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Effects of 2 Polyoxyethylene Alkyl Ethers on the Function of Intestinal P-glycoprotein and Their Inhibitory Mechanisms

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ABSTRACT

The purpose of this study was to investigate the effects of polyoxyethylene 10-oleyl ether and polyoxyethylene 9-lauryl ether, 2 polyoxyethylene alkyl ethers, on the transport and absorption of 2 P-glycoprotein (P-gp) substrates, quinidine and prednisolone, across the intestinal membrane and to elucidate the inhibitory mechanisms of intestinal P-gp by these polyoxyethylene alkyl ethers. For *in vitro* studies, we used a diffusion chamber method and the Caco-2 cell model. An *in situ* closed-loop method was used for *in vivo* study. The 2 polyoxyethylene alkyl ethers, nonionic surfactants, increased the intestinal absorptive transport of quinidine and prednisolone in the diffusion chamber studies, and absorptive permeability was enhanced in the *in vitro* Caco-2 cell study. Furthermore, these surfactants enhanced the rat intestinal absorption of prednisolone, and we observed no intestinal membrane damage in the presence of these surfactants. Furthermore, these surfactants increased membrane fluidity in intestinal brush border membranes and inhibited P-gp ATPase activity. For *in vitro* and *in vivo* studies, these surfactants enhanced the intestinal absorption of quinidine and prednisolone, 2 P-gp substrates. The alteration in intestinal membrane fluidity and the inhibition of P-gp ATPase activity by these 2 polyoxyethylene alkyl ethers may be confirmed as mechanisms of P-gp inhibition.

Introduction

One major reason for failure of cancer therapy is multidrug resistance (MDR). MDR is inherent in some types of tumors, whereas it is usually acquired in others. MDR decreases the intracellular concentration of anticancer drugs.[1,2] The overexpression of membrane transporter proteins, including P-glycoprotein (P-gp) and the multidrug resistance proteins which belong to the ATP-binding cassette transporter family, is confirmed as one of the mechanisms of MDR.[3]

P-gp, which is an ATP-dependent membrane glycoprotein of 170 kDa, is expressed not only in MDR tumor cells, such as adenocarcinoma cells and leukemia cells,[4,5] but also in various normal tissues, including brain, the intestinal brush border membranes, liver, kidney, testes, and adrenal glands.[6,7] In the intestine, P-gp regulates the intestinal absorption of drugs and other xenobiotics, thus affecting the oral bioavailability of drugs.[8] In order to overcome the activity of the P-gp efflux transporter and improve the bioavailability of anticancer drugs or P-gp substrates, many pharmacological modulators have been found to inhibit the function of P-gp efflux transport.[9] However, P-gp modulators such as verapamil and cyclosporin A have marked pharmacological activities. Moreover, these modulators not only inhibit the activity of P-gp but also reduce the function of breast cancer resistance protein and multidrug resistance protein 1.[10,11] The competitive or noncompetitive blocking of the binding of a substrate to the P-gp drug-binding domain was confirmed as the primary mechanisms of inhibition of P-gp by these inhibitors.[12] Therefore, P-gp modulators which have lower pharmacological activities are important to discover.

Other pharmaceutical excipients, including fatty acids and bile salts such as Labrasol and sodium deoxycholate, could inhibit the function of P-gp, thereby enhancing the absorption of drugs such as P-gp substrates, in *in vitro* and *in vivo* studies.[13] In recent years, many studies have focused on the effects of the nonionic surfactants such as Pluronic 85, Tween 80, and polyethylene glycols (PEGs) on intracellular accumulation and intestinal absorption of P-gp substrates,[14-16] which have been determined in the everted

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gut sacs of rat jejunum and ileum and the human colon adenocarcinoma (Caco-2) cell line.

Polyoxyethylene alkyl ethers, which belong to the category of nonionic surfactants, are widely used as emulsifying, solubilizing, and wetting agents in topical and cosmetic formulations to improve the dissolution and absorption of poorly soluble drugs.[17,18] Yu et al.[19] demonstrated that polyoxyethylene glycol dodecyl ether effectively enhanced the absorptive direction of bis-(12)-hupyridone (B12H) in Caco-2 cells, and Lo[17] found that polyoxyethylene lauryl ether significantly increased the intestinal absorption of epirubicin in rats. Another study indicated that both doxorubicin and paclitaxel-loaded lipid-based nanoparticles containing polyoxyethylene 20-stearyl ether were able to overcome P-gp-mediated drug resistance.[20] However, these studies did not examine the effects of various polyoxyethylene alkyl ethers on the intestinal transport and absorption of P-gp substrates in both *in vitro* and *in vivo* studies. In our previous reports, polyoxyethylene alkyl ethers increased the intestinal transport and absorption of rhodamine 123 in both *in vitro* and *in vivo* studies.[21] In that study, polyoxyethylene 10-oleyl ether (Brij97) and polyoxyethylene 9-lauryl ether (BL-9EX) with the structure of the oleyl ether and lauryl ether had greater effects on the absorption of rhodamine 123 than other polyoxyethylene alkyl ethers without these structures in the intestine. However, it has been reported that rhodamine 123, a typical P-gp substrate, might be primarily transported by the paracellular pathway rather than P-gp-mediated efflux absorptive transport.[22] Therefore, effects of polyoxyethylene alkyl ethers and other P-gp modulators on the intestinal absorptive transport of rhodamine 123 could not be observed clearly. On the other hand, quinidine, a class IA antiarrhythmic drug, is a well-known P-gp substrate as well as a P-gp inhibitor,[23] and prednisolone, which is effective for the treatment of rheumatoid arthritis and ulcerative colitis, is used as a P-gp substrate.[24] In the present study, therefore, we evaluated the effects of polyoxyethylene 10-oleyl ether and polyoxyethylene 9-lauryl ether on the intestinal absorption of quinidine and prednisolone via *in vitro* and *in vivo* studies.

