$; ns: not significantly different, compared with the control.

shown in Table 1, 10 mM SPM displayed the largest AUC value both in the jejunum and in the colon. On the other hand, 1 mM SPM and 10 mM PUT did not improve the absorption of FD4 so much both in the jejunum and the colon. The rank order of the absorption-enhancing ability of these polyamines was 10 mM SPM > 5 mM SPM ≧ 10 mM SPD ≧ 1 mM SPM ≧ 10 mM PUT in the jejunum and the colon. Overall, the absorption-enhancing effects of these polyamines were greater in the jejunum than those in the colon. These findings indicated that 10 mM SPM would be one of the most promising enhancers for improving the intestinal absorption of FD4 in rats.

3.2. Effect of SPM on the intestinal absorption of FDs with different molecular weights

We also examined the effect of 10 mM SPM on the absorption of FDs with various molecular weights (FD4, FD10 and FD70) from the jejunum and the colon. As shown in Fig. 4 (A: jejunum, B: colon), the absorption of FD10 as well as FD4 from the jejunum and the colon was enhanced in the presence of 10 mM SPM, while we observed no significant increase in the intestinal absorption of FD70 even in the presence of 10 mM SPM. Overall, the absorption-enhancing effects of SPM for improving the intestinal absorption of FDs from the jejunum and the colon decreased as the molecular weights of these compounds increased.

3.3. Effect of SPM on the intestinal membrane toxicity

In order to confirm the safety of SPM, the total amounts of protein and LDH released from the intestinal epithelial cells with or without SPM were examined to evaluate the local membrane toxicity caused by SPM. The data were shown in Fig. 5(A) and (B). The total amount of protein and the activity of LDH in the presence of 10 mM SPM at 4 h after intestinal administration were almost the same levels compared to PBS, although there was a remarkable increase in the amount of protein and the activity of LDH after intestinal administration of 3% Triton X-100 as a positive control.

On the other hand, morphological observation of the intestinal mucosa after the exposure of 10 mM SPM was also performed by an *in situ* closed loop method. Fig. 6 showed the typical morphological pictures of jejunum and colon at 4 h after the exposure of 10 mM SPM (Fig. 6A: jejunum, Fig. 6B: colon). The results clearly indicated that 10 mM SPM did not cause any

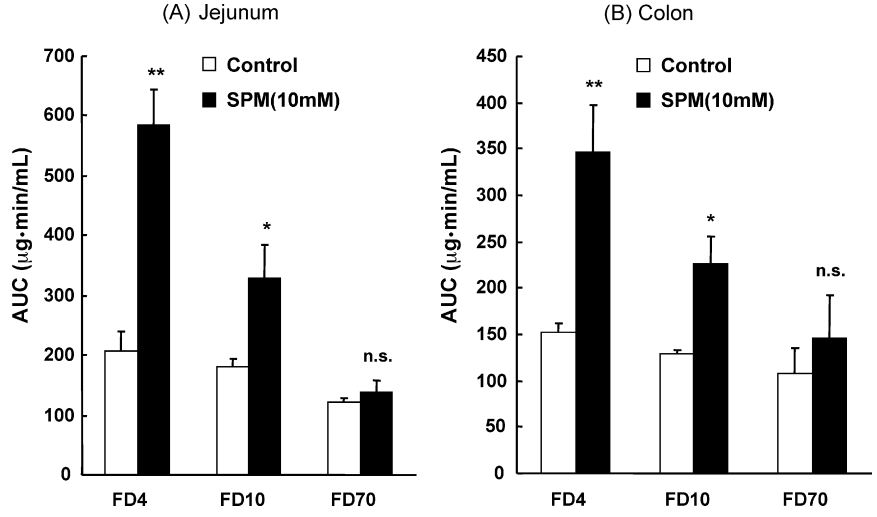

Fig. 4. Effect of 10 mM spermine (SPM) on the intestinal absorption of FDs with different molecular weights from (A) jejunum and (B) colon. The average molecular weights of FD4, FD10 and FD70 were 4400, 9300 and 69,000, respectively. Results are expressed as the mean ± S.E. of at least four experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, N.S. not significantly different, compared with the control.

