Effectiveness and Toxicity Screening of Various Absorption Enhancers in the Large Intestine: Intestinal Absorption of Phenol Red and Protein and Phospholipid Release from the Intestinal Membrane

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The effectiveness and local toxicity of absorption enhancers on the absorption of phenol red (PR) from the large intestine of rats were examined using an *in situ* loop method. The absorption enhancers used in this study were sodium glycocholate (GC-Na), sodium taurocholate (TC-Na), sodium deoxycholate (DC-Na), EDTA, sodium salicylate (Sal-Na), sodium caprate (Cap-Na), diethyl maleate (DM), *N*-lauryl-β-D-maltopyranoside (LM) and mixed micelles (MM), all used at a concentration of 20 mM. Local toxicity was also investigated by assessing protein and phospholipid release as biological markers.

DC-Na and MM were the most effective absorption enhancers, but they caused considerable release of proteins and phospholipids. GC-Na, TC-Na and LM, which caused little or only slight membrane damage, promoted PR absorption. Sal-Na, DM and EDTA did not enhance PR absorption. Overall, a correlation exists between the area under the curve of PR and protein and phospholipid release in the presence of absorption enhancers. However, GC-Na, TC-Na and LM promoted the absorption of PR with low toxicity. From these results, we concluded that GC-Na, TC-Na and LM are effective absorption enhancers which have low levels of toxicity at a concentration of 20 mM.

Key words intestinal absorption; absorption enhancers; phenol red; large intestine; local toxicity

The absorption of polar drugs is frequently limited by their poor intestinal permeability. A large number of absorption enhancers including surfactants, bile salts, chelating agents and fatty acids have been used to enhance the intestinal absorption of polar drugs.[1,2] But some of these adjuvants cause damage and irritate the intestinal mucosal membrane. Many studies have investigated their toxicity by means of hemolysis, protein release and morphological observation,[3-5] however, few have analyzed their effectiveness and toxicity at the same time. We earlier investigated the effects of absorption enhancers and their toxicity on the absorption of phenol red (PR) in the small intestine.[6]

Phenol red, which is poorly absorbed but is stable in the gastrointestinal tract, was chosen as a model polar drug in the present study and we compared the effectiveness and toxicity of absorption enhancers in a single experiment in an effort to rank their usefulness. The absorption enhancers used in this study were sodium glycocholate (GC-Na), sodium taurocholate (TC-Na), sodium deoxycholate (DC-Na), EDTA, sodium salicylate (Sal-Na), sodium caprate (Cap-Na), diethyl maleate (DM), *N*-lauryl-β-D-maltopyranoside (LM) and linoleic acid (LA)-HCO60 mixed micelle (MM), all used at a concentration of 20 mM. This concentration of absorption enhancers was selected to compare their promoting effects under the same conditions.

Morphological observation and hemolysis have been widely used to assess local toxicity of absorption enhancers. In some cases, the results obtained by hemolysis may not be extrapolated directly to the mucosal cells exposed to the enhancers.[2] Both these methods are not quantitatively to assess local toxicity to mucosal membrane. On the other hand, biological markers such as protein and phospholipid release are suitable for quantitative analysis. Because of this, we also examined protein and phospholipid release as an index of local toxicity in this experiment to select the effective and non-toxic absorption enhancers.

MATERIALS AND METHODS

Chemicals PR and DC-Na were obtained from Wako Pure Chemical Industries Co. (Osaka, Japan). GC-Na and TC-Na were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). LA of high purity grade (>99%) was provided by Nippon Oil & Fats (Tokyo, Japan). DM was purchased from Kanto Chemical Co. (Tokyo, Japan). Sal-Na and EDTA were obtained from Nacalai Tesque Inc. (Kyoto, Japan). HCO60 was donated by Nikko Chemical Co. (Tokyo, Japan). LM was supplied by Japan Fine Chemical Co. (Osaka, Japan). Cap-Na was purchased from Tokyo Kasei Industries Co. (Tokyo, Japan). All other chemicals were of reagent grade or better.

