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Effect of sodium ion on the adjuvant action of sodium salicylate in enhancing rectal ampicillin absorption in rats

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Summary

Sodium chloride facilitated the adjuvant action of sodium salicylate in enhancing rectal ampicillin absorption in rats. This action of sodium chloride involves facilitated absorption of salicylate by sodium ion. That is, enhancement of ampicillin absorption in the presence of salicylate correlated well with the increase of salicylate absortion with increasing concentration of sodium ion. The effect of sodium ion on the action of salicylate was confirmed using the sodium salts of ascorbate instead of sodium chloride. Since a mixture of sodium ascorbate and sodium salicylate showed less disruption of the rectal mucosal surface compared to a mixture of sodium chloride and sodium salicylate, sodium ascorbate seems to be suitable additive for inclusion with sodium salicylate for the formulation of rectal dosage forms of ampicillin.

Introduction

The use of surfactant (Touito et al., 1978; Shichiri et al., 1978) and non-surfactant (Nishihata et al., 1980, 1981a and 1982a; Kamada et al., 1981) adjuvants to enhance drug absorption, especially that of drugs which are poorly absorbed due to their low lipophilicity or high molecular weight, is well documented in the literature. Surfac-

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tants such as sodium lauryl sulfate, however, cause a lasting effect on rectal drug absorption which indicates possible damage to the mucosa (Nishihata et al., 1981b) whereas the enhancing action of such non-surfactant adjuvants as salicylte is eliminated by washing the exposed mucosa following pretreatment by salicylate (Nishihata et al., 1981b; Sithigorngul et al., 1983). Histological examination of rectal mucosa following chronic administration of sodium salicylate revealed no change in the normal appearance (Nishihata et al., 1982b). During studies of the mechanism involved in the adjuvant action of salicylate, the enhancing effect of certain agents (coadjuvants) on the promoting action of salicylate has been observed which increases the options available for devising effective drug delivery systems. These options of high ionic strength (Nishihata et al., 1982c) as well as formulation design (Nishihata et al., 1983a) also had a significant influence on the ability of salicylate to enhance rectal drug absorption. In this paper, methods to optimize the enhancing action of salicylate on the rectal absorption of water-soluble drugs are reported. The sodium ion dependency of salicylate's enhancing action, which was previously reported to exist (Nishihata et al., 1982c), and its relationship to microenema osmolarity as well as the coadjuvant action of the sodium salts of several ascorbate derivatives on salicylate-enhanced rectal absorption of ampicillin are described.

Materials and Methods

Chemicals

Sodium ampicillin was supplied by Toyo Jozo (Tokyo, Japan). Sodium salicylate, sodium ascorbate, sodium isoascorbate, sodium N-acetylascorbate, glucose, 2-deoxyglucose, and 3-*o*-methoxyglucose were purchased from Wako Pure Chemicals (Osaka, Japan). Other reagents used were of analytical grade.

Animals

Wistar male rats, 225–275g, were fasted for 16 h prior to experiments but water was given freely. During the experiment, rats were anesthetized with sodium pentobarbital (60 $mg \cdot kg^{-1}$) and were placed on a warm surface at 38°C.

Preparation of administered form

The drug solution used as microenema was prepared with 0.067 M sodium phosphate buffer at pH 7.0. Osmotic pressure of the drug solution was determined using a freezing point apparatus (Knauer Cryoscopic Unit No. 24.00, F.R.G.) which measured the freezing point depression.

Absorption study

Blood samples were taken from the jugular vein of the rat at the designated time intervals after rectal administration of a microenema. The blood sample was centrifuged at 3000 rpm for 10 min to obtain a plasma sample. An in situ rat rectal loop study using 4 cm of the rectum beginning at the anus was carried out as reported previously (Nishihata et al., 1984a). Drug absorption was determined by

measuring the amount of drug that disappeared from the loop. The wet weight of each loop used in this study was measured after experiments and was between 386 and 403 mg.

Histological study

A study to obtain histological observations was carried out by the method described in detail in a previous paper (Sithigorngul et al., 1983) using an in situ rat rectal loop method.

