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Comparison of the effects of sodium salicylate, disodium ethylenediaminetetraacetic acid and polyoxyethylene-23-lauryl ether as adjuvants for the rectal absorption of sodium cefoxitin

TOSHIAKI NISHIHATA*, HISAO TOMIDA, GREGORY FREDERICK, J. HOWARD RYTTING† AND TAKERU HIGUCHI

Pharmaceutical Chemistry Department, The University of Kansas, Lawrence, Kansas 66045 USA

Sodium salicylate, disodium ethylenediaminetetraacetic acid (EDTA) and polyoxyethylene-23-lauryl ether (POE) significantly enhanced the absorption of cefoxitin from the rectum but with the following differences. (1) The effectiveness of salicylate or EDTA was enhanced by sodium chloride, whereas the activity of POE was not. (2) Although the ratios of plasma cefoxitin peak values to cefoxitin dose were constant with POE or EDTA, the peak to dose ratios with salicylate decreased with increasing cefoxitin concentration. (3) Phlorizin and 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) inhibited the effectiveness of salicylate, but did not influence the adjuvant action of either POE or EDTA. (4) Although treatment with salicylate resulted in slightly less protein release than treatment with NaCl, both POE and EDTA increased the release of protein from the rectal mucosa. It appears that the effects of salicylate occur at the protein fraction of the rectal mucosa through a saturable process whereas the adjuvant action of POE and EDTA appears to involve some irreversible disruption of the membrane.

The coadministration of surfactants to increase the absorption of poorly absorbed drugs after both oral and rectal administration has been reported (Touitou et al 1978, 1980: Ichikawa et al 1980).

Recently the effect of sodium salicylate and several related compounds in enhancing the absorption of fairly water soluble drugs (including antibiotics such as cefoxitin and polypeptides such as insulin) from the small intestine and rectum, has been reported (Nishihata et al 1981a, b, 1982, 1983). Earlier, Kunze et al (1972) suggested that salicylic acid was absorbed more rapidly than would be expected from the pH-partition hypothesis. Salicylate is known to interact with divalent metal ions, and the inactivation of some metallo-enzymes by salicylate has been attributed to chelation (Grisolia et al 1970). However, since the stability constant for the salicylate-Ca^{2+} complex is low, 0·15 (Schubert 1954), chelation with the Ca^{2+} at the surface of the intestinal membrane probably does not account for the total enhanced absorption of salicylate.

Ethylenediaminetetraacetic acid (EDTA) was found (Kunze et al 1972) to increase the absorption of salicylate but to decrease the absorption of *m*- and *p*-hydroxybenzoic acids. Since EDTA is a strong chelating agent, it is possible that its absorption-enhancing ability may be related to its ability to chelate metal ions in the membrane, thereby increasing permeability. The present study compares the adjuvant action of salicylate, a weak chelating agent; EDTA, a strong chelating agent; and polyoxyethylene-23-lauryl ether, a surfactant, in an attempt to further understand the differences in the mechanisms of each of their activities.

MATERIALS AND METHODS

Sodium salicylate (Sigma), disodium EDTA (Sigma), 4,4-diisothiocyanostilbene-2,2′-disulfonic acid (Sigma), phlorizin (Sigma) and polyoxyethylene-23-lauryl ether (Brig 35, ICI Americas) were obtained commercially. Sodium cefoxitin was supplied by Merck Sharp and Dohme.

In the in-vivo studies, the drug and adjuvants were administered as microenemas prepared with distilled water. The pH was controlled at 5·5 to 6·0 by adding 0·01 M HCl or 0·01 M NaOH as necessary. Male Sprague-Dawley rats (200–225 g) were fasted for 16 h before the experiments. During the experiment, the rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}) and kept at 38 °C. Microenemas

* Present address: Faculty of Pharmaceutical Sciences, Osaka University, 1–6 Yamadaoka, Suita, Osaka 565, Japan.
† Correspondence.

were administered at a depth of 1 cm from the anus, via polyethylene tubing (PE 50), in a volume of 1·0 ml kg^{-1}. Blood samples were taken from the jugular vein at designated times and centrifuged at 3000 rev min^{-1} for 10 min to collect the plasma.

In-situ loop studies were carried out by the method of Levine et al (1955). Approximately, a 4 cm section of the rectum was tied at both ends to prepare the loop. After each experiment, the loop was weighed and found to have an average weight of 512 ± 37 mg. At an appropriate time following administration of the drug solution, the loop was removed from the rat's body and the contents collected by washing with distilled water. The collected solution was diluted to 10 ml with distilled water and the concentration of drug was measured to determine the amount of drug remaining in the loop.

