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Effect of Salicylate and Disodium Ethylenediaminetetraacetic Acid on the Rat Intestinal Absorption of Cefmetazole

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The enhancement by disodium ethylenediaminetetraacetic acid (EDTA) of cefmetazole absorption from rat jejunum and colon was dependent on the pH of the administered solution. The higher the pH of the administered solution, the greater the effect of EDTA. However, the enhancement by salicylate of the cefmetazole absorption was dependent on salicylate absorption rather than on the pH of the administered solution. The effectiveness of EDTA was similar at the colon and at the jejunum, while salicylate showed a significantly greater effect at the colon than at the jejunum.

Keywords—cefmetazole; colon; jejunum; absorption; EDTA; salicylate; pH dependency; ionic strength

Since it was first demonstrated that sodium salicylate enhances the rectal absorption of water soluble drug,¹⁾ it has also been reported that salicylate enhances the rectal absorption of various hydrophilic drugs and macromolecular compounds,²⁻⁶⁾ and that the effect of salicylate is partially suppressed by the presence of calcium ions in the administered dosage form.⁷⁾ Further, it has been demonstrated that the action of disodium ethylenediaminetetraacetic acid (EDTA) in enhancing the absorption of hydrophilic compounds involves the loss of calcium ions from the tight junctional area of intestinal epithelial cells, and can be completely suppressed by the presence of calcium ions in the administered dosage form.⁸⁾

The above findings raise the possibility in which the mechanism of action of salicylate and EDTA are similar. To examine this possibility, the effect of pH on the adjuvant action was examined, because it is known that the chelating activity of organic compounds is strongly pH-dependent.⁹⁾

Experimental

Materials—Sodium cefmetazole was supplied by Sankyo Co., Ltd. (Tokyo, Japan). EDTA and sodium salicylate were obtained from Nakarai Chemicals Co., Ltd. (Kyoto, Japan). Other reagents used were of analytical grade.

Animals—Wistar male rats, 200 to 225 g, were fasted for 16 h prior to experiments but water was given *ad lib*. During the experiments, rats were anesthetized with sodium pentobarbital (40 mg/kg, *i.p.*) and were kept on a hot surface at 38°C.

Drug Absorption Study from Rat Intestine—The drug absorption study from rat jejunum and colon was carried out by using the *in situ* loop method described previously.¹⁰⁾ About 4 cm segments of jejunum and colon were ligated after abdominal incision. Absorption of drugs was determined by following the disappearance of drugs from each loop 1 h after administration of 0.2 ml of solution. The pH of the administered solution was adjusted with 50 mM sodium phosphate buffer. At the end of the experiment, the pH of the fluid in each loop and the wet weight of each loop were also measured. The wet weights of jejunal loop and colonic loop used in this study were 348 ± 21 mg and 304 ± 17 mg, respectively. To study the stability of cefmetazole in the intestinal loop in the presence of adjuvant, 10 mM cefmetazole was incubated with 500 mg (wet weight) of the everted jejunal or colonic segment in 3 ml Krebs

Ringer solution (pH 7.4) in the presence or absence of 67 mM EDTA or 300 mM salicylate for 60 min at 37 °C. Since the recovery of cefmetazole from the medium was more than 95% whether adjuvant was present or not, the increased disappearance of cefmetazole from the loop in the presence of adjuvant represents increased absorption of cefmetazole from rat colonic or jejunal loop in the *in situ* loop study.

Uptake of Cefmetazole into Red Blood Cells—Red blood cells used were obtained from male beagle dogs. After collection of the blood, red blood cells collected by centrifugation were rinsed 3 times with 5 volumes of saline. Red blood cell suspension was prepared with isotonic buffer (50 mM sodium phosphate at pH 7.0) adjusted with sodium chloride. The uptake of cefmetazole into red blood cells was determined 10 min after incubation with sodium salicylate or EDTA at 38 °C. Measurement of cefmetazole in the red blood cells was carried out by the reported method.¹¹⁾ Uptake (percent) of cefmetazole into red blood cells was represented by the ratio of cefmetazole amount in the red blood cells to the initial amount of cefmetazole in the medium.