Assay

Determination of salicylate in plasma was carried out using a HPLC method described in an earlier paper (Nishihata et al., 1981b). Determination of ampicillin in plasma was carried out by microbioassay technique (Murakamin et al., 1981). The effect of sodium salicylate on the microbioassay of ampicillin was examined and it was confirmed that sodium salicylate up to 1.25 μmol · ml^{-1} in plasma did not affect the assay of ampicillin. Therefore, for the in situ loop study, microbioassays of ampicillin were carried out using samples which were diluted with distilled water to less than 0.938 μmoles of sodium salicylate · ml^{-1}.

Results and Discussion

Effect of sodium chloride on salicylate's enhancement of ampicillin absorption in rats

Rectal absorption of ampicillin in rats was not detectable (plasma concentrations

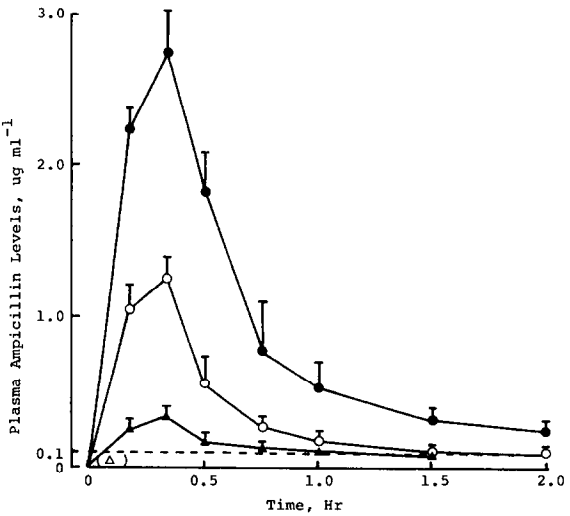

Fig. 1. Plasma ampicillin concentrations after rectal administration of 0.25 ml·kg^{-1} microenemas (containing the additives described below). The microenema contained 30 mg (89.5 μmoles) of sodium ampicillin·ml^{-1} (240–260 mOsm·kg^{-1} H_2O, △) and following additives: 0.27 M sodium chloride (800–820 mOsm·kg^{-1} H_2O, ▲), 0.27 M sodium salicylate (800–820 mOsm·kg^{-1} H_2O, ○) or 0.27 M sodium salicylate and 0.2 M sodium chloride (1200–1300 mOsm·kg^{-1} H_2O, ●). Each value represents the mean ± S.D. ($n \geqq 5$).

less than 0.1 $\mu g \cdot ml^{-1}$) when sodium ampicillin was administered alone at a dose of 30 mg (85.9 μmoles)$\cdot kg^{-1}$. Coadministration of sodium salicylate with ampicillin in a microenema increased the plasma ampicillin concentration significantly as shown in Fig. 1. The addition of sodium chloride to the microenema containing sodium ampicillin and sodium salicylate increased the plasma ampicillin concentration after rectal administration over those in which sodium ampicillin was administered only with sodium salicylate (Fig. 1). This is consistent with our previous report (Nishihata et al., 1982c) of a similar sodium chloride effect on absorption of other water-soluble compounds as enhanced by salicylate.

Sodium chloride coadministered rectally with ampicillin in the absence of sodium salicylate also increased the plasma ampicillin concentration (Fig. 1). Therefore, to further investigate the effect of both sodium salicylate and sodium chloride on the rectal ampicillin absorption, ampicillin absorption was examined by an in situ rat rectal loop method (Fig. 2). As the concentration of sodium chloride in the microenema was increased, rectal ampicillin absorption increased accordingly up to 0.45 M sodium chloride in the microenema, but at concentration of sodium chloride over 0.45 M there was no further increase in ampicillin absorption (Fig. 2). On the other hand, rectal ampicillin absorption increased with increasing concentration of sodium salicylate up to 0.9 M (Fig. 2). This may indicate that sodium chloride enhances rectal ampicillin absorption by disrupting the unstirred water layer which is the diffusion barrier as reported by Kowalewski et al. (1969). Since the maximum absorption of ampicillin observed in the presence of sodium chloride alone was