On the other hand, little is known about the P-gp inhibitory mechanisms of nonionic surfactants including polyoxyethylene alkyl ethers. Unlike the competitive inhibition exhibited by traditional inhibitors, the inhibitory actions of nonionic surfactants on P-gp function may be attributed to downregulation of P-gp expression, inhibition of P-gp ATPase, depletion of intracellular ATP, or alteration in membrane fluidity.[25-29] Recently, Tang et al.[18] demonstrated that polyoxyethylene 20-stearyl ether and polyoxyethylene 10-oleyl ether reduced verapamil-induced P-gp ATPase activity and intracellular ATP levels in resistant human lung cancer cells. However, this study did not evaluate the effects of polyoxyethylene alkyl ethers on membrane fluidity, although many nonionic surfactants that inhibit P-gp are known to affect membrane fluidity. Thus, it is important to evaluate the effects of polyoxyethylene alkyl ethers on membrane fluidity if they affect the function of P-gp.

In the present study, the effects of polyoxyethylene 10-oleyl ether and polyoxyethylene 9-lauryl ether on the intestinal absorption of 2 P-gp substrates, quinidine and prednisolone, were investigated in *in vitro* and *in vivo* studies. In addition, to determine whether these 2 polyoxyethylene alkyl ethers could enhance intestinal absorptive permeability of P-gp substrates via intestinal membrane damage, intestinal membrane damage was examined by surveying the release of protein and the activity of lactate dehydrogenase (LDH). Finally, we evaluated the inhibitory mechanisms of these 2 polyoxyethylene alkyl ethers on the P-gp efflux transport function by measuring P-gp ATPase activity and membrane fluidity.

Materials and Methods

Materials

Quinidine, prednisolone, bovine serum albumin (BSA), and LDH-Cytotoxic Test Wako were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polyoxyethylene 10-oleyl ether and polyoxyethylene 9-lauryl ether were obtained from Nikko Chemicals Company Ltd. (Osaka, Japan). For cell culture, MEM nonessential amino acid solution, fetal bovine serum, and Dulbecco's modified Eagle's medium were purchased from Life Technologies Corporation (Carlsbad, CA). Trypsin-EDTA, 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES), and antibiotic-antimycotic mixed stock solution (10,000 U/mL penicillin, 10 mg/mL streptomycin, 25 mg/mL amphotericin B, 0.85% saline) were prepared by Dojindo Laboratories (Kumamoto, Japan). 5(6)-Carboxyfluorescein (CF) was purchased from Eastman Kodak Company (Rochester, NY). 1,6-Diphenyl-1,3,5-hexatriene (DPH), cyclosporin A, and Hank's balanced salt were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO). Dansyl chloride (DNS-CL) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene-ρ-toluenesulfonate (Tma-DPH) were supplied by Santa Cruz Biotechnology Inc. (Dallas, TX). Human MDR1 membranes and ATPase assay reagent kit were supplied by GenoMembrane Inc. (Kanagawa, Japan). All other reagents were of analytical grade.

Preparation of Drug Solutions

Quinidine, prednisolone, and CF were dissolved in HEPES buffer (pH 7.4) containing 25 mM HEPES, 5.4 mM KCl, 140 mM NaCl, and 5 mM glucose. Excipients, including 0.01%-0.05% (v/v) polyoxyethylene 10-oleyl ether and polyoxyethylene 9-lauryl ether and 20 μM cyclosporin A, were added to the drug solution. In a diffusion chamber experiment, 0.1 mM quinidine, 0.2 mM prednisolone, and 10 μM CF were used, whereas in the Caco-2 cell experiment, 10 μM quinidine was used. In the *in vivo* absorption study, 5 mg/kg of prednisolone or quinidine was used, respectively. The concentrations of 2 polyoxyethylene alkyl ethers and these drugs were selected in line with the previous studies.[21]

Cell Culture

Caco-2 cells with passage 48-54 (Dainippon Sumitomo Pharma Company, Ltd.) were grown in 1% antibiotic-antimycotic mixed stock solution, 100 μM MEM nonessential amino acid solution, and Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The cells were seeded in density 1×10^5 on polycarbonate inserts (Transwells, 12 mm in diameter, 0.4-μm pores; Corning Inc., New York, NY) and cultured in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Every 2 days, the growth medium was changed. The transepithelial electrical resistance (TEER) was measured at 37°C with a Millicell®-ERS Epithelial Volt-Ohm Meter (Millipore, Billerica, MA) to evaluate the integrity of the cell monolayers. After 21 days, the TEER values of Caco-2 monolayers were above 500 $\Omega \cdot cm^2$ that were employed in transport experiments.

Transport of Drugs Across the Intestinal Membrane by an In Vitro *Diffusion Chamber System*

Transport of drugs across the intestinal membrane was examined by a diffusion chamber system (Corning Coster Corporation).[16,21] Male Wistar rats (230-260 g) were anesthetized with Somnopentyl® (sodium pentobarbital, 32 mg/kg body weight