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Enhancement of jejunal and colonic absorption of fosfomycin by promoters in the rat

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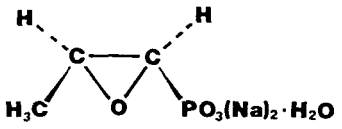
A means of enhancing absorption of the antibiotic, fosfomycin, has been investigated using promoters in rat jejunum and colon. Polyoxyethylene lauryl ether (BL-9EX), saponin, the sodium salts of fatty acids and mixed micelles were effective at 1% in increasing fosfomycin absorption. Of the sodium salts of saturated medium-chain fatty acids examined, the strength of this effect was in the order caprate > laurate > caprylate. Mixed micelles, consisting of fusogenic lipids and sodium taurocholate, enhanced fosfomycin absorption independently of the degree of unsaturation of the lipids; their effectiveness far exceeded that of sodium taurocholate alone. The action of these promoters was more evident in the colon than in the jejunum, except for the sodium salts of bile acids and disodium ethylenediaminetetraacetate (EDTA-2Na). The effects of glycocholate and taurocholate were essentially the same at both absorption sites, but that of EDTA-2Na was much greater in the jejunum than the colon. Improved fosfomycin absorption was observed at more than 0·5% sodium caprate concentrations in both the jejunum and colon. BL-9EX was effective at 0·1% in the jejunum or at 0·05% in the colon. The effectiveness at these low concentrations demonstrates the practicality of promoters for improving fosfomycin absorption with only minor membrane damage, especially in the colon.

Fosfomycin, (−)-(1*R*,2*R*)-(1,2-epoxypropyl)phosphonic acid, is a water-soluble and structurally simple antibiotic (I) that inhibits the initial stage of the synthesis of cell walls such as those of *Pseudomonas aeruginosa*, slime mould, *Staphylococcus* and *Escherichia coli* (Kahan et al 1974). Fosfomycin can be orally administered but only about 25–36% is absorbed by man (Hayakawa et al 1975; Kawabata et al 1975).



I

Structure of fosfomycin sodium.

Recently, much effort has been directed to improving the bioavailability of water-soluble and poorly absorbed drugs, e.g. greater rectal absorption of β-lactam antibiotics by enamine derivatives (Murakami et al 1981), salicylate (Nishihata et al 1982), diethyl maleate (DEM) (Nishihata et al 1984a), phenothiazine derivatives (Fix et al 1984), fatty acids (Nishimura et al 1985) and medium chain triglycerides (Sekine et al 1984) has been achieved.

We have investigated a means of enhancing fosfomycin absorption using various surfactants, chelating agents and other membrane-perturbing agents as promoters in rats. The absorption site specificity of the promoting action and the promotion mechanisms are discussed and a comparison is made of the jejunal and colonic absorption of fosfomycin.

MATERIALS AND METHODS

Materials

The disodium salts of fosfomycin were obtained from Meiji Seika Kaisha (Tokyo, Japan). Diethyl maleate (DEM), sodium salicylate, disodium salts of ethylenediaminetetraacetic acid (EDTA-2Na) and saponin were from Wako Pure Chemical Industries Ltd (Osaka, Japan). Sodium taurocholate, sodium glycocholate, sodium oleate, sodium linoleate and linolenic acid were purchased from Sigma Chemical Co. (St Louis, MO, USA). Sodium caprylate, sodium caprate and sodium laurate were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Sodium 5-methoxysalicylate and polyoxyethylene lauryl ether (BL-9EX) were kindly provided by Sankyo Co. Ltd (Tokyo, Japan) and Nikko Chemical Co.

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Ltd (Tokyo, Japan), respectively. Other reagents were of analytical grade or better.

Preparation of the drug solutions

Two mg (potency) mL^{-1} of fosfomycin in phosphate buffer solution (50 mM KH_2PO_4-Na_2HPO_4, pH 6·5) including 1% of the promoters was used as the solution for administration to rats. The effects of caprylate and BL-9EX were also examined at 0·1 to 0·5%. The osmotic pressure of the drug solution was finally adjusted to 280 mOsm with sodium chloride.

