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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.