EFFECTS OF PHENOTHIAZINES AND *N*-(6-AMINOHEXYL)-5-CHLORO-1-NAPHTHALENESULFONAMIDE ON RAT COLONIC ABSORPTION OF CEFMETAZOLE

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Rat colonic absorption of cefmetazole was increased significantly by calmodulin inhibitors such as phenothiazines and *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) when they were coadministered at a concentration between 1 and 100 μ M. The bioavailability of cefmetazole, determined by area under the blood concentration curve method, increased 20 to 30% by the coadministration of calmodulin inhibitors. It is speculated that the enhancing action of agents on rat colonic absorption of cefmetazole takes place by a paracellular route and coadministration of sodium ion increases their actions.

Keywords — calmodulin inhibitor; phenothiazine; *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7); colonic absorption; rat; cefmetazole

INTRODUCTION

There are many reports concerning the increase in intestinal permeability for poorly absorbed drugs by absorption-promoting adjuvants. We have previously reported that a decrease in reduced nonprotein sulfhydryl compounds in tissue, which is induced by agents such as diethyl maleate, is involved in a mechanism of increasing intestinal epithelial brush border membrane permeability for hydrophilic compounds.¹⁾ Furthermore, it has been reported that the enhancing action of disodium ethylenediaminetetraacetic acid on intestinal absorption of hydrophilic drugs occurs by removing the calcium ion from the tight junctional area, resulting in an increase in tight junctional permeability of intestinal mucosa.²⁾ However, there are still several adjuvants whose action can not be explained by the mechanism described above.

Since it has been reported that a calcium dependent protein, including calmodulin, may maintain the integrity of cell membrane and adhesion of cells,³⁾ it is interesting to investigate the role of calcium dependent protein on intestinal mucosal permeability. Since phenothiazines and *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) are known as calmodulin inhibitors, their action on rat colonic absorption of cefmetazole (poorly absorbed drug) was examined in a concentration range of 1 to 100 μ M in which phenothiazines⁴⁾ and W-7⁵⁾ are reported to act as calmodulin inhibitors.

MATERIALS AND METHODS

Materials — Prometazine hydrochloride, perfenazine hydrochloride and trifluoperazine malonate were supplied by Yoshitomi Pharmaceutical Industry Co., Ltd. (Osaka, Japan). Sodium cefmetazole was supplied by Sankyo Co., Ltd. (Tokyo, Japan). W-7 was obtained from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). Other reagents used were of analytical grade.

Animals — Wistar male rats, 200 to 250 g, were fasted for 16 h prior to experiments but water was given *ad libitum*. During the experiments, rats were anesthetized with sodium pentobarbital (30 mg/*i.p.*) and were kept on a hot plate at 37 °.

Absorption of Cefmetazole from Rat Colon — An absorption study of cefmetazole was performed by using the *in situ* rat colonic loop method described previously.⁶⁾ Briefly, about 3 cm segment of colon was ligated after abdominal incision. After administration of cefmetazole solution (15 mg sodium cefmetazole/ml) at a volume of 100 μ l/100 g-weight into the loop, blood samples were collected from the jugular vein at designated time intervals and centrifuged to obtain plasma. The administered cefmetazole solution was prepared with 0.01 M sodium phosphate buffer (pH 7.0) and adjusted to $\mu = 0.75$ ionic strength with sodium chloride. The ionic strength of $\mu = 0.75$ in the administered solution was selected to characterize the action of agents, since the enhancing action of pheno-

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thiazines, when the ionic strength of the administered solution was adjusted to $\mu = 0.15$, was not well determined. In the experiments using the administered solution with $\mu = 0.15$, when cefmetazole alone was administered, no plasma cefmetazole concentrations were observed and furthermore, plasma cefmetazole concentrations, even when cefmetazole was administered with phenothiazine, were also often less than the detectable concentration of $0.2 \mu\text{g/ml}$. Since the mucin layer may disturb drug absorption as described previously,⁷⁾ the ionic strength of the administered solution was adjusted with $\mu = 0.75$ with sodium chloride to disrupt the mucin layer.⁸⁾

To estimate the bioavailability of cefmetazole, after rat colonic administration, by determining plasma cefmetazole concentrations in comparison with those after intravenous administration of cefmetazole, cefmetazole solution prepared with saline was administered intravenously in the rat tail vein at a dose of 5 mg/kg .