Absorption experiments

Wistar male rats (220–260 g) were fasted overnight but had free access to water. The experimental method was conducted according to Doluisio et al (1969) and Shiga et al (1987). The jejunal loop, 10 cm in length from a point 15 cm from the gastric pyrolus, and the colonic loop, 10 cm in length right next to the caecum, were used. Blood samples (0·25 mL) were taken at 15 min intervals from the jugular vein cannula for 60 min following the injection of fosfomycin solution (3 mL) into the loop.

Analytical method

The blood samples were kept one night in cold storage and then centrifuged at 12 000 rev min^{-1} and 4 °C for 3 min to make the serum samples. Each serum sample was adequately diluted with the phosphate buffer and the fosfomycin serum concentration was assayed by the cup method using *Escherichia coli* K-12HW-8235. The sensitivity limit of the fosfomycin assay was about 0·5 μg mL^{-1} in serum.

Statistical analysis

The significance of differences in the serum fosfomycin concentrations in each promoter group versus the control was examined by the Student's *t*-test.

RESULTS

Fig. 1 shows the fosfomycin serum concentrations in the presence of 1% promoters. The absorption of fosfomycin in the control colon without a promoter was so low as to be beyond the sensitivity of the assay (Fig. 1b). The serum fosfomycin concentration in the jejunum in the control was 3·4 μg mL^{-1} 60 min after the administration (Fig. 1a). DEM, sodium salicylate and sodium 5-methoxysalicylate, all reported to promote the colorectal absorption of cefmetazole (Nishihata et al 1982, 1984a), failed to enhance fosfomycin absorption in the jejunum or colon. The enhancing effect of EDTA-2Na could be observed

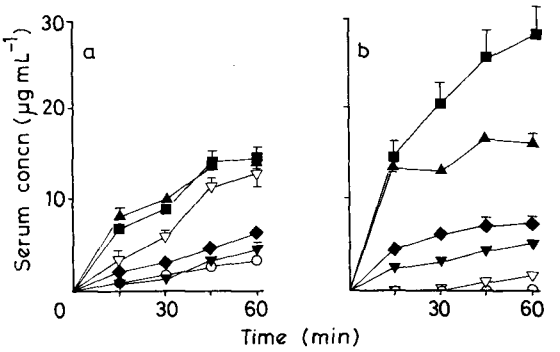

FIG. 1. Effects of 1% promoters on the jejunal (a) and colonic (b) absorption of fosfomycin. Each value represents the mean ± s.e. of four to eight rats. ■ BL-9EX; ▲ saponin; ▽ EDTA-2Na; ◆ glycocholate; ▼ taurocholate; ○ control (in the absence of promoters).

60 min after its administration in the colon (the antibiotic serum concn 1·3 μg mL^{-1}) but in the jejunum, the maximum serum concentration increased to 12·7 μg mL^{-1}. The effect of sodium glycocholate exceeded that of taurocholate being essentially the same in the colon and jejunum. The effect of BL-9EX was the most pronounced of all promoters at both absorption sites (Fig. 1). Saponin had a greater effect than either bile salts or EDTA-2Na.

Fig. 2 shows the effects of carbon chain length on the promoting action of 1% sodium salts of saturated medium-chain fatty acids. The strength of effect was shown in the order sodium caprate (C_{10}) > sodium laurate (C_{12}) > sodium caprylate (C_8) in the jejunum and colon. The effect of sodium caprate was essentially the same as that of BL-9EX in the colon, but in

