Pharmaceutical Research, Vol. 5, No. 6, 1988

Research Article

Enhancement of Colonic Drug Absorption by the Paracellular Permeation Route

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Received September 16, 1987; accepted January 19, 1988

Colonic absorption of poorly absorbable cefmetazole was shown to increase considerably by the addition of 1% sodium caprate, sodium laurate, and mixed micelles composed of sodium oleate and sodium taurocholate. At 0.25%, their effects were weaker but still significant. Colonic absorption of inulin was also increased by the promoters at a concentration of 0.25%. These results suggest that there is a common route between inulin and cefmetazole absorption, i.e., the paracellular route. Sodium taurocholate, sodium caprylate, and EDTA disodium salts (EDTA-2Na) at 1% enhanced cefmetazole absorption less than caprate, laurate, or mixed micelles, but no such effect was found at 0.25%. The colonic pore radius was determined from the equivalent pore theory using an everted sac procedure. Caprate, laurate, and mixed micelles at 0.25% caused this radius to increase significantly, thus making it possible for inulin to permeate the everted sac from the mucosal to the serosal side. The effects of taurocholate, caprylate, and EDTA-2Na for increasing colonic pore sizes and the degree of inulin permeation were less than those of caprate, laurate, or mixed micelles. The change in the paracellular route is thus considered to result from the increase in pore size.

KEY WORDS: colonic absorption; absorption promoter; cefmetazole; inulin; pore radius; paracellular route.

INTRODUCTION

There are two routes in transepithelial drug transport by passive diffusion, the transcellular route through the lipoidal cell membrane and the paracellular route from the tight junction to the lateral intercellular space. The tight junction in the small intestine is leaky but tight in the colon (1,2). Thus, the paracellular route contributes little to colonic drug absorption. However, since many poorly absorbed drugs are water soluble and less lipophilic, improvement in drug absorption by promoters via both hydrophilic paracellular and lipophilic transcellular routes may be important.

Ethylenediaminetetraacetic acid (EDTA) has chelating ability with metal ions and has been found to be a paracellular promoter by depleting calcium and magnesium in regions of the tight junction (3,4). Also, the sodium salts of medium-chain fatty acids, bile salts, and surface active agents have been found to promote the absorption of poorly absorbable drugs by chelating action (5–8). In addition, solubilization action as surfactants may relate to increase in the paracellular permeation.

The colorectum, which can serve as the site for either drug absorption or drug administration, is more capable of maintaining the promoter concentration above the effective level than the small intestine, where the arrival of drugs and promoters at the absorptive site requires gastric transition after oral administration. Thus, enhancement of membrane permeability through paracellular routes in the colon by various promoters should make possible greater absorption of water-soluble and poorly absorbable drugs.

In this study, the paracellular route was considered a water-filled channel, and the extent to which the pore size was increased by promoters was calculated using the theory of equivalent pore radius, as proposed by Solomon (9). Estimates of equivalent pore radius were obtained from water diffusive and osmotic permeability coefficients using the everted sac of a rat colon. We already reported that water absorption, particularly water influx from the lumen to the blood, is increased by promoters (10–12). Thus, change in pore size should become evident in water permeation experiments. In this study, cefmetazole was used to model a water-soluble, poorly absorbed drug and inulin, also water soluble and poorly absorbed. The poorly lipophilic inulin is considered to permeate exclusively by the paracellular route by virtue of its molecular size (radius, 12–15 Å). Thus, a study of this inulin permeation should provide some indication of pore size in this route. In addition to sodium taurocholate, sodium caprate (C_{10}), and EDTA disodium salts (EDTA-2Na), already reported to enhance effectively colonic absorption of cefmetazole (11), sodium caprylate (C_8), sodium laurate (C_{12}), and mixed micelles composed of so-

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0724-8741/88/0600-0341$06.00/0

dium oleate ($C_{18:1}$) and sodium taurocholate were used as promoters.