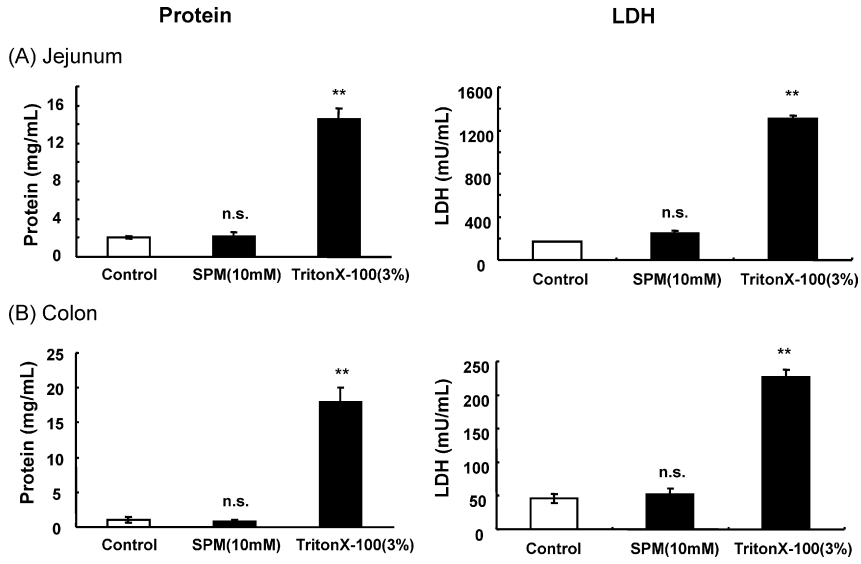

Fig. 5. The amount of total protein and the activity of lactate dehydrogenase (LDH) at 4 h after intestinal administration. Results are expressed as the mean ± S.E. of at least four experiments. $^{**}P < 0.01$, n.s.: not significantly different, compared with the control.

significant change in the morphology of the intestinal membrane after intestinal administration both in the jejunum and in the colon.

3.4. Effect of SPM on the transepithelial electrical resistance (TEER)

Finally, we investigated the absorption-enhancing mechanism of SPM for improving the intestinal absorption of poorly absorbable drugs. The TEER values of the rat jejunal membranes were measured by using a diffusion chamber method. As shown in Fig. 7, SPM decreased the TEER value in a concentration dependent manner and 10 mM SPM had almost the same effect to decrease the TEER values of the rat jejunal membranes compared with EDTA as a positive control. These findings suggest that SPM may loosen the tight junction of the epithelium, thereby increasing the intestinal absorption of drugs via a paracellular route.