Animal Experiments Absorption experiments were performed using an *in situ* closed loop method.[7] Briefly, male Wistar albino rats (Japan SLC, Inc., Hamamatsu, Japan) weighing 200—300 g were used.[6] The ileocaecal junction was ligated and 2 ml of the drug solution (2.5 mg/ml of PR in phosphate-buffered saline) was injected into the large intestinal loop. Blood samples were taken from the jugular vein at predetermined times for up to 240 min, and the plasma concentration of PR was determined on a spectrophotometer (Hitachi model U-2000, Tokyo, Japan). The peak concentration (C_{max}) and time to reach C_{max} (T_{max}) were determined directly from the concentration–time profiles. The area under the plasma concentration–time curve after the large intestinal administration was calculated by the trapezoidal method from time zero to the final sampling time.

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Assessment of Membrane Damage of the Large Intestine by Absorption Enhancers To evaluate the membrane damage, the release of proteins and phospholipids from the large intestinal membrane was measured as described.[6] The amount of protein released from the intestinal membrane was determined by the method of Lowry *et al.* using bovine serum albumin as the standard.[8] In the case of TC-Na, Sal-Na and EDTA, the amount of protein release was determined by a modified method of Bensadoun and Weinstein to avoid interfering with the assay.[9] The amount of phospholipid released from the intestinal membrane was assayed with a Nescauto PL kit (Nippon Shoji Co., Japan). Absorption enhancers used in this experiment did not interfere with the phospholipid assay.

Statistical Analyses Results are expressed as the mean ± S.E. and statistical significance was assessed by the Student's *t*-test.

RESULTS AND DISCUSSION

Effects of Absorption Enhancers on the Absorption of PR from the Large Intestine and their Local Toxicity The time course of PR concentration in the plasma after its administration into the large intestine with or without the various absorption enhancers is shown in Fig. 1. Table 1 shows the maximum PR levels, time to the maximum PR level, the *AUC*, protein release and phospholipid release. Sal-Na, DM and EDTA had only slight or no promotional effect on the absorption of PR. Conversely, plasma PR levels increased significantly after the coadministration of DC-Na or MM into the large intestine compared to the control (no additive), whereas only a slight increase was observed with GC-Na, TC-Na, LM or Cap-Na.

DC-Na caused a significant release of proteins compared with the control. A slight release of protein was observed in the presence of GC-Na, TC-Na, Cap-Na and MM. DM, EDTA, Sal-Na and LM had similar protein levels as in the controls. A significant release of phospholipids was observed in the presence of DC-Na and MM. Cap-Na, EDTA and LM caused minor release of phospholipids, while GC-Na, TC-Na, Sal-Na and DM had little or no effect and had values similar to the control. DC-Na caused the most release of proteins and phospholipids of all the absorption enhancers used. A minor release was observed in the case of GC-Na, TC-Na and LM.

In the bile salts, DC-Na significantly enhanced the absorption of PR from the large intestine and was the most effective absorption enhancer used in this experiment. However, it caused a significant release of proteins and phospholipids into the large intestinal lumen, which limits its clinical use. The absorption of PR from the small and large intestine was enhanced in decreasing rank order by DC-Na > GC-Na > TC-Na. These results are consistent with those from previous reports on nasal and intestinal absorption.[3,10-13]

The chelating agents, EDTA and Sal-Na, did not have a significant effect on PR absorption. Their absorption enhancing ratios derived from the *AUC* in the treated and control intestinal loops were 1.65 and 0.74, respectively. However, EDTA and Sal-Na promoted a 2.24-fold and 0.68-fold increase in the absorption of PR compared to the control values in the small intestine (data not shown). This result shows that EDTA is a more effective absorption enhancer in the small intestine than in the large intestine. Sal-Na did not significantly promote the absorption of PR from the rat small intestine.[11] Aungst and Rogers reported that EDTA and Sal-Na remarkably enhanced insulin absorption across the rectal membrane in rats.[14] Morishita *et al.* reported that EDTA promoted the absorption of insulin more remarkably in the colon than in the small intestine.[12] Our results do not agree with those results, although at present we cannot explain this discrepancy. Ishizawa *et al.*, however, reported that the enhancing effects of EDTA on the absorption of fosfomycin were higher in the small intestine than in the large intestine in the *in vivo* experiments, which concurs with our results.[15]

MM have a significant effect on the absorption of PR across the large intestine and were 9.0 fold more effective there than in the small intestine (data not shown). We previously showed that MM enhanced the absorption of streptomycin in the large intestine, and to a lesser degree in the small intestine, which is supported by these our results.[1] MM only caused minor protein release similar to the control levels, however, a significant release of phospholipids was observed in the large intestinal lumen.

