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The synergistic effects of concurrent administration to rats of EDTA and sodium salicylate on the rectal absorption of sodium cefoxitin and the effects of inhibitors

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Plasma levels of cefoxitin, as enhanced by rectal coadministration of sodium salicylate, were reduced by concurrent administration of $<0{\cdot}5$ mg mL^{-1} *N*-ethylmaleimide (NEM) or *p*-chloromercuriphenylsulphonic acid, sodium salt (*p*-CMP). Concentrations of these inhibitors above 1 mg mL^{-1} resulted in enhanced peak plasma values of cefoxitin. This did not occur after coadministration with either ethylenediaminetetraacetic acid (EDTA) or polyoxyethylene-23 lauryl ether (POE). Ouabain and 2,4-dinitrophenol (DNP) suppressed plasma cefoxitin levels in the presence of salicylate and the enhancing effects of EDTA and POE when EDTA and POE were administered at low doses. At higher concentrations of EDTA and POE, DNP had little effect, while ouabain had little effect on POE and only partially suppressed the effects of EDTA. Plasma concentrations of cefoxitin after coadministration with salicylate and POE together, or with EDTA and POE together, were about the same as expected from summing the plasma levels resulting from coadministration of each adjuvant individually at the same concentrations. However, combined administration of salicylate and EDTA with cefoxitin yielded plasma cefoxitin concentrations which were much higher than expected from the sum of their individual actions.

We previously suggested (Nishihata et al 1985) that the effectiveness of each of three adjuvants, sodium salicylate, disodium EDTA and polyoxyethylene-23-lauryl ether (POE), in enhancing the rectal absorption of sodium cefoxitin occurs by three different mechanisms: salicylate acting at the protein fraction in the membrane with the enhanced absorption of cefoxitin not occurring by simple diffusion; EDTA also mainly acting at the protein fraction but the enhanced absorption of cefoxitin seeming to occur through a simple diffusion mechanism; and POE acting primarily at the lipid layer of the membranes and enhancing rectal absorption of cefoxitin by a simple diffusion mechanism.

The present study was carried out to provide additional understanding of the mechanism of enhanced rectal absorption of cefoxitin by each of these adjuvants. Furthermore, the effects of various combinations of these adjuvants were studied as another potential pharmaceutical approach for enhancing rectal absorption of cefoxitin.

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MATERIALS AND METHODS

Sodium salicylate (Sigma), disodium EDTA (Sigma) and polyoxyethylene-23-lauryl ether (Brij 35, ICI Americas) were obtained commercially. Sodium cefoxitin was supplied by Merck Sharp and Dohme. Other chemicals used were reagent grade.

The microenemas administered were prepared with distilled water and adjusted to pH 5·5 to 6·0 by adding either 0·01 M HCl or 0·01 M NaOH as necessary. The volume administered was 1·0 mL kg^{-1}. Male Sprague-Dawley rats (200 or 225 g) were fasted for 16 h before use. During the experiment, the rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}) and were kept on a surface at 38 °C.

The in-vivo rectal absorption study, in-situ rectal loop study and the in-vitro rectal everted sac study were carried out by the methods described by Nishihata et al (1985).

The assay of cefoxitin was by a high pressure liquid chromatography technique described by Nishihata et al (1984b). The protein assay was carried out using an analytical kit from Sigma.

RESULTS

Fig. 1 shows the effects of *N*-ethylmaleimide (NEM) and *p*-chloromercuriphenylsulphonic acid, sodium

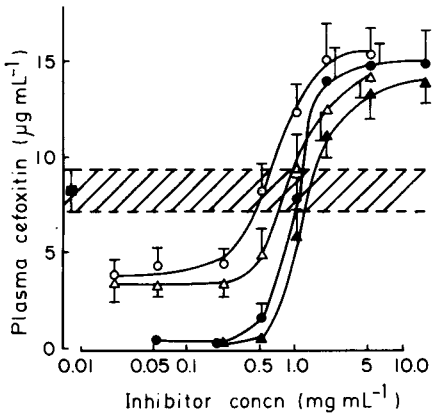

FIG. 1. The effects of 15 mg kg^{-1} *N*-ethylmaleimide (NEM, △ and ▲) and *p*-chloromercuriphenylsulphonate (*p*-CMP, ○ and ●) on the plasma peak cefoxitin levels after rectal administration in the presence (○, △) or absence (●, ▲) of sodium salicylate (100 mg kg^{-1}) are shown. As reference the effects of salicylate alone are also shown (■). Each value represents the mean with its standard deviation and n ≥ 4.

