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Enhancement of Rectal Absorption of Ampicillin by Sodium Salicylate in Rabbits

TOSHIAKI NISHIHATA,* TOMOKO SAKAKURA, MIRE HITOMI, MASARU YAMAZAKI and AKIRA KAMADA

Faculty of Pharmaceutical Sciences, Osaka University, 1–6 Yamadaoka, Suita, Osaka 565, Japan

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The enhancing action of sodium salicylate on rectal ampicillin absorption in rabbits showed a strong dependency on the concentration of sodium salicylate in the administered solution. Maximum bioavailability of ampicillin obtained when increasing sodium salicylate concentration was 60 to 70%. Although maximum bioavailability of ampicillin administered as a suppository was also 60 to 70%, the suppository formulation required a significantly lower dose of sodium salicylate compared to the solution, suggesting that the effective fluid volume available to dissolve drugs in rabbits might be very small. Further, sodium ion might enhance the adjuvant action of sodium salicylate.

Keywords——sodium salicylate; sodium ampicillin; rectal absorption; adjuvant; microenema; suppository; pH; sodium ion

Recently, it has been reported that sodium salicylate markedly enhances the rectal absorption of many drugs which penetrate poorly through the rectal membrane owing to unfavorable physicochemical properties such as low lipophilicity[1-3] and/or large molecular size.[4,5] Although it has been also reported that the adjuvant action of salicylate depends on its concentration in the perfusate in *in situ* rat rectal perfusion, it is necessary to clarify which factor is essential for the adjuvant action of salicylate, its amount or its concentration in the formulation, and how its enhancing action is influenced by the formulation factors in *in vivo* studies. Furthermore, in a study of rectal absorption of insulin, it was shown that an aqueous microenema preparation of insulin with salicylate showed a better bioavailability than did a triglyceride suppository, owing to poor dissolution of insulin from the suppository.[5] The dissolution process of insulin seems to be the limiting step for insulin absorption after administration of the suppository, dissolution of poorly lipophilic small molecular drugs such as β-lactam antibiotics may not regulate their absorption due to their very high water solubility. Thus, it is necessary to clarify the availability of microenema preparation of other nonmacromolecular drugs such as ampicillin, which is also unfavorable for rectal delivery because of its poor permeability through the rectal mucosa.[6]

In the present study, as a preformulation study, factors influencing the adjuvant action of salicylate on the rectal absorption of ampicillin were studied. Further, a comparison between suppository and microenema formulations was made.

Experimental

Materials——Sodium ampicillin was supplied by Toyo Jozo Co., Ltd. (Tokyo, Japan) and sodium salicylate was purchased from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). Other reagents used were of analytical grade.

Preparations for Rectal Delivery——Microenemas were prepared by dissolving sodium salicylate and sodium ampicillin in distilled water or 0.05 M phosphate buffer at various pH values. Suppositories were prepared by a fusion method as described in a previous paper using triglyceride base (Witepsol H-15, Chemische Werk, Witten,

Germany).[7]

Animal Study——Albino male rabbits, 3.0 to 3.5 kg, were fasted for 16 h prior to experiments. To restrict movement, rabbits were confined in boxes in a crouching posture throughout the experimental period. Administration of the microenema was carried out with a plastic syringe attached to polyethylene tubing (PE 50). After rectal administration of one of the formulations, blood was taken from the marginal ear vein at designated time intervals and the whole blood was centrifuged at 3000 rpm for 10 min. The plasma samples were kept at 0 °C until assay. The concentration of ampicillin was determined by a microbiological assay method as described in a previous paper[8] and salicylate concentration was determined by an HPLC method as also described in a previous paper.[9]

Results and Discussion

It has been reported that ampicillin is not absorbed well from rabbit rectum because of its low lipophilicity.[8] In a preliminary experiment, ampicillin was not detected in plasma (less than 0.2 μg/ml) after the rectal administration of sodium ampicillin at a dose of 15 mg/kg as either a microenema or a suppository without sodium salicylate.