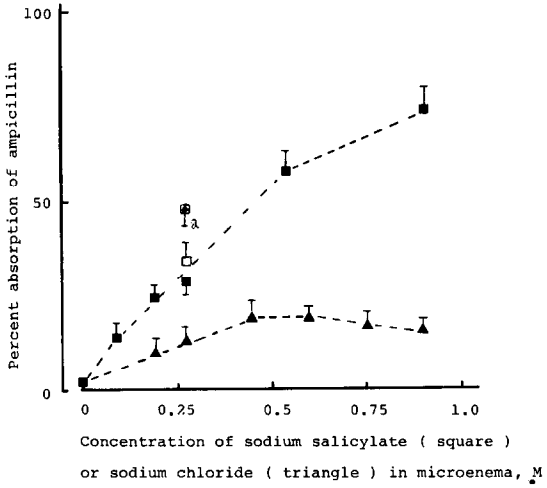

Fig. 2. The effect of concentration of sodium salicylate (square) and sodium chloride (triangle) in the microenema on the rectal absorption of ampicillin 20 min after administration of 0.25 ml microenema containing 30 mg (89.5 μmoles) of sodium ampicillin$\cdot ml^{-1}$. ■ represents the result obtained after administration with 0.27 M sodium salicylate and 0.2 M sodium chloride (see inTable 1). □ represents the results obtained when a microenema containing 0.27M sodium salicylate was administered after pretreatment with 0.45 M sodium chloride (see in Table 1 and text). Each value represents the mean ± S.D. ($n \geq 3$). $^aP < 0.05$ versus □ (Student's *t*-test).

about 20% compared to 75% ampicillin absorption obtained in the presence of 0.9 M sodium salicylate, a change in the permeability of the epithelial membrane may not be the major effect of sodium chloride. The addition of sodium chloride in a microenema containing sodium ampicillin and sodium salicylate also increased the rectal absorption of ampicillin more than administration of ampicillin in the absence of sodium chloride (Figs. 1 and 2). However, when a 0.45 M sodium chloride solution (0.25 ml · loop^{-1}) was administered in the loop and then washed out with 10 ml of 0.067 M phosphate buffer for 5 min 15 min after administration of the

TABLE 1

THE EFFECTS OF OSMOTIC PRESSURE IN MICROENEMAS ADJUSTED WITH VARIOUS ADDITIVES ON THE RECTAL ABSORPTION OF SODIUM SALICYLATE AND SODIUM AMPICILLIN IN RATS USING AN IN SITU RECTAL LOOP METHOD

The absorption was determined by measuring the percent of the drug that had disappeared from the loop 20 min after administration of 0.25 ml of the microenema. The concentration of sodium salicylate and sodium ampicillin were 0.27 M and 30 mg (85.9 μmoles)·ml^{-1}, respectively ($n \geq 3$).

Additive (concentration)	Osmotic pressure (mOsm·kg^{-1} H_2O)	Percent absorbed (mean ± S.D.)	
		Sodium salicylate	Sodium ampicillin
(1) no additive	800– 820	40.2 ± 5.3	28.6 ± 3.1
(2) pretreatment [a] with 0.45 M sodium chloride	800– 820	44.6 ± 6.1	33.4 ± 4.8
(3) sodium chloride (0.2 M)	1200–1300	58.3 ± 6.2 [b]	47.3 ± 4.3 [b]
(4) glucose (0.4 M)	1200–1300	37.5 ± 4.6	24.7 ± 3.9
(5) 2-deoxyglucose (0.4 M)	1200–1300	39.4 ± 5.8	25.3 ± 2.4
(6) 3-*o*-methoxyglucose (0.4 M)	1200–1300	36.8 ± 7.3	26.2 ± 3.6
(7) choline chloride (0.2 M)	1200–1300	41.7 ± 6.2	32.8 ± 4.6
(8) potassium chloride (0.2 M)	1200–1300	48.1 ± 3.1 [c]	40.7 ± 4.8 [e]
(9) sodium ascorbate (0.2 M)	1200–1300	56.4 ± 7.2 [b]	45.7 ± 6.3 [b]
(10) sodium chloride (0.7 M)	2100–2300	45.1 ± 3.8	33.4 ± 3.2
(11) glucose (1.4 M)	2100–2300	29.6 ± 4.3 [d]	18.6 ± 3.2 [e]
(12) 2-deoxyglucose (1.4 M)	2100–2300	28.3 ± 5.4 [d]	19.1 ± 4.1 [e]
(13) 3-*o*-methoxyglucose (1.4 M)	2100–2300	26.2 ± 7.5 [d]	17.3 ± 3.6 [d]