Assay—Assay of cefmetazole and salicylate was carried out by using high performance liquid chromatography as described previously.^{11,12)}

Results and Discussion

Disappearance of cefmetazole (CMZ) from rat jejunal and colonic loop was less than 10% at 1 h after administration without EDTA and salicylate, and did not depend on the pH of the administered solution from pH 5 to 9 (Figs. 1A and 2A). The presence of EDTA in the administered solution increased the disappearance of CMZ significantly from both jejunum and colon, especially at above pH 7.0 (Figs. 1B and 2B). Since this pH dependency of EDTA action enhancing CMZ absorption seems to correspond to the pH dependency of chelating activity of EDTA with calcium ion,⁹⁾ the above result supports the view that the enhancing action of EDTA on intestinal drug absorption occurs through the intestinal epithelial tight junction which becomes leaky as a result of the removal by EDTA of calcium ions from the tight junctional area.⁸⁾ The above suggestion is further supported by the evidence that coadministration of calcium ions suppressed the action of EDTA in enhancing the intestinal absorption of CMZ (Figs. 1B and 2B).

Since the ionic strength in the administered solution was different with and without EDTA and it has been reported that ionic strength affects the absorption of ampicillin,¹³⁾ the ionic strength of the administered solution was adjusted to 0.8 ($=\mu$) with sodium chloride. Absorption of CMZ in either the presence or the absence of EDTA was increased by increase of the ionic strength of the administered solution (Figs. 1A, B and 2A, B). Even at high ionic strength, the enhancing action of EDTA on the CMZ absorption showed pH dependency.

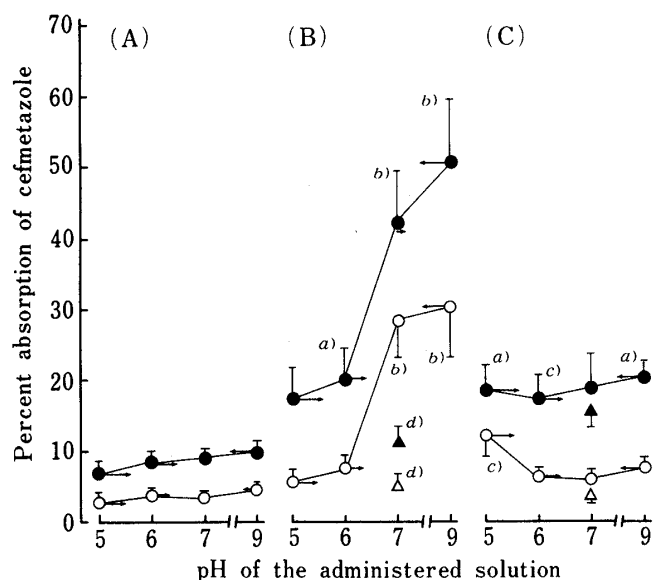


Fig. 1. Cefmetazole Absorption from Rat Jejunal Loop 1 h after Administration of 0.2 ml of 10 mM CMZ Solution with No Additive (A), 67 mM EDTA (B), or 300 mM Sodium Salicylate (C)

Open symbols and closed symbols represent the results obtained in the absence and presence of sodium chloride, used to prepare high ionic strength solution ($\mu=0.8$), respectively. Circles and triangles represent the results obtained in the absence and presence of calcium gluconate (50 mM for (B) and 300 mM for (C)), respectively. Arrows represent the change of pH of the solution in the intestinal lumen at 1 h after administration.

Each value represents the mean \pm S.D. ($n \geq 4$). a) $p < 0.05$ versus no adjuvant; b) $p < 0.005$ versus no adjuvant; c) $p < 0.1$ versus no adjuvant; d) $p < 0.005$ versus without calcium gluconate.