An in-vitro everted sac technique, as described by Barr & Riegelman (1970), was used to determine the protein released from the mucosal membrane. In these studies a 5 ml solution containing adjuvant was incubated with the rectal everted sac at 37 °C for 1 h. Following this, the solution was dialysed with 500 ml of distilled water at 4 °C for 12 h. This volume of water was used because a high concentration of salicylate interfered with the assay for protein.

Following dialysis, the amount of protein in the sample was determined using an analytical kit supplied by Sigma. The assay of cefoxitin was carried out by high pressure liquid chromatography as described previously (Nishihata et al 1983).

RESULTS

Each of the three adjuvants studied, polyoxyethylene-23-lauryl ether (POE), sodium salicylate, and disodium ethylenediaminetetraacetic acid (EDTA), significantly increased plasma cefoxitin levels after rectal administration as a microenema (Fig. 1). A good linear correlation was found for the plasma peak values and the areas under the curve of cefoxitin concentration over 3 h, as determined using the trapezoidal method (Fig. 2). Therefore, peak plasma values were used as an indicator for relative adjuvant effectiveness.

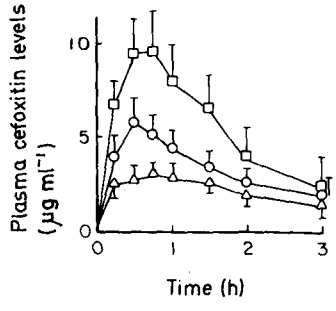

FIG. 1. Plasma concentration of cefoxitin after rectal administration of a dose of 15 mg kg^{-1} ml^{-1} in a microenema containing 60 mg kg^{-1} sodium salicylate (○), 44 mg kg^{-1} disodium EDTA (△) or 10 mg kg^{-1} POE (□). Error bars represent standard deviations with n ≥ 6.

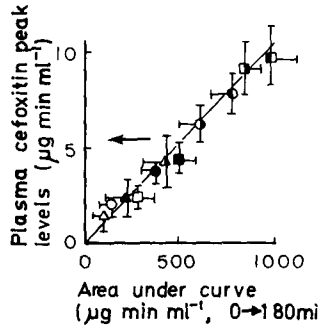

FIG. 2. Relationship between peak concentrations of cefoxitin in plasma and the area under the curve of cefoxitin concentration in plasma 3 h after rectal administration of cefoxitin at a dose of 15 mg kg^{-1} in microenemas containing the following additives: sodium salicylate at doses of 30·0 mg kg^{-1} (◐), 45·0 mg kg^{-1} (●), 60·0 mg kg^{-1} (◑) and 75·0 mg kg^{-1} (○); disodium EDTA at doses of 22·25 mg kg^{-1} (△), 44·5 mg kg^{-1} (▲) and 66·75 mg kg^{-1} (▲); or POE at doses of 5·0 mg kg^{-1} (□), 6·0 mg kg^{-1} (■), 8·0 mg kg^{-1} (◨) and 10·0 mg kg^{-1} (◧). Error bars represent the standard deviations with n ≥ 6.

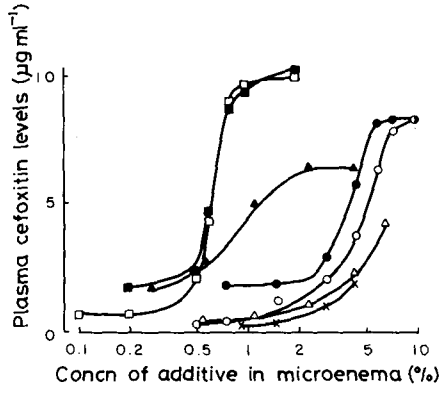

FIG. 3. Dose response profiles for sodium salicylate (○, ●), disodium EDTA (△, ▲), POE (□, ■) and sodium chloride (×) on the plasma peak cefoxitin concentration after rectal administration of cefoxitin at a dose of 15 mg kg^{-1} ml^{-1} in a microenema. The open symbols (○, △, □) represent microenemas without sodium chloride, and the closed symbols (●, ▲, ■) represent microenemas with sodium chloride to adjust the ionic strength to 0·75. The error bars represent standard deviations with n ≥ 4).