Little or no absorption enhancing effect in the large intestine was observed in the presence of DM, and this substance also failed to enhance PR absorption in the small intestine. Shiga *et al.* reported that DM did not enhance the absorption of PR from the colon.[16] A concentration of 20 mM may not be enough for DM to enhance PR absorption. This might partially explain our results. It is clear that DM is not a suitable absorption enhancer to PR at a concentration of 20 mM in the small and large intestine.

LM, an alkylsaccharide, was recently found to lower surface tension and to have an absorption enhancing activity in the gastrointestinal tract. It enhanced 3.7 fold the absorption of PR in the large intestine but had no such effect in the small intestine.[6] We also showed that rectal absorption of carboxyfluorescein was improved approximately 10 fold *vs.* the control by the coadministration of 10 mM LM. The enhancing effect of LM is reversible and no histological change was observed in rectal mucosa,[17] which concurs with the present findings. These results indicate LM has a suitable adjuvant with good absorption promoting effects and low toxicity.

Correlation between Absorption Enhancing Effect and Membrane Damaging Effect The relationship between protein release and the *AUC* value of PR in the presence of various absorption enhancers is shown in Fig. 2A. Overall, there exists a correlation between these parameters, however, LM seems the most suitable enhancer due to its low toxicity and good absorption enhancing effect. Figure 2B also shows the correlation between the *AUC* value of PR and the release of phospholipids into the luminal fluid. The *AUC* correlates well with phospholipid release. GC-Na, TC-Na and LM promoted the absorption of PR with low toxicity as shown. These data

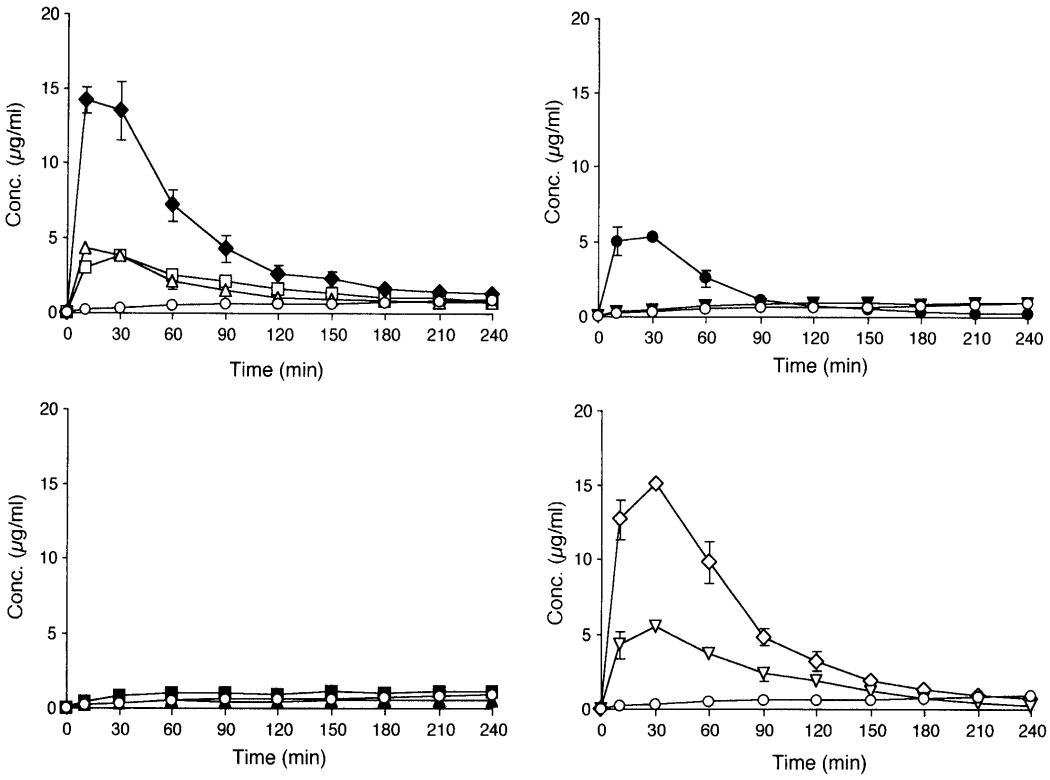

Fig. 1. The Effects of Various Absorption Enhancers (20 mM) on Plasma Concentrations of PR Following Its Administration into the Large Intestine

Results are expressed as the mean ± S.E. of 4 or 5 experiments. Control (○); GC-Na (△); TC-Na (□); DC-Na (◆); Cap-Na (●); DM (▼); EDTA (■); Sal-Na (▲); LM (▽); MM (◇).