salt (*p*-CMP) on the plasma levels of cefoxitin as enhanced by coadministration of sodium salicylate (100 mg kg^{-1}) rectally. Both inhibitors at concentrations of 200 μg mL^{-1} or less given with salicylate significantly reduced the peak plasma values of cefoxitin whereas at concentrations above 1 mg mL^{-1} there was an enhancement of peak plasma cefoxitin values. In the absence of salicylate, concentrations of 200 μg mL^{-1} or less of NEM or *p*-CMP did not increase cefoxitin plasma levels, but at 1·0 mg mL^{-1} or greater, these were increased significantly. Neither NEM nor *p*-CMP (50 μg mL^{-1}) had significant effects on the peak plasma values of cefoxitin after coadministration with either EDTA or POE.

During the in-situ rectal loop study, NEM and *p*-CMP (50 μg mL^{-1}) reduced the disappearance of cefoxitin following administration with salicylate but had no effect when coadministered with EDTA or POE (Table 2). Tables 1 and 2 also show that 2,4-dinitrophenol (DNP) and ouabain suppressed the plasma cefoxitin levels, as well as the extent of disappearance of cefoxitin from the rectal loop, after administration of cefoxitin with salicylate, while the enhancing effects of EDTA and POE were suppressed by them when EDTA and POE were administered at low doses.

The effects of administering various combinations of the three types of adjuvants were varied. Plasma levels of cefoxitin were slightly less after coadministration with salicylate and POE, and with EDTA and POE than would be expected from summing the plasma levels obtained after coadministration of each adjuvant individually at the same concentrations. However, the combined administration of salicylate and EDTA with cefoxitin yielded plasma cefoxitin levels which were much higher than would be expected from summing the levels obtained when the adjuvants were administered separately with cefoxitin (Fig. 2). The increase was particularly

Table 1. Effects of NEM, *p*-CMP, DNP, and ouabain on the plasma peak concentrations of cefoxitin following rectal administration of 15 mg mL^{-1} cefoxitin in microenemas containing adjuvants.

Adjuvant concn in microenema	Plasma peak concn of cefoxitin, μg mL^{-1} [a] (peak time min) Control	NEM (50 μg mL^{-1})	*p*-CMP (50 μg mL^{-1})	DNP (50 μg mL^{-1})	Ouabain (20 μg mL^{-1})
Sodium salicylate, (0·625 M)	8·2 ± 1·1 (30 min)			5·4 ± 0·9* (30 min)	5·0 ± 1·2* (30 min)
Disodium EDTA (0·130 M)	2·3 ± 0·9 (45)	2·1 ± 0·4 (45)	2·0 ± 0·7 (45)	1·0 ± 0·5** (30)	0·4 ± 0·2* (30)
Disodium EDTA (0·196 M)	4·2 ± 1·3 (45)	3·9 ± 1·3 (45)	4·5 ± 1·6 (45)	4·5 ± 1·6 (45)	0·8 ± 1·2* (30)
Disodium EDTA (0·392 M)	9·4 ± 1·8 (30)	9·1 ± 2·3 (30)	10·1 ± 2·8 (30)	7·9 ± 2·6 (30)	4·7 ± 1·3* (30)
POE (5 mg mL^{-1})	2·3 ± 0·7 (30)	2·0 ± 0·8 (30)	2·6 ± 0·7 (30)	1·2 ± 0·5** (30)	1·0 ± 0·4* (30)
POE (10 mg mL^{-1})	9·7 ± 1·8 (45)	9·1 ± 1·4 (45)	9·4 ± 1·6 (45)	9·6 ± 0·8 (45)	8·9 ± 1·4 (45)

[a] Error limits represent standard deviations (n ≥ 4).
* $P < 0·001$ using Student's *t*-test.
** $P < 0·05$.

Table 2. Effects of NEM, *p*-CMP, DNP and ouabain on the disappearance of cefoxitin from an in-situ rat rectal loop 30 min after administration of 15 mg mL^{-1} kg^{-1} cefoxitin in microenemas containing various adjuvants.