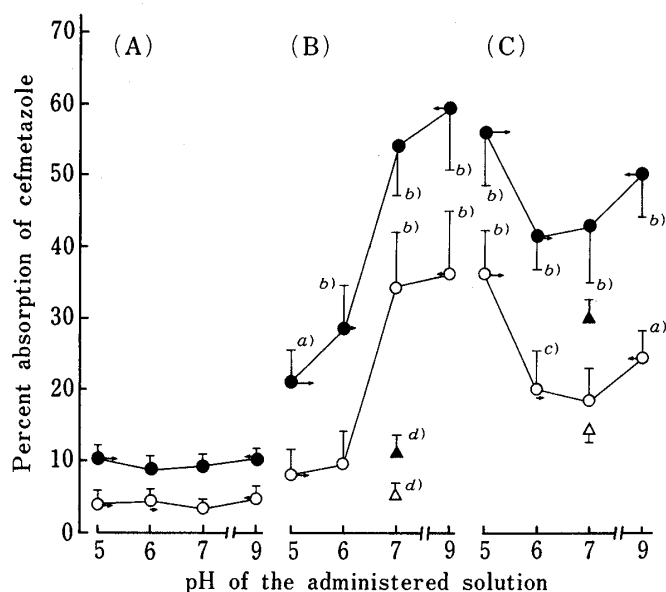


Fig. 2. Cefmetazole Absorption from Rat Colonic Loop during 1 h after Administration of 0.2 ml of 10 mM Cefmetazole Solution with No Additive (A), 67 mM EDTA (B), or 300 mM Sodium Salicylate (C)

All symbols are the same as in Fig. 1.

Each value represents the mean \pm S.D. ($n \geq 4$). a) $p < 0.05$ versus no adjuvant; b) $p < 0.005$ versus no adjuvant; c) $p < 0.1$ versus no adjuvant; d) $p < 0.005$ versus without calcium gluconate.

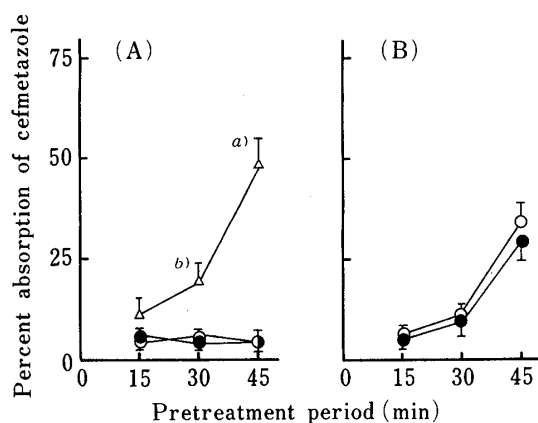


Fig. 3. Effect of Pretreatment with 67 mM EDTA (Δ in A, and \circ and \bullet in B) or 300 mM Sodium Salicylate (\circ in A) on the Rat Colonic Absorption of Cefmetazole during 1 h after Administration of 0.2 ml of Cefmetazole Solution (10 mM)

The pretreatment solution containing 0.15 M sodium chloride (\bullet in A), 67 mM EDTA (Δ in A, and \circ and \bullet in B), or 300 mM sodium salicylate (\circ in A) was perfused at a flow rate of 1 ml/min for various periods using the *in situ* perfusion method described previously¹⁾ and then a washing solution was perfused for 10 min before the administration of 0.2 ml of cefmetazole solution into the loop ligated at both ends.

In (A), saline was used as a washing solution. In (B), 0.15 M sodium chloride solution containing 50 mM calcium gluconate was used as a washing solution. Closed symbols in (B) represent the results obtained when the administered cefmetazole solution contained 50 mM calcium gluconate. Each value represents the mean \pm S.D. ($n \geq 4$). a) $p < 0.001$ versus saline pretreatment, b) $p < 0.05$ versus saline pretreatment.

The enhancing action of high ionic strength (adjusted with sodium chloride) may be due to disruption of the mucin layer, since it has been reported that treatment of the intestinal lumen with a high concentration of sodium chloride destroys the diffusion barrier composed of mucin.^{14,15)} Further, we have reported¹³⁾ that the rat colonic absorption of salicylate and the enhancing action of adjuvant on the rat colonic absorption was suppressed by high osmotic pressure in the administered solution (adjusted with 2-deoxyglucose, glucose or 3-O-methoxyglucose). Since high osmolarity in the administered solution appears to suppress drug intestinal absorption, probably by causing apparent net water outflux to the lumen, it is considered that the action of sodium chloride in disrupting the mucin layer is more important than the increase in osmotic effect under the conditions used in this study.