[a] Method of pretreatment with 0.45 M NaCl is described in text.
[b] $P < 0.001$ versus (1).
[c] $P < 0.1$ versus (1).
[d] $P < 0.005$ versus (1).
[e] $P < 0.01$ versus (1).

sodium chloride (this pretreatment process was carried out to disrupt the unstirred water layer and the solution remaining in the loop was removed with a cotton fiber before administration of ampicillin microenema), coadministration of sodium salicylate increased the rectal ampicillin absorption but not as much as was observed when a combination of sodium chloride and sodium salicylate were used (Fig. 2 and Table 1). This result seems to indicate that the effect of sodium chloride when coadministered with sodium salicylate facilitates the action of salicylate through some metabolism other than disrupting the unstirred layer as suggested above.

To determine whether the action of sodium chloride on salicylate's adjuvant activity is due to hypertonicity, three monosaccharides (glucose, 2-deoxyglucose and 3-*o*-methoxyglucose) were each used in place of sodium chloride to render the microenema hypertonic. 2-Deoxyglucose and 3-*o*-methoxyglucose were employed since neither compound is consumed as an energy donor in living cells.

The osmotic pressure of a microenema containing 0.27 mmoles sodium salicylate and 30 mg (85.9 μmoles) sodium ampicillin in 1.0 ml of 0.067 M phosphate buffer was 800–820 mOsm · kg^{-1} H_2O. When the osmotic pressure in the above microenema was adjusted to 1200–1300 mOsm · kg^{-1} H_2O with sodium chloride (final concentration in the microenema was 0.2 M), the enhancing action of salicylate on ampicillin absorption was significantly facilitated, whereas monosaccharides (final concentration in microenema was 0.4 M) added to adjust microenema osmolarity, did not influence the enhancing action of salicylate (Fig. 3). The adjustment of osmolarity of a microenema containing 0.54 mmoles sodium salicylate and 30 mg (85.9 μmoles) sodium ampicillin in 1 ml (1200–1300 mOsm · kg^{-1} H_2O) to 2100–2300

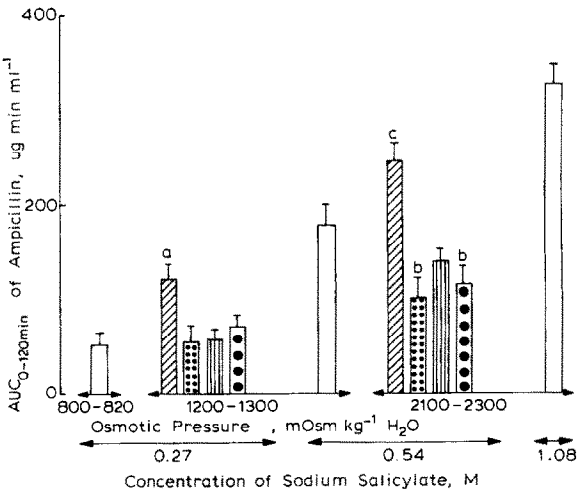

Fig. 3. The effect of osmotic pressure adjusted with the following additives: sodium chloride (□), glucose (▨), 2-deoxyglucose (▥), and 5-*o*-methoxyglycose (▩), on the area under the plasma ampicillin concentration curve for 120 min ($AUC_{0\text{-}120min}$) after rectal administration of a 0.25 ml · kg^{-1} microenema containing various concentrations of sodium salicylate (□). Each value represents the mean ± S.D. ($n \geq 4$). $^aP < 0.001$ versus without additive; $^bP < 0.05$ versus without additive; $^cP < 0.1$ versus without additive.