MATERIALS AND METHODS

Chemicals

The drugs and promoters used in this study and their sources are as follows: sodium taurocholate, sodium oleate, and fluoroisothiocyanate dextran (FITC-dextran) from Sigma Chemical Co., St. Louis, Mo.; sodium caprate, sodium laurate, and sodium caprylate from Tokyo Kasei Kogyo, Tokyo; EDTA-2Na from Wako Pure Chemical Industries, Ltd., Osaka, Japan; [^{3}H]inulin and *n*-[^{14}C]butanol from New England Nuclear, Boston, Mass.; and tritiated water from Amersham, Bucks, England. Cefmetazole was kindly supplied by Sankyo Co., Tokyo. Mixed micelles, composed of sodium oleate and sodium taurocholate of equal concentration (%; w/w), were prepared by dissolving both compounds together in 50 m*M* isotonic phosphate buffer solution (Na_2HPO_4 + KH_2PO_4, pH 6.5) under a nitrogen gas atmosphere to avoid the oxidation of sodium oleate. Other reagents were of analytical grade or better.

In Situ *Absorption Experiment*

Male Wistar rats (200 ± 20 g) fasted overnight were anesthetized by administering ethyl carbamate (1.1 mg/kg) intraperitoneally. Drug absorption was examined by the rat *in situ* colonic loop technique, which is able to keep the physiological condition and is economical in the experiment with radioactive compounds. The proximal and distal ends of the colon (about 8 cm in length) were cannulated according to Doluisio *et al.* (13), as described in a previous paper (12). After flushing the cannulated loop with 0.9% NaCl at 37°C until the eluted solution was clear, a 10-ml plastic syringe was connected to each cannula. The right jugular vein was also cannulated to obtain blood samples (14). The luminal solution consisted of a 50 m*M* isotonic phosphate buffer solution (Na_2HPO_4 + KH_2PO_4, pH 6.5) with 1 or 0.25% of a promoter containing either 1% cefmetazole or 0.001% inulin. To the luminal inulin solution, 3.60 μCi/ml [^{3}H]inulin was added. Five milliliters of the luminal solution was injected into the intestinal lumen. Blood (0.3 ml) was then collected from the jugular vein in heparinized tubes immediately as a blank sample and every 15 min for 60 min. The samples were centrifuged for 2 min with a Beckman microfuge to obtain the plasma. Before high-performance liquid chromatographic (HPLC) assay for cefmetazole, the plasma was treated with 6% trichloroacetic acid of the equal volume to precipitate plasma protein and the supernatant was used as the plasma sample.

Measurement of in Situ *Exsorption*

Exsorption of inulin from the blood to the intestinal lumen was measured by administering 0.1 ml of a 20 μCi/ml [^{3}H]inulin solution into the femoral vein during single-pass perfusion through the colonic segment at a rate of 1 ml/min. The perfusate consisted of a 50 m*M* isotonic phosphate buffer (pH 6.5) with 0.25% of one of the promoters studied. Samples of the luminal solution were collected every 10 min for 60 min.

Measurement of in Vitro *Water Transport and the Aqueous Diffusion Layer*

An everted sac of a rat colon (7 ± 1 cm) was prepared and cannulated at both ends in the same manner as the *in situ* Doluisio technique (12,13). After attaching a 10-ml plastic syringe to each cannula, the sac was suspended in 40 ml of phosphate buffer solution (pH 6.5) containing a 0.25% promoter (mucosal solution) stirred by the constant bubbling of O_2–CO_2 gas (95:5) at 37°C. Five milliliters of isotonic phosphate buffer (pH 6.5) was injected into the serosal side to conduct water transport experiments. Osmotically induced water flow rate was measured by the change in the FITC-dextran concentration (initial concentration, 25 μg/ml) in the mucosal solution. The osmotic gradient was made by adjusting the mucosal solution so as to be hypotonic (100–130 mOsm/kg), isotonic (280 mOsm/kg), and hypertonic (490–500 mOsm/kg) with sodium chloride. The diffusion rates of tritiated water (final concentration of radioactivity, 5 μCi/ml) and *n*-[^{14}C]butanol (final concentration of radioactivity, 1 μCi/ml) in the mucosal solution were determined under isotonic conditions at both the mucosal and the serosal sides. Soon after injection of the serosal solution (time 0), 2 ml of the mucosal solution and 1 ml of the serosal solution were removed. Two milliliters and 1.5 ml of these solutions, respectively, were also sampled at 15 and 30 min. The concentrations of tritiated water, *n*-[^{14}C]butanol, and FITC-dextran were corrected for volume change by this sampling.