Pretreatment of intestinal loop with EDTA enhanced CMZ absorption for a period that depended on the pretreatment period with EDTA, and the suppressing effect of calcium ions administered after the pretreatment with EDTA decreased when the pretreatment period was long. These results may indicate that coadministration of calcium ions with EDTA results in

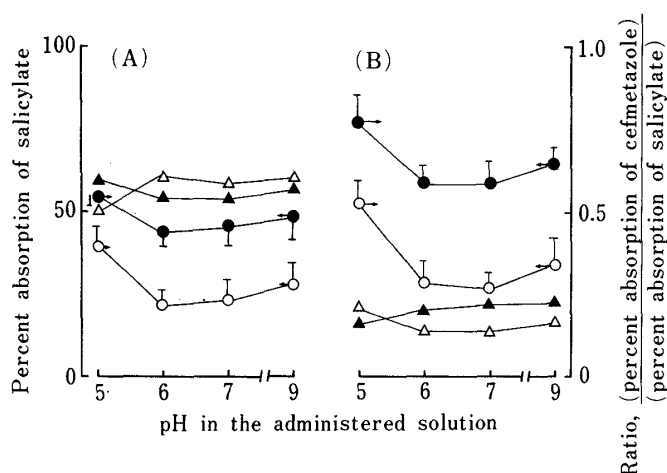


Fig. 4. Colonic (A) and Jejunal (B) Absorptions of Salicylate during 30 min after the Administration of 0.2 ml of Salicylate Solution (300 mM) in the Absence (○) and Presence (●) of Sodium Chloride, Used to Prepare the High Ionic Strength Solution ($\mu=0.8$), into Each Loop

The administered solution also contained 10 mM cefmetazole.

The ratio of cefmetazole absorption (not presented) to salicylate absorption during 30 min after administration into each loop in the absence (△) and presence (▲) of sodium chloride to increase the ionic strength ($\mu=0.8$).

Each value represents the mean \pm S.D. ($n \geq 4$).

complexation of EDTA with calcium ion, making the EDTA ineffective for removing calcium ions from the tight junctional area, and prolonged pretreatment with EDTA causes irreversible change of the tight junctional structure (Fig. 3).

On the other hand, the presence of sodium salicylate in the administered solution also increased the disappearance of CMZ from jejunum and colon. However, although it has been reported⁹⁾ that the chelating activity of fatty acid and salicylate is strong only at around pH 10, and is insignificant below pH 9.0, a significant adjuvant action of salicylate enhancing colonic CMZ absorption occurred between pH 5 and 9 (Fig. 1C). However, the salicylate action at the jejunum was significant only at pH 5.0 in the administered solution. When the ionic strength of the administered solution was adjusted to $\mu=0.8$, the enhancing action of salicylate on CMZ absorption was marked in both the colon and the jejunum (Figs. 1C and 2C).

Since absorption of salicylate from the jejunum in 1 h at high ionic strength was about 90% at pH 5 to 9 with no significant variation with pH, the absorption of CMZ and salicylate 30 min after administration was measured to study the relationship between the absorptions of salicylate and CMZ. As shown in Fig. 4, the absorption of salicylate showed pH dependency, with greater absorption at pH 5.0. However, the differences of salicylate absorption at various pHs in the administered solution were quite small when the ionic strength of the administered solution was $\mu=0.8$. The ratio of CMZ absorption to salicylate absorption was almost constant at pH 5.0 to 9.0 in the administered solution, though the ratio for the colon was greater than that for the jejunum. The results (Fig. 4) seem to indicate that the action of salicylate in enhancing intestinal CMZ absorption depends on salicylate absorption. The reduced variation of salicylate absorption with pH when the ionic strength of the administered solution was increased with sodium chloride may be due to the enhancing action of sodium ion on the ionization of salicylate, since it has been reported that absorption of the ionized form was increased with increase of sodium ion concentration in both the jejunum¹⁶⁾ and the colon.¹³⁾ This increased absorption of salicylate at high ionic strength (adjusted with sodium chloride) may also be partly due to the action of sodium chloride in disrupting the mucin layer diffusion barrier.

Coadministration of calcium ion with salicylate only slightly suppressed the action of salicylate (not statistically significant; Figs. 1C and 2C). The disappearance of the salicylate action after pretreatment of the intestinal loop with salicylate occurred rapidly even without coadministration of calcium ion (Fig. 3). These findings may suggest that the action of salicylate in enhancing the intestinal CMZ absorption occurs after the incorporation of salicylate into the intestinal tissue, and decreases with the disappearance of salicylate from the intestinal tissue flowing into the blood system.