mOsm · kg^{-1} H_2O with sodium chloride (final concentration in microenema was 0.45 M) also facilitated the adjuvant action of salicylate. However, when the osmolarity of microenema was adjusted to 2100–2300 mOsm · kg^{-1} H_2O with each monosaccharide (final concentration in microenema was 0.9 M), salicylate-enhanced absorption of ampicillin decreased somewhat. The above results indicate that the facilitating action of sodium chloride on the adjuvant action of sodium salicylate is not due to an increase in osmolarity of the microenema administered.

Other studies in our laboratories (unpublished data) have shown that the administration of hypertonic microenema prepared with mannitol up to 5-times isotonicity did not affect the apparent net water flux in the rat rectal compartment but that a further increase in osmotic pressure over 5-times isotonicity in the administered solution did cause an apparent net water outflux. Since the osmolarity of an isotonic microenema is about 280 mOsm · kg^{-1} H_2O, an increase in osmolarity to 2100–2300 mOsm · kg^{-1} H_2O is an 8-fold increase over the isotonic level. This high osmotic pressure may cause apparent net water outflux and may provide a possible explanation for the suppressing effect of monosaccharides on salicylate's absorption promoting activity. Water outflux may have a diluting effect on the concentration of ampicillin and salicylate in the rectal compartment resulting in decreased effective-

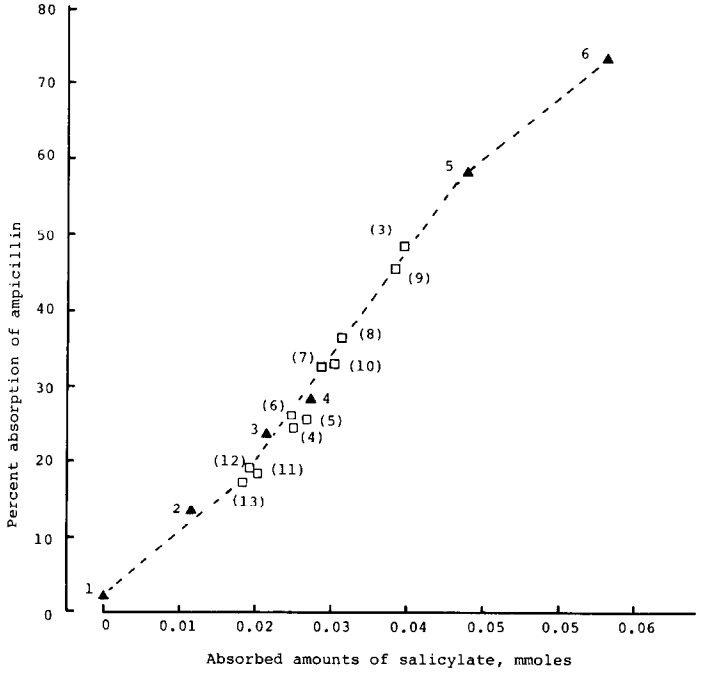

Fig. 4. Relationship between the amount of salicylate absorbed and the percent absorption of ampicillin after administration of a 0.25 ml microenema. Results were obtained from the data in Fig. 2 and Table 1 using the in situ loop method. ▲ represents values from Fig. 2 and numbers represent the concentration of sodium salicylate: 1 = 0 M; 2 = 0.09 M; 3 = 0.18 M; 4 = 0.27 M; 5 = 0.54 M; 6 = 0.9 M. □ represents values from Table 1 and each number corresponds to the same number in Table 1.

ness of sodium salicylate since it has been reported that enhanced rectal absorption of water-soluble compounds depends on the concentration of salicylate at the absorption site (Nishihata et al., 1981b). It was also observed that an increase in microenema volume with a constant dose of sodium salicylate and ampicillin decreased the rectal absorption of ampicillin (Nishihata et al., 1984b).