Measurement of in Vitro *Inulin Transport*

The everted sac prepared as described above was used. To the mucosal solution, 0.001% inulin containing 3.6 μCi/ml [^{3}H]inulin was added. The cumulative appearance of inulin in the serosal solution was determined at 15 and 30 min and expressed as a ratio of the concentration of inulin in the serosal solution to that in the mucosal solution.

Assay

Cefmetazole plasma concentration was measured by HPLC as reported by Sekine *et al.* (15). The sensitivity limit of this determination was 1 μg/ml. FITC-dextran concentration was determined as reported by Shiga *et al.* (10). To determine [^{3}H]inulin, tritiated water, and *n*-[^{14}C]butanol, a 10-ml scintillation cocktail (0.3 g of POPOP, 12 g of DPO, 2 liters of toluene, and 1 liter of triton X-100) was added to 0.1 ml of the plasma sample or 0.5 ml of the mucosal and serosal samples. All samples were counted in a liquid scintillation counter (Aloka 903, Tokyo). The decrease in the counting efficiency by quenching was automatically corrected by the external standard source.

Calculation of the Permeability Coefficient

Osmotic Water Permeability. Osmotically induced water flux (V_n; mol/min) is given by

$$V_n = -P_f \cdot A \cdot \Delta\pi \quad (1)$$

where $\Delta\pi$ is the osmotic difference (mOsm/kg) between the mucosal and the serosal sides, A is the surface area of the colonic membrane (cm^2), and P_f is the osmotic permeability

coefficient (cm/min). As shown in Eq. (1), P_f is obtained from a slope of the linear line produced by the relation between V_n and $\Delta\pi$ as the product of A and P_f since the correct value of A is unknown.

Tracer Permeability. The flux of tritiated water (J_d; mol/min) is given by

$$J_d = -P_d \cdot A \cdot (C_m - C_s) \quad (2)$$

where C_m and C_s are the mucosal and serosal concentrations of tritiated water, respectively (mol/ml), and P_d is the diffusive permeability coefficient of tritiated water (cm/min). Solving Eq. (2), the following equation is derived:

$$\ln\{1 - [(V_m + V_s)/V_m] \cdot (C_s/C_o)\} = -[(1/V_m) + (1/V_s)] \cdot P_d \cdot A \cdot t \quad (3)$$

where V_n and V_s are mucosal and serosal volumes, respectively, and C_o is the initial radioactivity concentration of tritiated water in the mucosal side. Thus, P_d is obtained from a slope of the linear relation between the left side in Eq. (3) and time as the product of A and P_d in the same manner as P_f.

Correction for the Unstirred Water Layer. According to Holz and Finkelstein (16), the effective thickness of an unstirred water layer adjacent to the epithelial cell membrane can be determined with *n*-butanol whose membrane permeation is considered diffusion limited. The diffusion coefficient of *n*-butanol in water ($D_{n\text{-butanol}}$) was taken from Lyons and Sandquist (17). This method has already been confirmed to indicate reliably the effective thickness of the unstirred water layer by Smulders and Wright (18). The diffusive permeability of water $(P_d)_m$ can be determined exactly by

$$1/(P_d)_{obs} = [1/(P_d)_m] + (\delta/D_{3_{HHO}}) \quad (4)$$

where $(P_d)_{obs}$ is the P_d value of tritiated water obtained by Eq. (2), δ is the unstirred layer thickness (cm), and $D_{3_{HHO}}$ is the diffusion coefficient of tritiated water (cm²/sec) (19). The δ value is obtained from the relation that $(P_d)_{obs}$ approximately equals $D_{n\text{-butanol}}/\delta$ since $(P_d)_m$ is greater than $D_{n\text{-butanol}}/\delta$ for *n*-butanol. The δ value indicates the thickness per unit surface area (cm/cm²), δ/A, since $(P_d)_{obs}$ is obtained as the product of A and P_d in Eq. (3) also for *n*-butanol.

Estimate of the Equivalent Pore Radius

The computation of the equivalent pore radius (r) is given by

$$\lambda = (8 \cdot \eta_w \cdot D_w/k') \cdot (P_f/P_d - 1) \quad (5)$$

$$r = -a_w + (2 \cdot a_w^2 + \lambda)^{1/2} \quad (6)$$

where η_w is the water viscosity (dyn · sec/cm²), D_w is the water diffusion coefficient (cm²/sec) (18), k' is the conversion factor ($k' = RT/V_w$) (dyn/cm²), R is the gas constant (dyn/deg/mol), T is the absolute temperature, and V_w is the partial molar volume of water (cm³/mol). a_w is the radius of the water molecule (1.5 Å) (9).