Adjuvant concn in microenema	Percent loss of cefoxitin from rectal loop[a]				
	Control	NEM (50 μg mL^{-1})	*p*-CMP (50 μg mL^{-1})	DNP (50 μg mL^{-1})	Ouabain (20 μg mL^{-1})
Sodium salicylate (0·625 M)	51·5 ± 7·2	31·4 ± 5·3*	30·8 ± 6·2*	34·2 ± 4·6*	33·5 ± 6·5*
Disodium EDTA (0·130 M)	19·6 ± 2·6	21·5 ± 5·6	19·2 ± 5·1	13·5 ± 3·2**	7·2 ± 4·6**
Disodium EDTA (0·196 M)	30·3 ± 6·1	32·5 ± 7·1	26·4 ± 7·3	28·6 ± 9·3	14·9 ± 2·6
Disodium EDTA (0·392 M)	64·4 ± 11·3	61·9 ± 8·6	60·7 ± 9·2	52·4 ± 7·6	30·6 ± 5·1*
POE (5 mg mL^{-1})	18·3 ± 4·6	17·2 ± 4·3	20·8 ± 4·8	12·6 ± 1·5**	11·8 ± 2·7**
POE (10 mg mL^{-1})	59·8 ± 8·3	62·3 ± 4·8	58·6 ± 4·6	57·8 ± 5·7	54·8 ± 6·9
No adjuvant	8·5 ± 2·3	6·2 ± 2·1	5·1 ± 2·9	–	5·4 ± 3·9

[a] Error limits represent standard deviations (n ≥ 4).
* $P < 0·001$ using Student's *t*-test.
** $P < 0·05$.

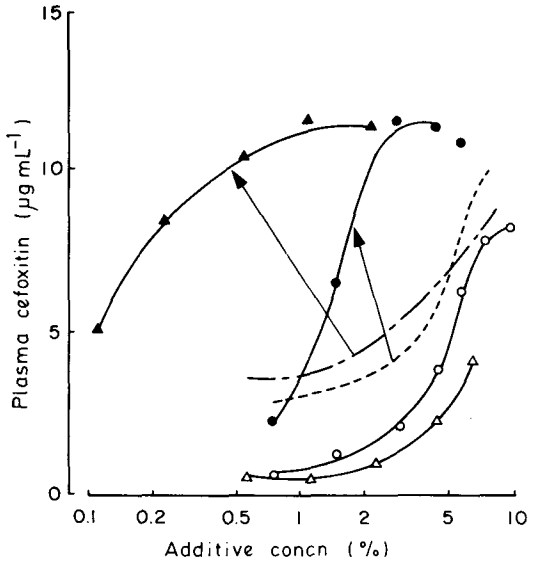

FIG. 2. Peak plasma levels of cefoxitin found following administration of sodium salicylate and disodium EDTA in combination. Effects are shown of various concentrations of salicylate (○) and EDTA (△) alone and with 22·5 mg mL^{-1} EDTA (●) and 45 mg mL^{-1} kg^{-1} salicylate (▲) added. The expected plasma levels of cefoxitin obtained from summing the effects of EDTA and salicylate given individually are shown by the dotted lines.

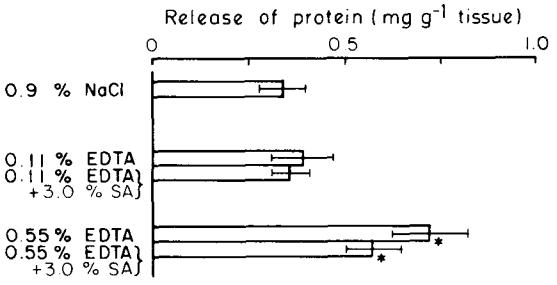

FIG. 3. Effects of disodium EDTA and combinations of disodium EDTA and sodium salicylate on the release of protein from mucosal membrane using an everted rat rectal sac technique following 1 h incubation at 30 °C. The error bars represent standard deviations with n ≥ 4. * represents $P < 0·001$ using Student's *t*-test with 0·9% NaCl as reference.

dramatic when a low dose of EDTA was administered with salicylate.

Fig. 3 shows the extent of protein release from mucosal membranes using an in-vitro everted sac technique. The presence of 0·11% EDTA (3×10^{-3} M) and 3·0% sodium salicylate (0·187 M) resulted in protein release to about the same extent as 0·9% NaCl (saline), while 0·55% EDTA (0·015 M) with 3% sodium salicylate showed only moderate increases in protein release compared with saline.