The rectal absorption of sodium salicylate was not affected when the microenema (containing 0.27 M sodium salicylate and 30 mg (85.9 μmoles) of sodium ampicillin · ml^{-1}) osmolarity was increased from 800–820 to 1200–1300 mOsm · kg^{-1} H_2O with monosaccharide (0.40 M: final concentration) but was significantly suppressed when the osmolarity was adjusted with a monosaccharide to 2100–2300 mOsm · kg^{-1} H_2O as shown in Table 1. The adjustment of osmolarity to 1200–1300 mOsm · kg^{-1} H_2O with sodium chloride (0.20 M), however, caused an increase of sodium salicylate absorption compared to that obtained without sodium chloride (Table 1). Rectal absorption of salicylate administered after pretreatment with 0.45 M sodium chloride was increased only slightly compared to that without pretreatment. The relationship between the amount of sodium salicylate absorbed and the percent of sodium ampicillin absorbed was plotted in Fig. 4 from the results shown in Fig. 2 and Table 1. From the relationship in Fig. 4, it appears that an increase in salicylate absorption increases ampicillin absorption from the rectum. The enhancing action of sodium chloride appears to be due to increase of the salicylate absorption from the loop (luminal surface area is quite constant in this study, since the wet weight of each loop was nearly constant as described in the experimental section).

The suppressed action of the monosaccharides when administered in microenemas with 2100–2300 mOsm · kg^{-1} H_2O is due to decreased salicylate absorption. Salicylate's adjuvant action on absorption of ampicillin was found to be directly dependent on the amount of sodium salicylate absorbed.

Coadjuvant action of sodium ascorbate derivatives on salicylate-enhanced rectal absorption of ampicillin

The data reported above suggests that the adjuvant action of sodium salicylate is influenced by the presence of sodium ion (Nishihata et al., 1982c). In this section, we further examine this sodium ion dependency by using the sodium salt of ascorbate derivatives.

Coadministration of potassium chloride in a microenema with ampicillin and salicylate enhanced salicylate absorption and increased the adjuvant action of salicylate on ampicillin absorption (Fig. 5 and Table 1). The action of the potassium ion was weaker than that of sodium ion. Since it has been reported that intestinal absorption of salicylate in the presence of sodium ion was greater than in the presence of potassium ion (Mayersohn and Gibaldi, 1969), the reduced action of potassium chloride compared to sodium chloride may be due to reduced absorption of salicylate in the presence of potasium ion compared to that in the presence of sodium ion (Fig. 4). Further, choline chloride coadministered in a microenema neither affected the adjuvant action of salicylate nor enhanced salicylate absorption (Table 1, and Figs. 4 and 5). This may confirm that the presence of sodium ion itself is an important factor in the facilitation of salicylate's adjuvant action although it