RESULTS

The plasma concentration data in Fig. 1 show the effects of 1% promoters on colonic absorption of cefmetazole.

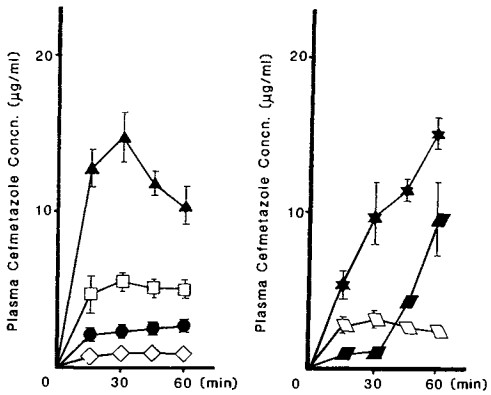

Fig. 1. Effects of 1% promoters on colonic absorption of cefmetazole. Each value represents the mean ± SE of 12 rats. For small SE, a bar is included in the symbols. (▲) Caprate; (□) laurate; (●) caprylate; (▭) taurocholate; (▰) EDTA-2Na; (★) mixed micelles; (◇) control.

Caprate and mixed micelles were the most effective, followed by laurate. Taurocholate and caprylate had the least effect. The action of EDTA-2Na could not be observed until 30 min following its injection into the lumen but it gave a higher plasma concentration of cefmetazole than laurate or taurocholate at 60 min.

The enhancing effects of 0.25% promoters on colonic absorption of cefmetazole are also shown in Fig. 2. Those of 0.25% caprate, mixed micelles, and laurate decreased from one-half to one-third those at 1% (Fig. 1) but were still significant. However, the other promoters failed to have any significant effect on cefmetazole absorption.

The effects of 0.25% promoters on inulin absorption and exsorption into the luminal solution following its intravenous administration *in situ* are shown in Figs. 3 and 4, respectively. In the control, neither absorption nor exsorption could be detected. Inulin absorption was enhanced considerably by caprate, laurate, and mixed micelles. EDTA-2Na, taurocholate, and caprylate showed lesser but significant enhancing effects on inulin absorption. The effect of EDTA-2Na was delayed as indicated in cefmetazole absorption. The cumulative exsorption amount of inulin increased by various promoters in the same manner as absorption; the effect of laurate was strongest.

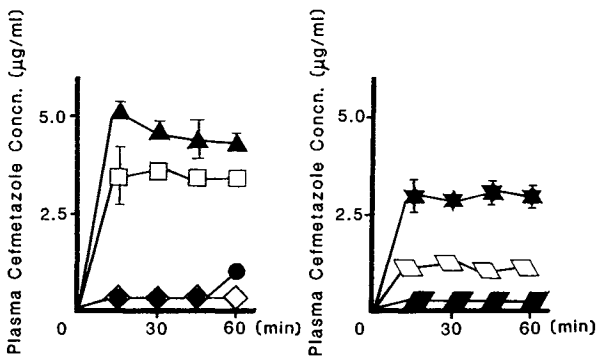

Fig. 2. Effects of 0.25% promoters on colonic absorption of cefmetazole. Each value represents the mean ± SE of six rats. For small SE, a bar is included in the symbols. All symbols are the same as those in Fig. 1.

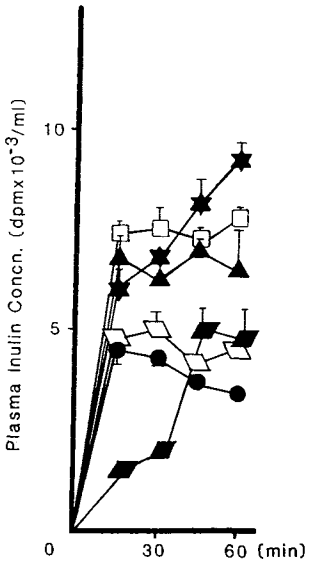

Fig. 3. Effects of 0.25% promoters on colonic absorption of inulin. For the control, no inulin absorption was detected. Each value represents the mean ± SE of four or five rats. For small SE, a bar is included in the symbols. All symbols are the same as those in Fig. 1.

