Effects of Various Absorption Enhancers on the Intestinal Absorption of Water Soluble Drugs by in Vitro Ussing Chamber Method: Correlation with an in Situ Absorption Experiment

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The effect of absorption enhancers on the small and large intestinal absorption of drug in rats was examined using an in vitro modified Ussing chamber method, and the results were compared with those from an in situ absorption experiment. Phenol red was chosen as a model drug, while the absorption enhancers used were sodium glycocholate (Na-GC), sodium taurocholate (Na-TC), sodium deoxycholate (Na-DC), EDTA, sodium salicylate (Na-Sal), sodium caprate (Na-Cap), diethyl maleate (DEM) and N-lauryl-β-D-maltopyranoside (LM), all used at a concentration of 20 mM. This modified Ussing chamber method showed that Na-DC and EDTA were the most effective absorption enhancers in the small intestine, whereas Na-DC, EDTA and LM were the most effective absorption enhancers in the large intestine. A good correlation exists between the area under the curve (AUC) (in situ loop model) and the cumulative amount of phenol red absorbed (in vitro modified Ussing chamber method). These results indicated that the in vitro modified Ussing chamber method can be used to evaluate the effects of various absorption enhancers in the intestine.

Key words absorption enhancer; phenol red; intestinal permeability; in vitro modified Ussing chamber method

The use of absorption enhancers has been shown to improve the absorption of poorly absorbable drugs including peptide and protein drugs.1,2 These absorption enhancers include surfactants, bile salts, chelating agents and fatty acids. It has also been shown that these enhancers enhanced the intestinal absorption of poorly absorbable drugs by various mechanisms.3,4 We examined the effects of these absorption enhancers on drug absorption from the small and large intestine by an in situ loop model.2,3 In vitro absorption experiments are very easy to design and manipulate, and these methods have frequently been used to identify the mechanisms of drug absorption from the gastrointestinal tract. However, the effects of various absorption enhancers have rarely been examined systematically by in vitro absorption experiments.

Phenol red was chosen as a model water-soluble compound in this study and the effects of various absorption enhancers on its absorption across the intestinal membrane were investigated by an in vitro modified Ussing chamber method. The absorption enhancers used were sodium glycocholate (Na-GC), sodium taurocholate (Na-TC), sodium deoxycholate (Na-DC), bile salts; sodium salicylate (Na-Sal) and EDTA, chelating agents; sodium caprate (Na-Cap), a medium-chain fatty acid; diethyl maleate (DEM), a protein modifier; and N-lauryl-β-D-maltopyranoside (LM), a medium-chain alkyI saccharide, all used at a concentration of 20 mM. We also compared results with those obtained from the in situ loop method.2,3

MATERIALS AND METHODS

Chemicals Na-TC was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A). Na-DC was obtained from Wako Pure Chemical Industries Co. (Osaka, Japan). DEM was obtained from Kanto Chemical Co. (Tokyo, Japan). Na-Sal and EDTA were obtained from Nacalai Tesque Inc. (Kyoto, Japan). LM was donated by Japan Fine Chemical Co. (Osaka, Japan). Na-Cap was purchased from Tokyo Kasei Industries Co. (Tokyo, Japan). All other chemicals were of reagent grade.

Animal Experiments Absorption experiments were performed on 200—300 g male Wistar albino rats (Japan SLC, Inc., Hamamatsu, Japan) by the in situ closed loop method. In the small intestine absorption experiments the ileoceleal junction was ligated and drug solution warmed at 37°C (2.5 mg/ml of phenol red in phosphate-buffered saline in the presence of 20 mM of various absorption enhancers) was injected into the small intestinal loop. Conversely, the large intestinal loop was prepared by cannulation with a 3 cm section of silicone tubing (3 mm i.d., 5 mm o.d.) at the proximal and distal ends of the large intestine. Phenol red solution (2 ml), at a concentration of 2.5 mg/ml, was introduced into the intestinal loop, which was closed by clipping the tubing with forceps. Blood samples were taken from the jugular vein at predetermined times for up to 240 min, and the plasma concentrations of phenol red were determined spectrophotometrically. Each plasma sample (200 µl) was alkalized with 3 ml of 1 M NaOH and determined spectrophotometrically at 560 nm.

In Vitro Transport Experiments The transport of phenol red across the small and large intestinal membranes was evaluated by the method of Yamamoto et al.5) Male Wistar albino rats weighing 200—250 g were used for these transport experiments. The intestine of each rat was excised, and 13 cm of the top of the small intestine and the final 10 cm portion of the intestine were cut away. The residual intestine was designated as the small intestine, and its central portion was used. The first 3 cm of the large intestine was removed and the next 5 cm was used as the large intestine. The underlying muscularis was removed.