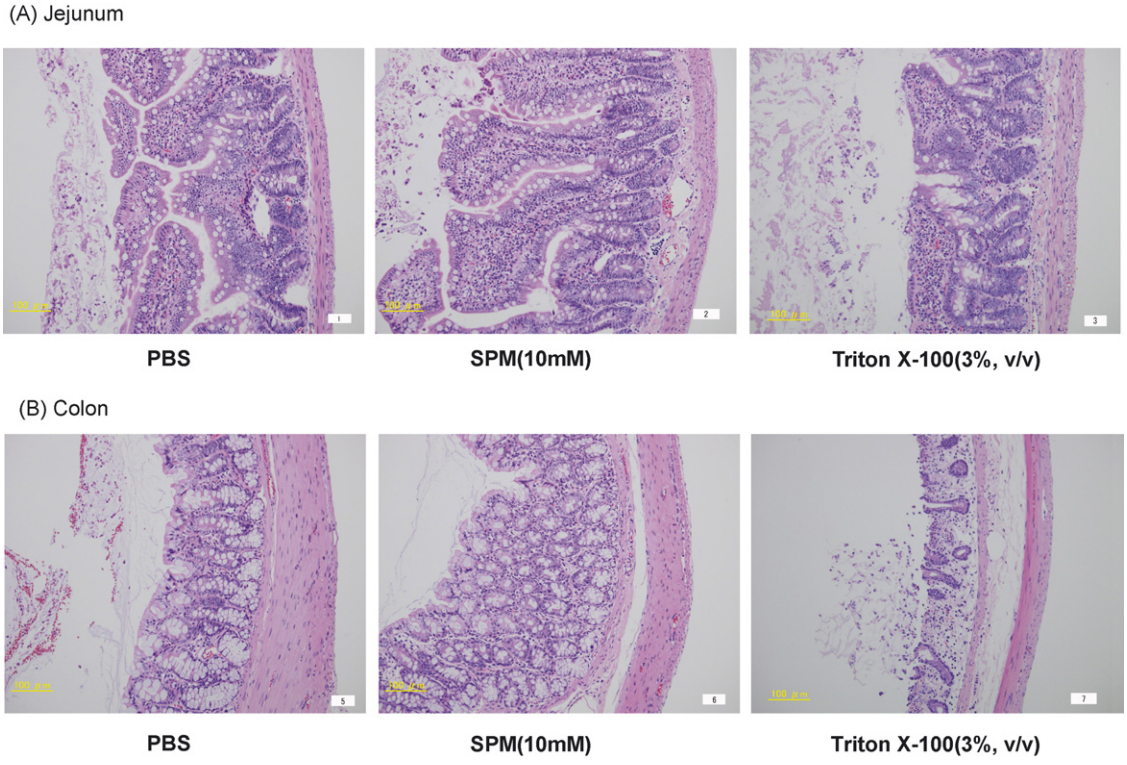

Fig. 6. Morphological observation of (A) jejunum and (B) colon at 4 h after intestinal administration of PBS, 10 mM SPM and 3% Triton X-100 to rats, respectively.

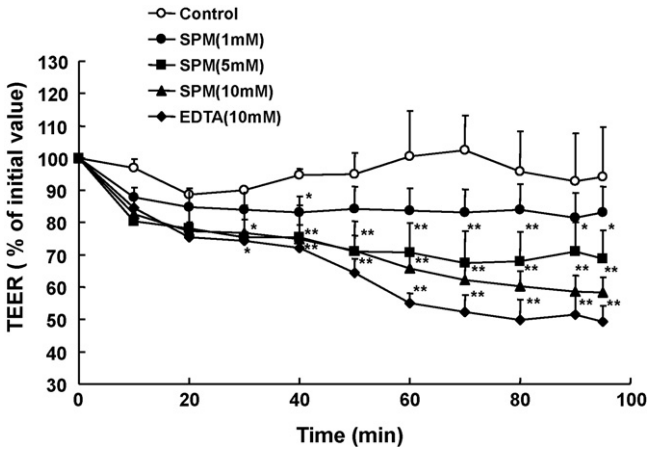

Fig. 7. Effect of spermine (SPM) with different concentrations on the transepithelial electrical resistance (TEER) values of the rat jejunal membranes. Results are expressed as the mean ± S.E. of at least three experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the control. Keys: (◯) control, (●) 1 mM spermine (SPM), (■) 5 mM spermine (SPM), (▲) 10 mM spermine (SPM), (♦) 10 mM EDTA.

4. Discussion

In the present study, we found that the absorption of FD4 from jejunum was enhanced significantly by SPM and SPD and the enhancing ability of SPM was larger than that of SPD, while PUT hardly improved the absorption of FD4 so much. Miyake et al. (2006a) reported that the absorption-enhancing effect of SPM was greater than that of SPD for improving the intestinal absorption of rebamipide. Furthermore, we recently demonstrated that the absorption-enhancing effect of SPM in the lung was greater than that of SPD for improving the pulmonary absorption of FD4 and insulin (He et al., 2007). Therefore, our present results were consistent with these previous findings. The reason for the different absorption-enhancing ability of these polyamines is not clarified at present. However, these polyamines have different numbers of amine groups (Fig. 1) and different cationic charges in their molecules, such difference may affect the absorption-enhancing ability of these polyamines in this study.

We demonstrated that there exist regional differences in the absorption-enhancing effects of these polyamines for improving the intestinal absorption of FD4 in rats. That is, the absorption of FD4 from the jejunum was improved in the presence of 5–10 mM SPM and 10 mM SPD, while the absorption of FD4 from the colon was improved only by 10 mM SPM. The absorption of FD4 co-administrated with SPD and PUT in the colon was almost the same as that in the control. This result was remarkably different from the previous findings that the absorption-enhancing effects of many absorption enhancers in the large intestine were generally much greater than those in the small intestine (Yamamoto et al., 1997). However, we recently found that the absorption-enhancing effects of NO donors in the small intestine was almost same as those in the large intestine for improving the intestinal absorption of insulin in rats (Fetih et al., 2005). Therefore, this finding suggests that there exist some absorption enhancers, which show greater or similar absorption-enhancing effects in the small intestine compared with those in the large intestine. This regional difference in the absorption-enhancing effect between the small and large intestine may be due to the physiological difference in the thickness of mucous layers, tightness and number of the tight junctions and membrane components, although the reason is still unknown.

