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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 anesthesized 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

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