As shown in Fig. 3, the effectiveness of the adjuvants showed a sigmoidal dependence upon their concentration. It appears that there is a minimum effective concentration and a maximum concentration after which no significant increase in

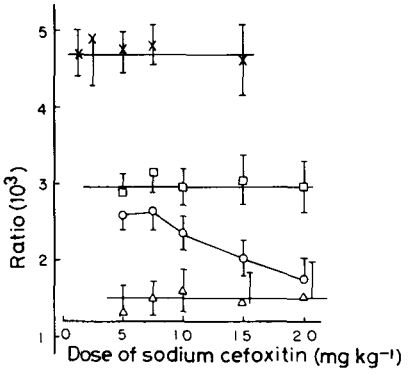

FIG. 4. Ratio of the plasma peak values of cefoxitin to dose of cefoxitin, plotted as a function of cefoxitin concentration, 15 min after i.v. injection (×) and after rectal administration of a microenema containing 60 mg kg^{-1} of sodium salicylate (○), 66·75 mg kg^{-1} disodium EDTA (△) or 8 mg kg^{-1} POE (□). The error bars represent standard deviations with n ≥ 4.

permeability occurs. In those experiments where sodium chloride was added to adjust the ionic strength to 0·75, the adjuvant action of salicylate and EDTA was enhanced, however, the added sodium chloride did not affect the action of POE markedly. The action of EDTA was especially affected by the presence of sodium chloride.

Fig. 4 shows the ratio of plasma cefoxitin peak values to dose of cefoxitin, as a function of the dose of cefoxitin following intravenous and rectal administration with the various adjuvants. The ratio after rectal administration in the presence of salicylate decreased with increasing cefoxitin dose. However, the ratios after i.v. injection of cefoxitin and after rectal administration of cefoxitin with POE or EDTA remained constant with increasing cefoxitin dose.

The enhanced peak plasma cefoxitin concentrations after rectal administration with salicylate as an adjuvant were significantly reduced by the co-administration of 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) or phlorizin (Table 1). However, the enhanced plasma peak values of cefoxitin after rectal administration with either POE or EDTA was not suppressed by either inhibitor.

Table 1. Effect of 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) and phlorizin on the plasma peak values of cefoxitin following its rectal administration (15 mg kg^{-1} ml^{-1}) in microenemas containing each of the following adjuvants.

Concentration of adjuvant in microenema	Plasma peak values of cefoxitin (μg ml^{-1}) Control	DIDS (500 μg ml^{-1})	Phlorizin (1 mg ml^{-1})
No adjuvant	0·2	0·2	0·2
Sodium salicylate (60 mg ml^{-1})	6·2 ± 1·1	2·7 ± 0·7*	3·8 ± 0·6*
Disodium EDTA (66·75 mg ml^{-1})	4·2 ± 1·3	4·5 ± 0·7	4·8 ± 1·5
POE (8 mg ml^{-1})	9·1 ± 1·4	9·4 ± 1·6	9·6 ± 1·2

* $P < 0·001$, Student's t-test (n ≥ 6).

Furthermore, as shown in Table 2, the disappearance of cefoxitin from the rectal loop measured by the in-situ loop technique was reduced by the presence of either DIDS or phlorizin in the microenema after administration of cefoxitin with salicylate. The disappearance of cefoxitin when POE or EDTA were adjuvants was not affected by either DIDS or phlorizin.

Table 2. Effect of DIDS and phlorizin on the disappearance of cefoxitin from a rectal loop 30 min following administration of cefoxitin at a dose of 15 mg kg^{-1} ml^{-1} in microenemas containing the following adjuvants using an in-situ rat rectal loop technique.

Concentration of adjuvant in microenema	% Disappearance of cefoxitin from rectal loop Control	DIDS (500 μg ml^{-1})	Phlorizin (1 mg ml^{-1})
No adjuvant	4·5 ± 1·8	4·7 ± 1·1	4·2 ± 1·5
Sodium salicylate (60 mg ml^{-1})	48·5 ± 6·2	18·3 ± 4·2*	29·5 ± 5·8*
Disodium EDTA (66·75 mg ml^{-1})	36·9 ± 9·6	40·5 ± 7·3	38·7 ± 7·2
POE (8 mg ml^{-1})	62·8 ± 8·3	61·7 ± 6·8	64·9 ± 9·1

* $P < 0·001$, Student's t-test (n ≥ 6).

The effects of each of the adjuvants on the release of protein from the rectal membrane as shown by the everted rectal sac technique are in Fig. 5. Compared with 0·9% NaCl (saline), salicylate did not influence the release of protein. However, both EDTA and POE significantly increased protein release. 1·5% sodium salicylate resulted in less protein release than treatment with saline.