Our present study also demonstrated that the absorption-enhancing effect of SPM for improving the intestinal absorption of FD4 increased as its concentration increased from 1 to 10 mM. This finding indicated that the absorption-enhancing effect of SPM for improving the intestinal absorption of FD4 was concentration dependent over the range of 1–10 mM. Osman et al. (1998) reported that the effects of SPM on the intestinal permeability to different-sized molecules generally seemed to depend on the intestinal region and on the polyamine concentration. Therefore, our result is consistent with the previous report. On the other hand, Saumon and Martet (2001) reported that SPM produced a large and sustained increase in the permeability of the alveolar barrier to mannitol and in alveolar liquid absorption in isolated rat lungs when they used 10 mM SPM in their studies. In addition, Mendizabal and Naftalin (1992) also examined the effect of 10 mM SPM on water absorption and permeability of polyethylene glycol 4000 in rat descending colon. Therefore, the concentration of SPM used in our study was almost equal to the previous reports. Furthermore, Miyake et al. (2006a) reported that 50 mM SPM caused intestinal mucosal damage such as exfoliation and shrinkage of epithelial cells. Therefore, based on these findings, higher concentrations of SPM (more than 10 mM) may not help to improve the intestinal absorption of drugs from the standpoint of mucosal toxicity.

The important issue of absorption enhancers in clinical use is their potential local toxicity. The membrane damage of these absorption enhancers is usually estimated by measuring the biological markers including protein and LDH released from the intestinal epithelial cells. Generally, protein is an index of membrane damage, because protein is one of the components of biological membrane and is released if the biomembrane was damaged. LDH is the cytosolic enzyme and its presence in the luminal fluid is generally regarded as evidence of cell membrane damage. We also observed the morphological changes of intestinal mucosa in the presence or absence of 10 mM SPM. As shown in Fig. 5, 10 mM SPM did not increase the amount of protein and LDH, although we observed a significant increase in these biological markers in the presence of 3% Triton X-100, as a positive control. Furthermore, we found no significant change in the intestinal membrane with 10 mM SPM by the morphological observation. Miyake et al. (2006a) reported that 10 mM SPM and SPD did not cause any significant change in stomach and duodenum by histopathological studies. Taken these findings together, it was suggested that 10 mM polyamines did not cause any significant membrane damage to the intestinal epithelium.

When these polyamines can be used in clinical application, we should compare their effectiveness and toxicity with the conventional absorption enhancers. Among these conventional absorption enhancers, only sodium caprate is clinically used in a commercial rectal suppository as an absorption enhancer of sodium ampicillin in Japan. However, our previous study

demonstrated that sodium caprate were not so effective to improve the small intestinal absorption of poorly absorbable drugs, although it had a great absorption-enhancing effect in the large intestine (Uchiyama et al., 1996; Yamamoto et al., 1997). Therefore, polyamines would be useful absorption enhancers when they are applied in the small intestine compared with the conventional absorption enhancers including sodium caprate.

The mechanisms whereby the intestinal absorption of poorly absorbable drugs enhanced by SPM were not fully understood. One possible mechanism for improving the intestinal absorption of FD4 by polyamines, especially SPM is that they can loosen the tight junction of the intestinal epithelium, thereby increasing the intestinal absorption of poorly absorbable drugs via a paracellular pathway. Indeed, our present studies indicated that the TEER values of the rat jejunal membranes were gradually decreased in the presence of SPM and this effect was concentration dependent (Fig. 7). Therefore, it may be plausible that these polyamines may increase the intestinal absorption of poorly absorbable drugs via a paracellular pathway by loosening the tight junction of the intestinal epithelium. On the other hand, Mendizabal and Naftalin (1992) supposed that SPM could cause a breakdown in the colonic barrier function by activating matrix metalloproteinases in the submucosa. Osman et al. (1998) demonstrated that the effect of SPM on drug permeability varied according to the intestinal region studied and the colon was less permeable than the small intestine, which could be explained by the structural differences between the large and small intestines. Because of their polycationic characters, the polyamines may interact with negatively charged structures and membrane components of the cell or affect membrane-bound enzymes, which in turn may result in alteration of transport properties. Though the mechanism of polyamines to improve the absorption is still unknown, the interaction with acidic molecules such as acidic mucopolysaccharides on the surface of membrane and phosphatidylserine might be important because SPM is well known to interact with DNA, RNA and ATP, etc., and the binding constants of SPM for macromolecules and ATP are larger than those of SPD (Miyake et al., 2006a).

In conclusion, these results suggest that SPM at 10 mM increased the absorption of FD4 after intestinal administration and did not cause any significant membrane toxicity. These findings indicated that it would be a promising safe absorption enhancer applied for improving the intestinal absorption of poorly absorbable drugs.

Acknowledgements

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