Assay of Cefmetazole— Assay of cefmetazole in rat plasma was performed by a high performance liquid chromatographic method described previously⁹⁾ with an assay minimum limitation of $0.2 \mu\text{g/ml}$.

RESULTS

As reported previously,⁹⁾ rat colonic absorption of cefmetazole was very low when cefmetazole was administered alone (Fig. 1). However, plasma cefmetazole concentration was increased significantly in the presence of phenothiazine at a concentration in which it has been reported to act as a calmodulin inhibitor⁴⁾ (Fig. 1). W-7, another type of calmodulin inhibitor, also increased the plasma cefmetazole concentration significantly after colonic administration of cefmetazole (Fig. 1).

The concentration dependency of the action of phenothiazines was observed between 1 and $100 \mu\text{M}$. Although the plasma concentration profile of cefmetazole, in the presence of W-7, was similar to those in the presence of phenothiazines, the effect of W-7 seemed to be somewhat stronger than that of phenothiazines (Fig. 2). In comparison of *AUC* (area under the blood concentration curve) of cefmetazole, the maximum enhancing actions of promethazine and perphenazine were observed at a concentration of 5 to $50 \mu\text{M}$ and 1 to $50 \mu\text{M}$, respectively (Fig. 3A,B),

FIG. 1. Plasma Cefmetazole Concentration as a Function of Time after Colonic Administration at a Dose of 15 mg/kg with No Agents ($\square$), Perphenazine ($\circ$, $50 \mu\text{M}$ in Administered Solution), Promethazine ($\bullet$, $50 \mu\text{M}$), Trifluoperazine (Δ , $50 \mu\text{M}$), or W-7 ($\blacktriangle$, $50 \mu\text{M}$)

Each value represents the mean $\pm$ S.E. ($n > 4$). In the experiments without addition of any additive ($\square$), S.E. of each mean was less than 6%.

and the observed plasma cefmetazole concentrations were decreased when each of the phenothiazines was coadministered at the concentration over those described above. Furthermore, no plasma cefmetazole concentration was observed when either 1 mM promethazine or $100 \mu\text{M}$ perphenazine (Table I) was coadministered with cefmetazole. However, within the concentration range used in this study, strong enhancing action of trifluoperazine occurred in accordance with the increase in its concentration in the administered solution (Fig. 3C). For W-7, the concentration-dependency is unclear (Fig. 3D).

Bioavailability of cefmetazole after colonic administration was determined by *AUC* method in comparison to plasma cefmetazole concentrations for 120 min after intravenous injection, using the trapezoidal rule.¹⁰⁾ As can be seen in Table I, bioavailability of cefmetazole after colonic administration with each calmodulin inhibitor used in this study was about 20 to 30%.

DISCUSSION

It is clear in this study that an adjuvant action of phenothiazines and W-7 in enhancing rat colonic absorption of cefmetazole occurred at a

FIG. 2. Plasma Cefmetazole Concentration as a Function of Time after Colonic Administration in Solution at a Dose of 15 mg/kg with Various Concentration of Prometazine (A) or W-7 (B)
 (A) ○, 1 μM; ●, 5 μM; △, 20 μM; ▲, 100 μM. (B) ○, 1 μM; ●, 5 μM; △, 25 μM; ▲, 100 μM.
 Dotted lines represent the results shown in Fig. 1. Each value represents the mean ± S.E. ($n > 4$).

concentration within 1 to 100 μM, in which they also act as calmodulin inhibitors as reported.⁴⁾

Since maximum action of prometazine and perfenazine was observed at 5 to 50 μM and 1 to 50 μM, respectively, their action mechanism seems to be different from that of other adjuvants such as strong chelating agents and surfactants which generally act strongly with the increase in their concentration.

Furthermore, since we observed¹¹⁾ that phenothiazines did not decrease the concentration of reduced nonprotein sulfhydryl compounds in colonic tissue, their action mechanism also appears to be different from diethyl maleate.¹⁾ Thus, it was considered that an enhancing action of calmodulin inhibitors used in this study on the rat colonic absorption of cefmetazole occurred in mechanism(s) different from those involved as strong chelating agents, surfactants or diethyl maleate.