Table 1. Effects of Absorption Enhancers on the Absorption of Phenol Red and Their Local Toxicity in the Large Intestine

	C_{max} (μg/ml)	T_{max} (min)	AUC (μg/ml·min)	Protein release (mg)	Phospholipid release (mg)
Control	0.95±0.10	217.5±14.4	136.6± 11.6	1.25± 0.32	0.07±0.01
GC-Na	4.28±0.28	10.0± 0.0	381.2± 47.6**	3.98± 0.82*	0.12±0.02$^{n.s.}$
TC-Na	3.83±0.32	30.0± 0.0	455.8± 14.2***	7.43± 1.96*	0.14±0.04$^{n.s.}$
DC-Na	15.26±1.43	15.0± 5.0	1283.2±114.0***	56.56±16.20*	0.46±0.12*
EDTA	1.30±0.06	150.0±32.4	225.4± 2.4***	4.75± 1.43$^{n.s.}$	0.18±0.01***
Sal-Na	0.57±0.06	60.0± 0.0	100.5± 11.6$^{n.s.}$	2.07± 0.59$^{n.s.}$	0.14±0.04$^{n.s.}$
Cap-Na	5.85±0.43	20.0± 5.8	369.5± 7.8***	7.82± 1.26**	0.17±0.01***
DM	1.07±0.29	120.0±27.4	180.5± 36.6$^{n.s.}$	2.41± 0.48$^{n.s.}$	0.10±0.01$^{n.s.}$
LM	5.52±0.30	30.0± 0.0	509.0± 30.3***	2.70± 0.78$^{n.s.}$	0.14±0.01**
MM	15.28±0.53	25.0± 5.0	1230.8± 88.6***	9.45± 1.36**	0.44±0.04***

*** $p<0.001$, ** $p<0.01$, * $p<0.05$, n.s., not significantly different, compared with the control. Each value represents the mean ± S.E. of 4 experiments.

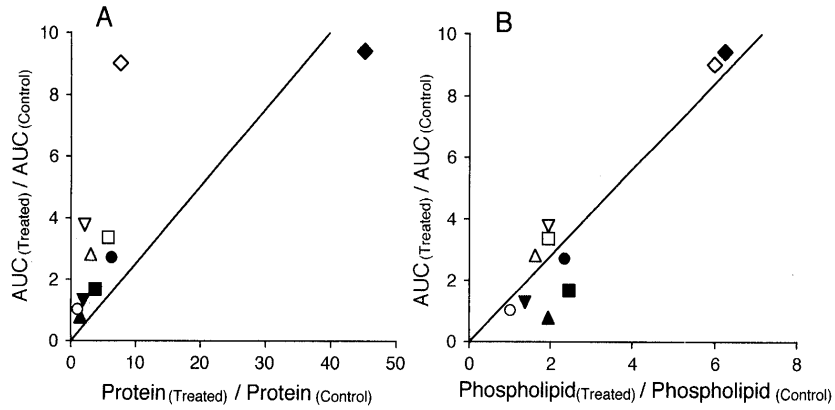

Fig. 2. Correlation between AUC and Protein (A), Phospholipid (B) Release in the Absence or Presence of Various Absorption Enhancers (20 mM)

Results are expressed as the mean ± S.E. of 4 or 5 experiments. Control (○); GC-Na (△); TC-Na (□); DC-Na (◆); Cap-Na (●); DM (▼); EDTA (■); Sal-Na (▲); LM (▽); MM (◇).

suggest that intestinal damage may occur in the presence of some absorption enhancers. We found that DC-Na and MM cause significant release of these biological markers. As shown in Figs. 2A and 2B, The *AUC* of PR is correlated with intestinal damage caused by these absorption enhancers, however, GC-Na, TC-Na and LM had comparatively good absorption promoting effects and low toxicity. They caused phospholipid and protein release to levels similar to control.

In summary, the effectiveness of various absorption enhancers and their local toxicity in the large intestine was investigated by an *in situ* loop method. A good correlation exists between the *AUC* of PR and the release of biological markers. However, GC-Na, TC-Na and LM effectively enhanced the intestinal absorption of PR with low levels of toxicity at a concentration of 20 mM. It can be concluded that these are thus effective in enhancing the absorption of drugs from the large intestine.

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