Coadministration of sodium salicylate produced a significant absorption of ampicillin (Fig. 1). The plasma ampicillin levels showed a dependency on the dose of salicylate when the drug was administered as the microenema.

As shown in Fig. 2, a linear relationship between the peak plasma level and the area under the curve of plasma ampicillin concentration ([AUC]) for 120 min after administration was observed for both microenema and suppository containing sodium ampicillin and sodium salicylate. Such a correlation is generally observed for rectal absorption of drugs enhanced by adjuvants. Thus, to enhance the rectal absorption of drugs and to sustain plasma levels without producing unnecessarily high peak levels in plasma for clinical or subclinical purposes, it is necesssary to develop a rectal delivery formulation with controlled release rate and extent of enhancing action of adjuvant.

Thus, the enhancing activity of adjuvant should be considered on the basis of two parameters, the peak plasma level and the value of [AUC]. However, in this study, the enhancement of rectal absorption of ampicillin by sodium salicylate was evaluated in terms of

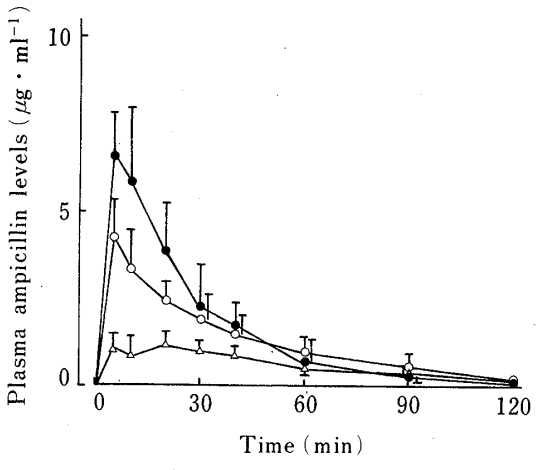

Fig. 1. Plasma Ampicillin Profile as a Function of Time after Rectal Administration of Solution (Dosage Volume; 0.15 ml/kg) at a Dose of 15 mg of Sodium Ampicillin/kg with the Following Dose of Sodium Salicylate; 30 (△), 45 (○) or 60 (●) mg/kg

Each value represents the mean ± S.D. ($n=4$).

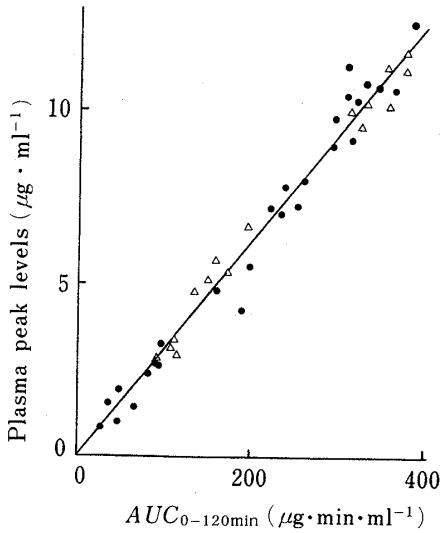

Fig. 2. Relationship between Plasma Peak Ampicillin Level and [AUC] of Plasma Ampicillin for 2 h after Rectal Administration of Sodium Ampicillin at a Dose of 15 mg/kg with Various Doses of Sodium Salicylate in Solution (●) or Suppository (△)

$y=0.032x-0.086$, $r=0.9831$.