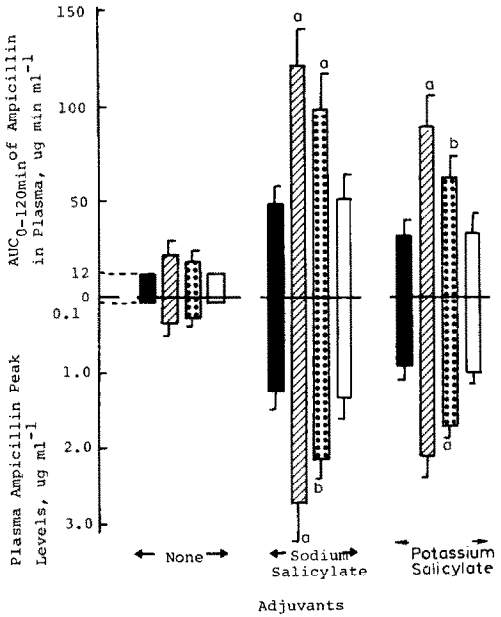

Fig. 5. The effects of sodium chloride (0.2 M, ▨), potassium chloride (0.2 M, ▩) and choline chloride (0.2 M, □) as additive on the rectal absorption of ampicillin as indicated by plasma peak concentration and AUC after administration of 0.25 ml microenemas containing 30 mg (89.5 μmoles) of sodium ampicillin · ml^{-1}. Three kinds of microenemas were examined; with no adjuvant (none), with sodium salicylate (0.27 M), and with potassium salicylate (0.27 M). The symbol, ■, represents the result obtained with an adjuvant but without other additives. The osmotic pressure of the microemnema was adjusted to 1200–1300 mOsm·kg^{-1} H_2O with additive. The osmotic pressure with no additive was 250–280 mOsm·kg^{-1} H_2O and was 800–820 mOsm·kg^{-1} H_2O for microenema containing sodium or potassium salicylate. Each value represents the mean ± S.D. ($n \geqq 4$). $^{a}P < 0.001$ versus no additive; $^{b}P < 0.05$ versus no additive.

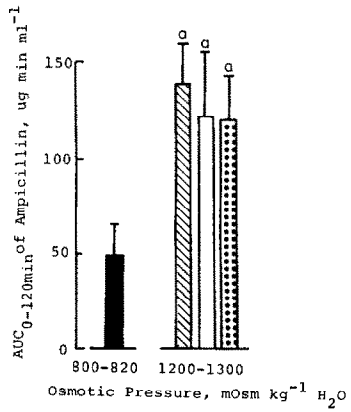

Fig. 6. The effect of sodium ascorbate (▧), sodium isoascorbate (□) or sodium N-acetylascorbate (▩) on the rectal absorption of ampicillin as indicated by AUC after administration in microenema containing 30 mg (89.5 μmoles) of ampicillin · ml^{-1} and 0.27 M sodium salicylate (■) with a volume of 0.25 ml · kg^{-1}. $^{a}P < 0.001$ against additive.

TABLE 2

THE EFFECTS OF VARIOUS ADDITIVES IN THE MICROENEMA ON THE SURFACE INTEGRITY OF THE RAT RECTAL MUCOSA AS DETERMINED BY LIGHT MICROSCOPY

Additive	Percent disrupted surface [a]
(1) Control [b]	0.91 ± 0.75
(2) Sodium salicylate [c]	1.14 ± 0.47
(3) Sodium salicylate [c] and sodium chloride [d]	7.38 ± 2.13 [e]
(4) Sodium salicylate [c] and sodium ascorbate [d]	3.61 ± 0.58 [e,f]

[a] The percent disrupted surface refers to the length of measured surface which was discontinuous as a percentage of the total distance measured.
[b] Control; administration of buffer solution without additive.
[c] Concentration of sodium salicylate; 0.27 M.
[d] Concentration of sodium chloride and sodium ascorbate; 0.30 M.
[e] $P < 0.001$ versus (1).
[f] $P < 0.01$ versus (3) (Student's t-test, n = 6).

should be noted that rectal administration of potassium salicylate also enhanced plasma ampicillin concentration (Fig. 5). This enhancement using potassium chloride, however, was markedly less when using the sodium salt of salicylate. To further study the sodium ion dependency of the adjuvant action of salicylate, sodium ascorbate, sodium isoascorbate and sodium N-acetylascorbate were added in salicylate/ampicillin microenema as coadjuvants. Since the coadjuvant action of these three sodium salts of ascorbate derivatives was similar to that of sodium chloride (Fig. 6), it may be that the presence of sodium ion is responsible for the effect of the analogous sodium ascorbate.

Although it has been reported that salicylate in the concentrations needed for adjuvant effectiveness does not cause damage to the rectal mucosa (Nishihata et al., 1982b), a hypertonic solution prepared with sodium chloride caused a disrupting in the rectal mucosa. Sodium ascorbate, however, had a much smaller effect (Table 2). This may suggest that sodium ascorbate may be more suitable as a coadjuvant for salicylate than sodium chloride is.

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