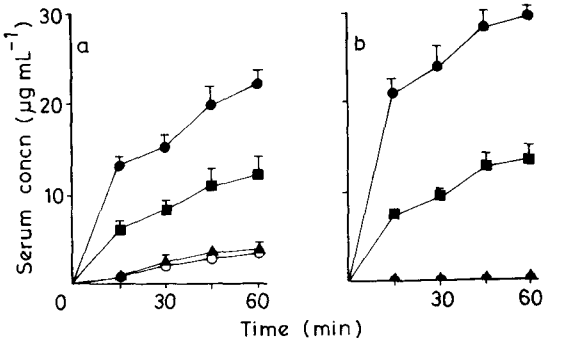

FIG. 2. Effects of carbon chain length in 1% sodium salts of saturated medium-chain fatty acids on the jejunal (a) and colonic (b) absorption of fosfomycin. Each value represents the mean ± s.e. of four to five rats. ▲ caprylate (C_8); ● caprate (C_{10}); ■ laurate (C_{12}); ○ control.

the jejunum, the former was the greater (Figs 1, 2). Caprylate exerted hardly any effect.

The mixed micelles consisting of 1% of both sodium taurocholate and unsaturated fatty acids, i.e., sodium oleate ($C_{18:1}$), sodium linoleate ($C_{18:2}$) or linolenic acid ($C_{18:3}$) were also used as promoters (Fig. 3). Their effects greatly exceeded that of taurocholate alone. The degree of unsaturation of these fatty acids resulted in similar effects on promoter action.

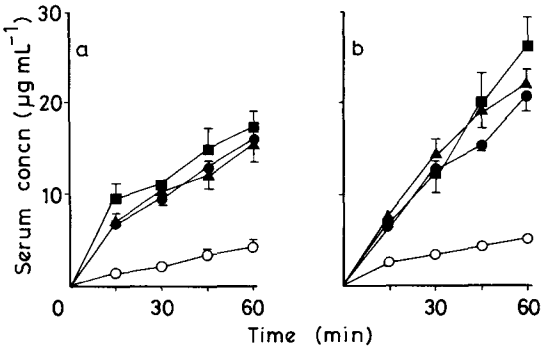

FIG. 3. Effects of mixed micelles containing 1% unsaturated fatty acid and 1% taurocholate on the jejunal (a) and colonic (b) absorption of fosfomycin. Each value represents the mean ± s.e. of four to five rats. ▲ Oleate ($C_{18:1}$); ■ linoleate ($C_{18:2}$); ● linolenic acid ($C_{18:3}$); ○ control (taurocholate alone).

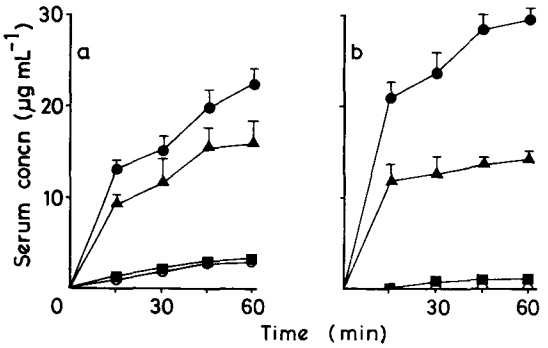

FIG. 4. Concentration effects of caprate on the jejunal (a) and colonic (b) absorption of fosfomycin. Each value represents the mean ± s.e. of four to five rats. ● 1%; ▲ 0·5%; ■ 0·1%; ○ control.

The effect of promoter concentration on fosfomycin absorption was examined for sodium caprate and BL-9EX, their effects being greatest at 1%, especially in the colon (Figs 1, 2). Figs 4 and 5 show serum fosfomycin concentrations in the presence of sodium caprate and BL-9EX, respectively. Sodium caprate had a significant effect at 0·5% and at 1% it