Since it has been reported¹⁷⁾ that the actions of diethyl maleate and diethyl ethoxymethyl-

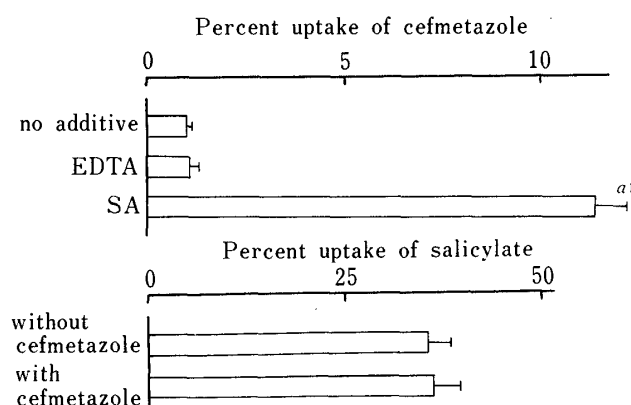


Fig. 5. Effects of 30 mM EDTA and 200 mM Sodium Salicylate (SA) on the Uptake of Cefmetazole into Red Blood Cells (Hematocrit: 0.5 ± 0.01) after a 10 min Incubation at 37°C

Initial concentration of cefmetazole in the medium was 10 mM. Percent uptake of cefmetazole was determined from the ratio of cefmetazole amount in red blood cells to the initial amount of cefmetazole in the medium.

Each value represents the mean \pm S.D. ($n \geq 4$). *a)* $p < 0.01$ versus no additive (Student's *t*-test).

enemalonate in enhancing colonic CMZ absorption are related to the concentration of the reduced nonprotein sulfhydryls in the colonic tissue, and also¹⁸⁾ that salicylate decreased the concentration of reduced nonprotein sulfhydryls in the intestinal tissue, salicylate may act mainly by decreasing the reduced nonprotein sulfhydryls after incorporation into the intestinal tissue. It is not clear why the adjuvant action of salicylate at the jejunum was weaker than at the colon, in spite of greater uptake of salicylate at the jejunum. It is possible that rapid metabolism relating to homeostasis of reduced nonprotein sulfhydryls in the *in vivo* jejunal tissue may maintain the barrier function of the epithelial cell membrane, since we have reported¹⁹⁾ that the adjuvant action of salicylate in an *in vitro* everted sac study with no nutrients in the medium (to avoid synthesis of glutathion) was not very different in the jejunum and the colon.

It should be noted that although the action of EDTA was not so very different at the jejunum and at the colon (Figs. 1B and 2B), the effect of salicylate at the colon was greater than at the jejunum (Figs. 1C and 2C). This result may indicate that the action of salicylate involves a different mechanism from that of EDTA, as suggested above. The action of EDTA may occur at the mucosal side of epithelial cells, by removal of calcium ions from the tight junctional area, while salicylate action may involve an alteration of metabolism relating to the reduced nonprotein sulfhydryls in the intestinal epithelial cells after incorporation of the drug into the cells.

When the effects of salicylate and EDTA on the uptake of CMZ into red blood cells were examined, it was found that salicylate increased the uptake of CMZ but EDTA did not (Fig. 5). Even though there are characteristic differences of the membrane between intestinal mucosal membrane and red blood cells membrane, we may suggest that salicylate increases cefmetazole transport through the epithelial cell membrane, while EDTA only increases cefmetazole transport through the tight junctional region of the intestine. The suggestion that salicylate enhances cefmetazole absorption by the transcellular route is supported by the previous report²⁰⁾ which showed that salicylate enhances the transport of trypan blue by the transcellular route, as confirmed by a microscopic technique. Although it is known²¹⁾ that EDTA at high concentration solubilizes the protein of red blood cell membrane (related to hemolysis), the concentration used in this study did not cause hemolysis during the experimental period. Therefore, the enhancing action of EDTA seems to occur at the tight junctional region, as suggested previously.⁸⁾

In the present study, it was shown that the action of EDTA in enhancing CMZ absorption is dependent on the pH in the administered solution, suggesting that the action of EDTA depends on the chelating activity. The action of salicylate, however, seems to require absorption of the drug.

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