It has been reported¹²⁾ that an increase in permeability through the paracellular route by disodium ethylenediaminetetraacetic acid was inhibited by the treatment with ouabain and it was

also suggested¹²⁾ that the diffusion of solutes through the paracellular route requires a driving force attained by high osmotic pressures in the intercellular space between epithelial cells. Furthermore, it has been demonstrated¹²⁾ that only the adjuvant action which increases the permeability through the paracellular route was inhibited by ouabain action based on its inhibiting Na^+/K^+ adenosine triphosphatase (ATPase). Since it is reported¹³⁾ that phenothiazines have an action inhibiting Na^+/K^+ ATPase, suppression of adjuvant action of prometazine and perfenazine at a concentration more than 100 μM may be due to their own Na^+/K^+ ATPase inhibiting action; *i.e.* it is speculated that both calmodulin inhibitors increase permeability through the paracellular route but their increasing action is suppressed by their own Na^+/K^+ ATPase inhibition at high concentrations. As described in the experimental section in which low ionic strength in administered solutions did not induce strong action of phenothiazines, transport of sodium ion into intercellular space may be required to induce the strong enhancing action of calmodulin inhibitors.

FIG. 3. Effect of Prometazine (A), Perfenazine (B), and Trifluoperazine (C), W-7 (D) on the AUC for 120 min after Colonic Administration of Cefmetazole in Solution at a Dose of 15 mg/kg

Each value represents the mean $\pm$ S.E. ($n > 4$). a) $p < 0.01$ versus no additives. b) $p < 0.05$ versus 1 μM of prometazine. c) $p < 0.01$ versus 5 and 20 μM of prometazine. d) $p < 0.05$ versus 1, 10 and 50 μM of perfenazine. e) $p < 0.05$ versus 20 and 50 μM of trifluoperazine.

Since there is no observation on the $\text{Na}^+\text{-K}^+\text{ATPase}$ inhibiting action by W-7, it can be considered that the action of W-7 was not suppressed at high concentrations. Since a slight decrease of W-7 adjuvant action was observed at 50 μM , we plan to investigate the action of W-7 in *in vitro* experiments.

On the other hand, although trifluoperazine acts as an $\text{Na}^+\text{-K}^+\text{ATPase}$ inhibitor, it has also been reported¹³⁾ that only trifluoperazine and chlorpromazine inhibited Ca^{2+} ion influx into cells at 100 μM , followed by an increase in cell membrane permeability. The reason why there is no observation of the decreasing-action of tri-

fluoperazine at 100 μM may be due to the increase of cell membrane permeability for cefmetazole with inhibition of Ca^{2+} ion influx by trifluoperazine, resulting in an increase of intracellular transport.

Although it has been reported¹⁴⁾ that some phenothiazines at significant high concentrations (over mM order) increase the rat rectal absorption of antibiotics, the mechanism of the enhancing action at high concentrations may act as surface active agent.¹⁵⁾

In this study, we clarified that calmodulin inhibitors such as phenothiazines and W-7 at low concentrations increased rat colonic mucosal

TABLE I. Bioavailability^{a)} of Cefmetazole after Rat Colonic Administration at a Dose of 15 mg/kg

Coadministered agent	Concentration (μM)	Bioavailability of cefmetazole (%)
None		7.73
Trifluoperazine	25	15.57
	50	17.95
	100	21.29
	1	14.87
Prometazine	5	24.51
	20	21.13
	50	20.13
	100	12.62
	1000	Not detected
	1	20.49
Perfenazine	10	22.14
	50	20.49
	75	12.40
	100	Not detected
W-7	1	24.22
	5	25.88
	25	34.41
	50	25.02
	100	33.60

Bioavailability was determined by AUC method with the data shown in Fig. 3.

$$a) \text{ Bioavailability} = \frac{[AUC]_{\text{colon}} \times (\text{Dose})_{\text{i.v.}}}{[AUC]_{\text{i.v.}} \times (\text{Dose})_{\text{colon}}} \times 100$$

[AUC]_{colon} and (Dose)_{colon} represents the colonic administration. [AUC]_{i.v.} and (Dose)_{i.v.} represents the intravenous injection. [AUC]_{i.v.} at a dose of 5 mg/kg for 120 min after administration was 165.42 (μg · min/ml).

membrane permeability. Although further investigations should be performed to determine how calcium-dependent-protein in the epithelial cells regulates colonic mucosal membrane permeability, the results in this study suggest that intestinal mucosal membrane permeability is regulated by several physiological factors.

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