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prior to mounting in a modified Ussing chamber, in which a surface area of 0.2826 cm$^2$ was exposed. Two or one-half milliliters of glutathione bicarbonate Ringer’s (GBR) solution, pre-adjusted to pH 7.4, was added to the reservoir bathing the serosal side. An equal volume of 2.5 mg/ml phenol red solution in the presence of 20 mM of the various absorption enhancers was added to the mucosal side. Each side of the chamber was aerated with 95% O$_2$ and 5% CO$_2$ gas in order to mix each solution and to maintain the viability of the membrane. The temperature was maintained at 37°C during the experiment. At predetermined times up until 3 h, 200 μl of solution was sampled from the serosal side, and this was immediately replaced with an equal volume of buffer solution. The concentration of phenol red in these samples was determined spectrophotometrically. Each sample (200 μl) was alkalinized with 3 ml of 1 M NaOH and determined spectrophotometrically at 560 nm.

The apparent permeability coefficient ($P_{app}$) of each compound was calculated from the linear portion of a plot of penetrant accumulated versus time.

The viability of intestinal membrane during the test period was monitored by measuring the transport of trypan blue dye. There was minimal transport of dye during the incubation.

Statistical Data Analyses Results are expressed as the mean ± S.E. Statistical analyses were performed by the Student’s t-test.

RESULTS

Penetration of Phenol Red across the Intestinal Membrane in the Presence of Various Absorption Enhancers

An in vitro modified Ussing chamber was used to examine the transport of phenol red across the intestinal membrane in the presence or absence of various absorption enhancers. Table 1 shows % of dose and the $P_{app}$ in the small intestinal membrane. Neither Na-Cap nor LM enhanced the permeability of phenol red. In contrast, remarkable increases in permeability were observed in the presence of 20 mM Na-DC and EDTA compared with the controls, whereas only slight increases were observed in the presence of Na-TC, Na-GC, Na-Sal and DEM. The $P_{app}$ values of phenol red across the small intestinal membrane in the presence of Na-DC and EDTA were 6.8 and 4.0 times higher than in the controls.

Table 2 shows % of dose and the $P_{app}$ in the large intestinal membrane. DEM did not enhance the permeability of phenol red, while the absorption was increased remarkably in the presence of 20 mM Na-DC, EDTA or LM compared with controls, and slight increases in permeability were observed in the presence of Na-GC, Na-TC, Na-Sal or Na-Cap. The $P_{app}$ values of phenol red across the large intestinal membrane in the presence of Na-DC, EDTA or LM were 16, 11 or 11 times higher than in the controls. The effects of absorption enhancers were thus more significant in the large intestine than in the small intestine. Of the three bile salts used in this study (Na-DC, Na-TC and Na-GC), Na-DC most significantly enhanced the permeability of phenol red. The dihydroxy bile salt Na-DC was a more effective absorption enhancer than the more polar trihydroxy bile salts Na-TC and Na-GC because of their hydrophobicity.

Na-DC and EDTA significantly enhanced the intestinal permeability of phenol red in this study. Yamamoto et al. $^5$ reported that Na-DC and EDTA caused remarkable damage to the small intestinal membrane at the same concentration as used in this study. Consequently, these absorption enhancers at these concentrations may damage the intestinal membranes, thereby increasing the intestinal absorption of phenol red. Na-Sal and DEM, however, exhibited little or no significant absorption enhancement in the intestinal membranes, and perhaps must be used at higher concentrations to enhance the absorption of a drug.

The promotional effects of absorption enhancers used in this study were more effective in the large intestine than in the small intestine. Muranishi$^3$ suggested that the decreasing rank order of the sensitivity of the site where enhancers were administered is rectum $>$ colon $>$ small intestine $>$ stomach $>$ skin. This report also suggested that the administration site can affect the effectiveness of absorption enhancers. Our results were consistent with these findings.

Correlation between the Area under the Phenol Red Absorption Curve (AUC) (in Situ) and the Cumulative Amount (in Vitro) of Phenol Red Transported across the Intestinal Membrane

As seen in Fig. 1, a good correlation was
obtained between the area under the phenol red absorption curve (AUC) \textit{(in situ)} and the cumulative amount \textit{(in vitro)} in the small and large intestinal membrane. The correlation coefficient was 0.951 for the small intestine and 0.941 for the large intestine. The \textit{in situ} loop method was found to be useful for evaluating the effectiveness of various absorption enhancers in the intestine. Our results indicate that an \textit{in vitro} modified Ussing chamber method can also be beneficial for evaluating the effects of various absorption enhancers in the intestine.

REFERENCES