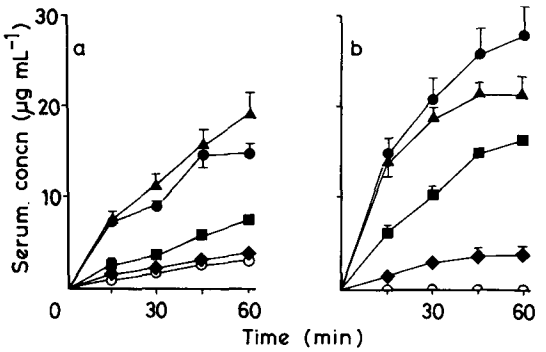

FIG. 5. Concentration effects of BL-9EX on the jejunal (a) and colonic (b) absorption of fosfomycin. Each value represents the mean ± s.e. of four to five rats. ● 1%; ▲ 0·5%; ■ 0·1%; ◆ 0·05%; ○ control.

was greater at both absorption sites (Fig. 4). The effect of BL-9EX was significant at 0·1% in the jejunum and at 0·05% in the colon (Fig. 5). The intensity of the action of BL-9EX at 0·5% was essentially the same as that at 1% at both absorption sites.

DISCUSSION

DEM has been reported to change glutathione metabolism in the membrane while salicylate interacts with membrane proteins or lipids resulting in enhanced membrane permeability (Nishihata et al 1984a, b; Kajii et al 1985, 1986). It is evident that such compounds are transcellular promoters. However, they failed to enhance fosfomycin absorption. The promoting effect of EDTA-2Na, a paracellular promoter, on fosfomycin absorption was less in the colon than jejunum and had a lag time in the colon similar to that noted for cefmetazole (Shiga et al 1987) (Fig. 1). The tight junction of the jejunum has been reported to be leaky but tight in the colon (Powell 1981). Thus, the difference in EDTA effect at these absorption sites may possibly arise from the degree of tightness of the tight junction.

Sodium taurocholate, sodium glycocholate and sodium caprate have lower, but significant, chelating ability than EDTA-2Na and thus may serve as paracellular promoters (Hunt 1983; Yata et al 1983). Their effects on fosfomycin absorption were found to exceed that of EDTA-2Na in the colon (Figs 1, 2). The effect of BL-9EX and saponin indicate their effectiveness as surface active agents on uptake. Bile salts and fatty acids have surface activity as well as chelating activity that may possibly be related to promoting action.

Among the sodium salts of saturated medium-

chain fatty acids, the order of the promoting effect was caprate (C_{10}) > laurate (C_{12}) > caprylate (C_8) in both the jejunum and colon. Hardly any action was noted for caprylate (Fig. 2). Regarding the effects of these promoters on rectal ampicillin absorption, the same order as with fosfomycin has been reported, but differences in the effects of caprate and caprylate on ampicillin absorption were much less than those on fosfomycin absorption (Nishimura et al 1985). It is thus apparent that promoting effects depend on the particular drug administered as well as the promoters themselves.

The mixed micelles were also effective transcellular promoters. They have been reported to interact with the polar head groups of membrane phospholipids, resulting in increased membrane permeability (Muranishi 1985). The present data have demonstrated that mixed micelles enhance fosfomycin absorption to a far greater degree than taurocholate alone. The degree of unsaturation of fusogenic lipids in the micelles had no effect on promotion, as was also noted with streptomycin (Muranishi 1985) (Fig. 3).

The concentration of promoters is a factor to be considered in avoiding membrane damage. Fosfomycin absorption was enhanced by sodium caprate and BL-9EX at low concentrations. The effective concentration of sodium caprate was 0·5% in both the jejunum and colon, and for BL-9EX, 0·1% in the jejunum and 0·05% in the colon (Figs 4, 5). Since the release of membrane proteins and phospholipids in the presence of promoters at such low concentrations did not significantly differ from that in their absence, no membrane damage was observed (data not shown).

Thus, these promoters appear capable of enhancing fosfomycin absorption with little or no membrane damage. Since maintenance of effective promoter concentration in the jejunum is practically difficult and the extent of promotion in the colon certainly exceeds that in the jejunum (Figs 1, 2), the administration of promoters into the rectal route may render their action more effective.

Acknowledgement

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