DISCUSSION

The three adjuvants, sodium salicylate, disodium EDTA and POE, significantly enhanced the absorption of cefoxitin from the rectum. However, the

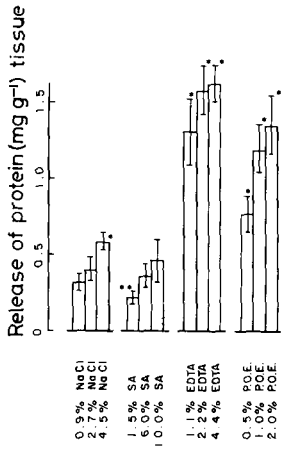

FIG. 5. The effects of various concentrations of sodium chloride (NaCl), sodium salicylate (SA), disodium EDTA (EDTA) and POE on the release of protein from mucosal membrane using an everted rat rectal sac technique following 1 h incubation at 30 °C. The error bars represent standard deviations with $n \geq 4$. * Represents $P < 0{\cdot}001$ and ** $P < 0{\cdot}05$ Student's t-test with 0·9% NaCl as reference.

following differences were observed. (1) The effectiveness of salicylate or EDTA was enhanced by sodium chloride, whereas the activity of POE was not. (2) Although the ratios of plasma cefoxitin peak values to cefoxitin dose following rectal administration with POE or EDTA were constant, the ratio after cefoxitin was given with salicylate decreased with increasing cefoxitin concentration. (3) Phlorizin and DIDS inhibited the effectiveness of salicylate, whereas the inhibitors did not influence the adjuvant action of either POE or EDTA. (4) Although treatment with salicylate resulted in slightly less protein release than treatment with NaCl, both POE and EDTA increased the release of protein from the rectal mucosa.

DIDS has been shown (Cabantchik et al 1978) to inhibit anion transport through the membrane of red blood cells by interacting with the amino groups in the protein fraction (op den Kamp 1981). Phlorizin is also known to inhibit active glucose transport in the small intestine (Alvarado & Crane 1964) and to bind with the Na^+ and D-glucose cotransport in the brush border membrane of the small intestine (Toggenburger et al 1982). Since both DIDS and phlorizin suppressed the adjuvant action of salicylate, it is tempting to suggest that salicylate acts at the protein fraction of the rectal surface membrane by interacting with components in the protein fractions such as amino groups.

Since it was reported by Reynolds & Trayer (1971) that EDTA causes the solubilization of protein from red blood cell ghosts, and it has been demonstrated in this study that EDTA causes the release of protein from the mucosal membrane, it appears that EDTA may also act at the protein fraction in the membrane. However, salicylate appears to cause a temporary change, possibly conformational, in the protein fraction by a reversible interaction with components of the protein fraction, whereas EDTA probably causes some longer-lasting or irreversible changes such as solubilizing or otherwise releasing protein from the membrane. Reynolds & Trayer (1971) demonstrated that 90% of the erythrocyte membrane protein is soluble in EDTA with a significant reduction of the phospholipid to protein ratio in the soluble species. This finding also suggests that the site of action of EDTA is the protein fraction.

Surfactants are known to dissolve lipid and it is possible that POE may cause an increased release of protein from the membrane by its lipid-dissolving action. This is substantiated by the report of Singer & Nicolson (1972) who found protein floating in a lipid bilayer. Thus the adjuvant action of POE may occur primarily at the lipid fraction of the membrane. The fact that sodium chloride enhances the adjuvant effects of salicylate and EDTA, which appear to act at the protein fraction of the membrane, but does not affect the action of POE, further suggests that POE may act at the lipid portion of the membrane.

The ratios of plasma cefoxitin concentration to dose of cefoxitin were relatively constant with increasing cefoxitin concentration when POE and EDTA were used as adjuvants, but the ratio decreased when salicylate was used. This suggests that cefoxitin permeation in the presence of POE and EDTA probably occurs as a simple diffusion since it follows Fick's Law and depends on the concentration of cefoxitin. However, simple diffusion does not account for cefoxitin absorption in the presence of salicylate. One explanation is that although permeation of cefoxitin, as enhanced by POE and EDTA, may occur through apparent non-barrier membranes which were damaged by POE and EDTA, penetration of cefoxitin enhanced by salicylate may occur through some specific interaction at the site of the membrane where DIDS may also act.

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