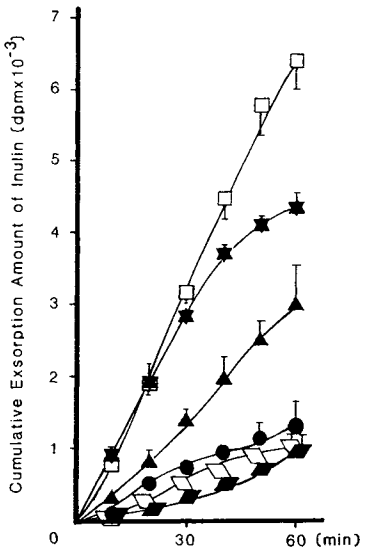

Fig. 4. Effects of 0.25% promoters on colonic exsorption of inulin after intravenous administration. Exsorption is expressed as the cumulative amount exsorbed from the blood into the lumen. For the control, no inulin exsorption was detected. Each value represents the mean ± SE of four to six rats. For small SE, a bar is included in the symbols.

The change in estimated equivalent pore radius in the presence of 0.25% promoters at 15 and 30 min is shown in Table I along with the thickness of the unstirred water layer and the diffusive and osmotic permeabilities of water. The surface area for calculating the thickness of the unstirred water layer was obtained assuming the lumen to be a cylinder with a smooth surface. The significance of differences was not tested for pore radius since it is calculated by the ratio of osmotic and diffusive permeabilities obtained from separate experiments, as shown in Eq. (4). However, a significant increase in the osmotic permeability was found in almost all promoters, resulting in an obvious increase in the estimated equivalent pore radius. The pore radius in the control, 8–9 Å, increased to 14–16 Å by caprate, laurate, and mixed micelles. Taurocholate and caprylate caused a lesser increase to 11–12 Å. EDTA-2Na had no effect on pore radius for the 15-min treatment but caused it to increase to 12 Å for the 30-min treatment. Caprate, laurate, and mixed micelles decreased the thickness of the unstirred water layer. In particular, caprate and laurate reduced it from one-third to one-quarter the control value.

The effects of 0.25% promoters on the cumulative permeation ratio of inulin from the mucosal to the serosal sides at 15 and 30 min are shown in Fig. 5. The cumulative permeation ratio of inulin for caprate, laurate, and mixed micelles including that for EDTA-2Na only at 30 min, 0.3–0.5% at 15 min and 0.8–1.4% at 30 min, exceeded the control level (about 0.1%). In the presence of caprylate and taurocholate, the ratio tended to exceed the control level but was not significantly different from the control even at 30 min.

The correlation between the areas under the plasma concentration curves (AUC) of cefmetazole (Fig. 2) or inulin (Fig. 3) calculated by trapezoidal rule and pore radius (Table I) in the presence of 0.25% promoters is shown in Fig. 6. In both cases, significant correlations were found in the confidence limit of more than 95%.

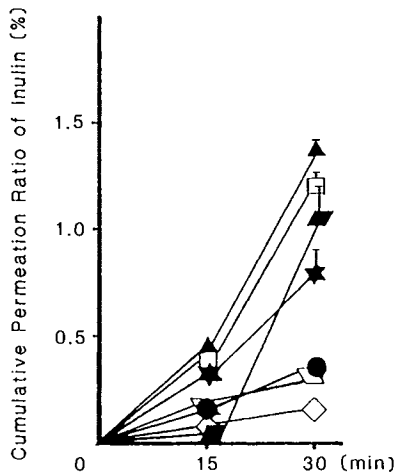

Fig. 5. Effects of 0.25% promoters on inulin permeation from the mucosal to the serosal side in the colonic everted sac. Inulin permeation is expressed as the ratio of the cumulative permeation amount to the initial amount in the mucosal solution. Each value represents the mean of five to seven rats. All symbols are the same as those in Fig. 1.