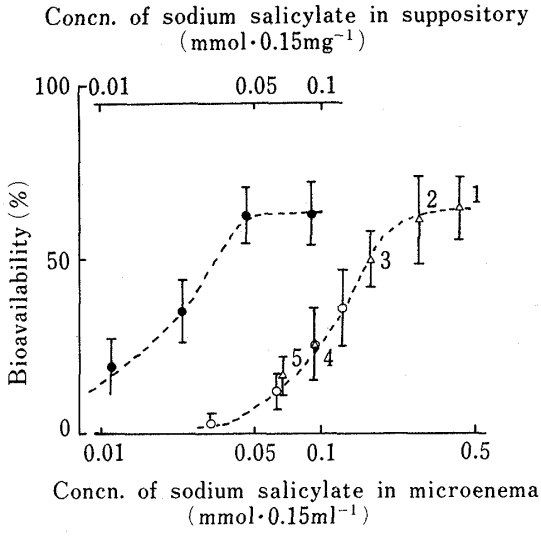

Fig. 3. Effect of Salicylate Concentration in Solution (○ and △) and Suppository (●) on the Bioavailability of Ampicillin after Rectal Administration of Sodium Ampicillin at a Dose of 15 mg/kg

Solution containing various concentrations of sodium salicylate (○) was administered at 0.15 ml/kg or solution (△) containing a constant sodium salicylate dose (0.0938 mmol/kg) was administered in the following volume; 0.033 (1), 0.05 (2), 0.083 (3), 0.15 (4) and 0.25 ml (5)/kg. Suppository (●) was administered at 0.15 g/kg. Each value represents the mean ± S.D. ($n=4$).

the bioavailability of ampicillin calculated from the following equation;

$$([AUC]_{rec} \times (\text{Dose})_{i.v.}/[AUC]_{i.v.} \times (\text{Dose})_{rec}) \times 100$$

where $[AUC]_{rec}$ and $[AUC]_{i.v.}$ represent $[AUC]$ after rectal and intravenous administrations of ampicillin, respectively. The value of $[AUC]_{i.v.}$ for intravenous administration of sodium ampicillin at a dose of 15 mg/kg to rabbits was 542.8 ± 62.5 μg min/ml with a half-life of 20.3 ± 2.1 min ($n=4$) in plasma.

The bioavailability of ampicillin after administration of the microenema increased in a sigmoidal manner with increase of the salicylate concentration (Fig. 3). A maximum bioavailability of 60 to 70% was obtained, which is significantly larger than that of 19.7% obtained for oral administration at the same dose in rabbits.[6]

A similar sigmoidal relationship was observed for the triglyceride suppository with a maximum bioavailability of 60 to 70% (Fig. 3). From these results, it may be concluded that the enhancing activity of salicylate in terms of maximum bioavailability is not influenced by the delivery formulation. However, the enhancing action of salicylate in the suppository was much stronger than that in the microenema. The reason may be as follows: since sodium salts of ampicillin and salicylate are highly water-soluble, these drugs are rapidly released from the suppository, maintaining a high concentration at the absorption site. Because the effective volume of luminal fluid in the rectum is considered to be very small in comparison with the volume of microenema administered, and rapid dissolution of the two drugs occurs after administration, the following equation was applied for estimation of the effective fluid volume in the rectum by comparing the amounts of salicylate required to cause the same degree of absorption of ampicillin from both formulations.

$$(\text{Dose})_{enema}/[(A)_{enema} + (X)] = (\text{Dose})_{supp}/(X)$$

where $(\text{Dose})_{enema}$ and $(\text{Dose})_{supp}$ represent the dose of sodium salicylate in microenema and suppository, respectively, $(A)_{enema}$ represents the dosage volume of microenema and (X) represents the estimated effective luminal volume of rectal fluid.

If the above equation is valid, the effective volume of the rectal fluid is about 30 μl/kg b.w. which is much smaller than the administered volume of microenema, 150 μl/kg b.w. To estimate the rectal luminal fluid volume more practically, a cotton fiber bar (3 mmϕ × 10 mm) was inserted into the rectum to 1 cm depth from anus and was removed and weighed at 30 min after insertion. The fluid weight adsorbed on the cotton was 36.8 ± 9.7 mg/kg b.w. ($n=5$). This results support the effective rectal luminal fluid volume obtained in the above study.