DISCUSSION

It has been suggested that sulphydryl blockers such as N-ethylmaleimide (NEM) and *p*-chloromercuriphenylsulphonate (*p*-CMP) inhibit the facilitated diffusion of drugs such as cyclocillin and amoxycillin by interactions in the protein fraction, particularly with sulphydryl groups within the mucosal membrane of the small intestine (Kimura et al 1978). Since the enhanced rectal absorption of cefoxitin when coadministered with sodium salicylate was suppressed

significantly by the presence of low doses of the sulphydryl blockers NEM and *p*-CMP, it is tempting to suggest that the adjuvant action of salicylate may involve facilitated diffusion of cefoxitin through the rectal mucosal membrane. The observation that NEM and *p*-CMP had little if any effect on the adjuvant action of disodium EDTA and polyoxyethylene-23-lauryl ether (POE) confirms the report (Nishihata et al 1985) that EDTA and POE caused irreversible damage to the mucosal membrane and enhanced the simple diffusion of cefoxitin by reducing the membrane barrier.

As can be seen from the data in Fig. 1, the suphydryl blockers appear to have different effects depending on concentration. At low concentrations ($<0{\cdot}5$ mg mL^{-1}) in the presence of salicylate, NEM and *p*-CMP reduce cefoxitin absorption compared with salicylate alone, whereas at higher concentrations (>1 mg mL^{-1}) cefoxitin plasma levels increase perhaps due to increased simple diffusion through the mucosal membrane in which the protein fraction has been disrupted. The integrity of the protein fraction in the membrane may involve interactions with S–S bonds, and sulphydryl blockers have been reported to disrupt S–S bonds in studies on the cause of ulcer formation (Szabo et al 1981).

Fujita et al (1971) reported that Na^+- or K^+-ATPase had little activity in the microvillus membrane but high activity in the basolateral membrane of epithelial cells in the rat small intestine. If similar Na^+-, K^+-ATPase activity is found in the rectal epithelial cells, the inhibitory effects of DNP and ouabain on cefoxitin absorption in the presence of adjuvants may occur when cefoxitin is transported through basolateral membranes. Since the action of the surfactant, POE, particularly at the higher concentrations studied, was not inhibited by DNP or ouabain (Tables 1, 2), it appears that its enhancing effects may involve increased simple diffusion due to disruption of the microvillus membrane by the surfactant without involving physiological factors (Nishihata et al 1985).

At low concentrations of EDTA, ouabain inhibited the action of EDTA almost completely whereas at higher EDTA concentrations only partial inhibition was observed. This suggests that at low concentrations of EDTA, the enhancement of cefoxitin absorption by EDTA occurs predominantly by increasing the permeability of the paracellular route of absorption, as has been reported by Palmora et al (1980). At higher concentrations of EDTA, enhancement of cefoxitin absorption by EDTA may also involve increased simple diffusion due to disruption of the microvillus membrane as in the case of POE.

Since all of the inhibitors studied, NEM, *p*-CMP, DNP and ouabain, suppressed the enhancing action of salicylate, salicylate may increase the absorption of cefoxitin via both the transcellular and paracellular routes. Ouabain and DNP may suppress the increase in paracellular permeability caused by salicylate, whereas *p*-CMP and NEM may suppress the increase in transcellular route permeability to cefoxitin found in the presence of salicylate. The adjuvant action of salicylate in enhancing transcellular route permeability may involve the binding of salicylate to the cell membrane (Kajii et al 1975; Nishihata & Higuchi 1984; Nishihata et al 1984a).

The study of the effects of administering combinations of the three adjuvants showed that of the three possible combinations, only the mixture of EDTA and salicylate had greater activity when administered together than the sums of their activities when administered individually. As shown in Fig. 2, even a very low concentration of EDTA significantly increased the effect of salicylate on plasma cefoxitin levels when administered concurrently. Nishihata et al (1985) suggested that the adjuvant action of EDTA and salicylate occurs in the protein fraction of the membrane whereas POE appears to affect the lipid layer of the membrane. If such is the case, the findings reported here may indicate that a combination of adjuvants which affect the same target with different mechanisms of action will synergistically enhance their adjuvant action. A possible reason for the increased effectiveness of salicylate and EDTA together may be that although EDTA does not penetrate the membrane barrier readily by itself, salicylate enhances the permeation of EDTA into the membrane where EDTA may have a profound effect on the characteristics of the protein fraction and consequently increase permeation. Furthermore, as shown in Fig. 3, 0·11% EDTA with 3·0% salicylate did not appear to cause severe damage to the mucosal membrane as reflected by the amount of protein released from the membrane. Used in conjunction with salicylate, EDTA can be effective at a sufficiently low concentration that substantial protein release from the mucosal membrane does not occur, in contrast to the situation with high concentrations of EDTA as reported earlier (Nishihata et al 1985).

It appears that a combination of two adjuvants such as salicylate and EDTA may have potential merit in enhancing rectal absorption of poorly absorbed drugs, such as cefoxitin, which have high water solubility.

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