Table I. Effects of 0.25% Promoters on the Pore Radius of the Colonic Membrane[a]

	δ (cm)[b]	$(P_d)_m \cdot A$ (cm^3/min)[c]	$P_f \cdot A$ (cm^3/min)[d]	Pore radius (Å)
Control	0.112 (0.003)	0.0447 (0.0025)	0.362 (0.046)	7.9 (0.3)
	0.0946 (0.0502)	0.0433 (0.0083)	0.388 (0.129)	8.5 (1.0)
Taurocholate	0.0798 (0.0061)**	0.0444 (0.0044)	0.612 (0.183)*	11.0 (0.5)
	0.0747 (0.0044)	0.0397 (0.0040)	0.618 (0.191)*	11.8 (0.7)
EDTA-2Na	0.0837 (0.0179)*	0.0451 (0.0058)	0.413 (0.251)	8.5 (0.7)
	0.119 (0.065)	0.0576 (0.0123)	0.928 (0.333)**	12.0 (0.2)
Caprylate	0.216 (0.009)**	0.0446 (0.0027)	0.601 (0.199)*	10.8 (0.5)
	0.0887 (0.010)	0.0397 (0.0041)	0.552 (0.117)*	11.1 (0.7)
Caprate	0.0290 (0.0035)**	0.0333 (0.0043)*	0.678 (0.106)**	13.8 (1.0)
	0.0301 (0.0058)**	0.0324 (0.0059)	0.721 (0.183)**	14.6 (0.4)
Laurate	0.0351 (0.0187)**	0.0397 (0.0150)	0.694 (0.129)**	13.7 (0.4)
	0.0240 (0.0046)**	0.0395 (0.0024)	0.689 (0.094)**	13.5 (0.3)
Mixed micelles	0.0722 (0.0040)**	0.0367 (0.0017)*	0.668 (0.124)**	12.8 (0.1)
	0.0607 (0.0081)**	0.0307 (0.0039)*	0.813 (0.135)**	16.0 (0.8)

[a] Values in the upper and lower rows for each promoter system are the mean and SD of more than five rats for the 15- and 30-min treatment, respectively. SD values are shown in parentheses.
[b] Thickness of the unstirred water layer calculated from the surface area, which was obtained assuming the lumen to be a cylinder with smooth surface.
[c] Product of the diffusive water permeability coefficient corrected for the unstirred water layer and surface area of the colonic membrane.
[d] Product of the osmotic water permeability coefficient and surface area of the colonic membrane.
* $0.01 < P < 0.05$ versus control (Student's t test).
** $P < 0.01$ versus control (Student's t test).

DISCUSSION

The colonic absorption of cefmetazole was enhanced by 1% promoters such as caprate, laurate, caprylate, mixed micelles, taurocholate, and EDTA-2Na (Fig. 1). Caprate and mixed micelles showed the strongest effect in this regard. At 0.25%, caprate, laurate, and mixed micelles still exerted such effects but the other promoters failed to bring about significant increases beyond the control cefmetazole absorption (Fig. 2). The colonic absorption of inulin is significantly increased by all promoters at 0.25% (Fig. 3). The effects of caprate, laurate, and mixed micelles were almost the same and exceeded those of the other promoters in the same manner as cefmetazole absorption enhancement. These findings indicate the possibility of a common permeation route in the colonic absorption of cefmetazole and inulin. Since inulin is a large molecular, water-soluble, and poorly lipophilic compound, it possibly permeates exclusively through a water-filled channel, and thus the common routes described above may be considered aqueous channels, i.e., the paracellular route. However, in contrast to cefmetazole absorption, inulin absorption was increased also by 0.25% caprylate, taurocholate, and EDTA-2Na. The electrically repulsive effects of negative charges in the channel wall on the paracellular permeation of negative ions of weakly acidic cefmetazole may not occur in the case of a neutral compound such as inulin (1), and this may explain in part the difference between cefmetazole and inulin absorption. Thus, a pore size exceeding a certain value may be necessary to prevent this repulsion of cefmetazole. It may be asked whether channels exist with a pore size large enough for inulin permeation but with sufficient electric repulsion to render cefmetazole permeation impossible. This hypothesis needs to be tested with further experimentation.