This finding may indicate that only a very small volume of luminal fluid in the rectal lumen is available to dissolve drugs administered as a suppository, and that the concentration of these drugs reaches unexpectedly high values at the absorption site. Thus, the concentration of salicylate at the absorption site may be one of the most important factors in the enhancement of rectal absorption of the drug by the adjuvant.

To clarify whether the enhanced rectal absorption of ampicillin is controlled by the concentration of salicylate alone. The effect of enema volume with the same concentration of sodium salicylate (0.0938 mmol/0.15 ml) at a fixed dose of sodium ampicillin (15 mg/kg b.w.) was studied. As shown in Fig. 4, increase in the volume of microenema resulted in an increasing absorption of ampicillin, but the results were still inferior to those obtained with the suppository. This effect of microenema volume may be explained as follows: increasing the volume caused enlargement of the effective absorption area for ampicillin without changing the concentration of adjuvant, while the concentration of ampicillin decreases at the absorption site.

Further, to clarify whether the dose of ampicillin can affect the absorption at a constant dose of salicylate in a fixed volume of enema, the relationship between the dose of ampicillin and the [*AUC*] was studied.

As shown in Fig. 5, increase of the ampicillin dose resulted in a linear increase of the value of [*AUC*] with some positive deviation at higher doses, suggesting passive absorption of ampicillin in the presence of sodium salicylate in the rectum. However, the bioavailability at each dose remained fairly constant at 25 to 30%. This finding appears to support the importance of salicylate concentration in the rectal lumen for its adjuvant action. A positive deviation from the linear relationship at higher doses of ampicillin may be explained on the basis of the ionic strength of sodium ion in the microenema, because the ionic strength (adjusted with sodium chloride) in the perfusate influenced the rectal absorption of cefmetazole and L-dopa in *in situ* perfusion experiments.[9]

Thus, a further study was performed to clarify the effect of ionic strength (adjusted with sodium chloride). For this purpose, a constant dose of sodium salts of salicylate and ampicillin in various volumes of microenema was administered with or without sodium

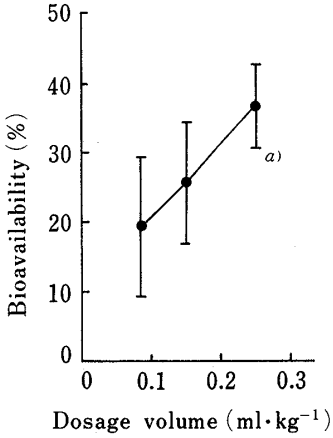

Fig. 4. Effect of Dosage Volume of Solution Containing 0.0938 mmol of Sodium Salicylate/0.15 ml on the Bioavailability of Ampicillin after Rectal Administration of a Solution at a Dose of 15 mg of Sodium Ampicillin/kg

Each value represent the mean ± S.D. ($n=4$). *a*) $p<0.1$ *versus* dosage volume of 0.083 ml/kg (Student's *t*-test).

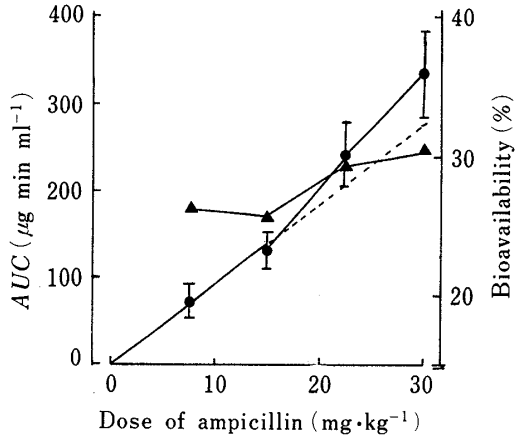

Fig. 5. Relationship between Dose of Sodium Ampicillin and [*AUC*] of Plasma Ampicillin (●) or Bioavailability of Ampicillin (▲) after Rectal Administration of Solution Containing 0.0938 mmol of Sodium Salicylate/0.15 ml at a Dosage Volume of 0.15 ml/kg

Each value represents the mean ± S.D. ($n=4$).