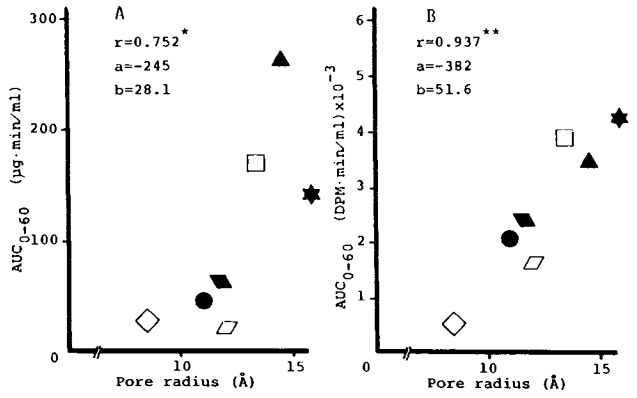

Fig. 6. Correlation between the area under the plasma concentration curves (AUC_{0-60}) of cefmetazole (A) or inulin (B) and the estimated pore radius in the presence of 0.25% promoters. AUC_{0-60} was obtained from Figs. 2 and 3 by trapezoidal rule. All symbols are the same as those in Fig. 1. $Y = a + bX$ where $a = Y$ intercept and b = slope; r represents the correlation coefficient. (**) $P < 0.01$; (*) $0.01 < P < 0.05$.

Inulin exsorption from the blood into the lumen was increased by all promoters at 0.25% (Fig. 4). This exsorption, which occurred essentially in the same manner as absorption, indicates that these promoters possibly function by penetrating the membrane interior as well as the membrane surface. Laurate increased inulin exsorption much more than caprate, in contrast to their action on its absorption. The stronger effects of laurate may be related to its own greater absorbability, compared to caprate (20).

The promotion mechanism was examined from changes

in membrane pore size (radius) in the presence of promoters (Table I). The pore size in the control was 8–9 Å, corresponding to that of the rectal membrane calculated from the Levitt equation (21) using the molecular radius of antipyrine and its reflection coefficient (22). The degree to which pore size increased in the presence of promoters corresponded to the extent to which they caused cefmetazole and inulin absorption to increase (Figs. 2 and 3 and Table I). Caprate, laurate, and mixed micelles, effective at 0.25% on cefmetazole and inulin absorption *in situ*, caused the pore size to increase more than taurocholate or caprylate, neither of which was effective at the low concentration. The former three increased the pore size to 14–16 Å, and the latter two to 11–12 Å (Table I). The effect of EDTA-2Na on pore size could be detected only after 30 min. Essentially the same delay was noted for cefmetazole and inulin absorption *in situ* (Figs. 1 and 3 and Table I). The permeation ratio of inulin (molecular radius, 12–15 Å) from the mucosal to the serosal side *in vitro* was increased by the promoters, which indicates that the pore radius listed in Table I is sufficient for inulin permeation (Fig. 5). Caprate, laurate, and mixed micelles increased inulin permeation more than the others. The increase in its permeation ratio corresponded well with that in pore size (Fig. 5 and Table I). These results suggest that promotion of absorption by these agents is mediated by a change in the paracellular route, as indicated above.

The significant correlation between the AUC of cefmetazole or inulin and the estimated pore radius in the presence of 0.25% promoters (Fig. 6) also supports the idea that the increases in absorption of cefmetazole and inulin were mediated by the change in the paracellular permeation, i.e., the increase in the pore radius.

The thickness of the unstirred water layer in the unperfused loop somewhat exceeded that previously reported (Table I) (23,24). Caprate and laurate decreased this thickness from one-third to one-quarter the control value. These promoters are effective for enhancing cefmetazole and inulin absorption *in situ* (Figs. 1–3) and inulin permeation *in vitro* (Fig. 5), suggesting a decrease in the resistance of the unstirred water layer to represent an additional promotion mechanism.

In the present study, mainly the change in the paracellular permeation was examined. The promoters that increased the colonic absorption of cefmetazole enhanced inulin absorption and increased pore size in the aqueous channel. The increase in pore size corresponded to that in the amount of inulin permeating from the mucosal to the serosal side. From the present data, it appears that cefmetazole absorption is increased by the paracellular route. The membrane damage plays a minor role in the promotion mechanism since the released amount of membrane protein and phospholipids in the presence of promoters was not related to the intensity of their promoting action, and the electron micrographs did not show a significant change in the brush border region after promoter treatment (unpublished data). To examine the change in the transcellular permeation as the other promotion mechanism, the interaction between the promoters and membrane protein and lipid is now under study.

ACKNOWLEDGMENTS

This work was supported by a Grant-in Aid for Scientific Research provided by the Ministry of Education, Science and Culture of Japan (Nos. 59460197 and 60571094). The authors wish to thank Mr. Toyohiro Sawada for his technical assistance.

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