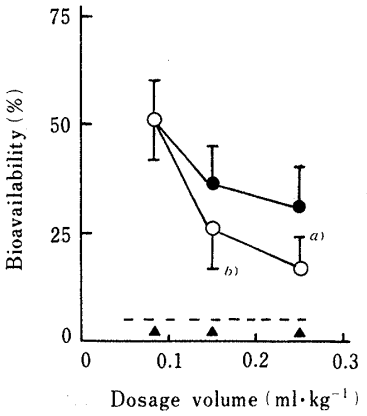

Fig. 6. Effect of Ionic Strength (Adjusted with Sodium Chloride) of the Administered Solution on the Bioavailability of Ampicillin after Rectal Administration of Solution at Various Dosage Volumes with a Constant Dose of Ampicillin (15 mg/kg) and Sodium Salicylate (0.0938 mmol/kg)

●, constant ionic strength; ○, ionic strength was not adjusted; ▲, no sodium salicylate. Each value represents the mean ± S.D. ($n=4$). *a*) $p<0.05$ *versus* dosage volume of 0.1 ml/kg *b*) $p<0.1$ *versus* dosage volume of 0.1 ml/kg.

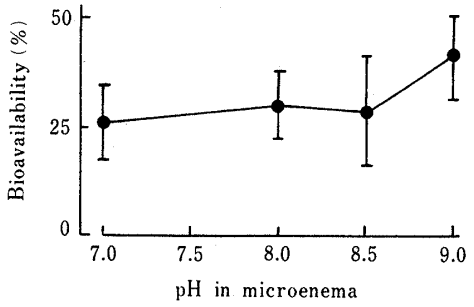

Fig. 7. Effect of pH (Adjusted with 0.05 M Phosphate Buffer) on the Bioavailability of Ampicillin after Rectal Administration of a Solution Containing 15 mg of Sodium Ampicillin and 0.0938 mmol of Sodium Salicylate in 0.15 ml at a Dosage Volume of 0.15 ml/kg

Each value represents the mean ± S.D. ($n=4$).

chloride (Fig. 6). The results without sodium chloride support the importance of adjuvant concentration at the absorption site observed in the above experiment (Fig. 4). At a constant ionic strength (adjusted with sodium chloride) equivalent to that of 0.083 ml of microenema/kg, the bioavailability profiles were slightly higher than those without sodium chloride. As shown in the figure, the effect of sodium chloride at fixed ionic strength without salicylate was negligible irrespective of the volume of a microenema, and a low bioavailability of less than 5% was obtained at any dosage volume.

To explain the effect of sodium chloride on the enhancing action of salicylate, the effect of osmotic pressure of the microenema as well as in the rectal luminal fluid should be considered. However, since it has been reported that addition of sugar[10] or chloine chloride[11] did not facilitate the adjuvant action of sodium salicylate, the effect of sodium chloride on the salicylate action may be due to the action of sodium ion.

Since the microenema containing sodium ampicillin was alkaline with a pH of more than 8, the effect of pH of the microenema as well as that of the rectal fluid after administration should be considered. As shown in Fig. 7, the absorption of ampicillin enhanced by salicylate was 9, but no other pH effect was apparent. This result indicates that the enhancing activity of salicylate is retained over a wide range of pH values. The observed slight increase of adjuvant action of salicylate at pH 9 is probably due to slight damage to the rectal mucosal tissue by the alkaline solution, since we have reported[12] that permeation of trypan blue through the rectal mucosal membrane was slightly facilitated at pH 9 compared with that at pH 7 and, although 2% salicylate did not cause any rectal tissue damage except for shortening of the glycocalyx as pH 7, it might cause at slight damage at pH 9. In this work, we studied the factors influencing the adjuvant action of salicylate on the rectal absorption of ampicillin. We confirmed that triglyceride base is preferable to microenema for ampicillin formulation because a high adjuvant concentration is maintained at the absorption site. This result might be applicable to other highly water-soluble drugs in formulations with adjuvant.

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