INTERACTION OF TERTIARY AMINES AND QUATERNARY AMMONIUM COMPOUNDS WITH GASTROINTESTINAL MUCIN

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(Received May 26, 1986)

The binding property of some tertiary amines (chlorpromazine, promethazine and imipramine) and quaternary ammonium compounds (propantheline, mepenzolate and butylscopolamine) for gastric mucin was investigated. All tertiary amines tested bound gastric mucin to varying extents, and the binding of chlorpromazine was especially strong. Moreover, the absorption behaviors of chlorpromazine in rat intestinal loops was significantly inhibited in the presence of gastric mucin.

On the contrary, all quaternary ammonium compounds tested did not bind gastric mucin and the absorption of propantheline was not affected in the presence of gastric mucin.

These results suggested that gastrointestinal mucin produced a significant effect on the bioavailability of these tertiary amines after oral administration.

Keywords — gastric mucin; binding property; tertiary amine; quaternary ammonium compound; methylene blue; intestinal absorption

INTRODUCTION

It has been suggested that the mucin layer of the gastrointestinal surface has important physiological functions, such as lubrication, protection and waterproofing of mucosa and antibacterial action.¹⁾ After oral ingestion, a drug must pass through the mucin layer before reaching the intestinal epithelial membrane for absorption. It has also been reported that the mucin layer has a significant effect on absorption or bioavailability of various drugs.²⁾

It is well known that quaternary ammonium compounds (QAC) are poorly absorbed from the gastrointestinal tract. Certain peculiarities in the absorption behavior of QAC has been reported,^{2f, 3)} such as the complete cessation of rapid initial absorption even though 70—90% of the administered dose still remained in the intestine. Levine *et al.*^{3a)} suggested that the cessation of absorption was due to the formation of nonabsorbable complexes of QAC with acid components of intestinal mucin. Later, however, Turnheim and Lauterbach^{3b, 4)} found an active secretory process for QAC in guinea pig intestinal epithelium. They explained that the cessation of absorption was due to the establishment of an equilibrium between absorptive and secretory fluxes. Thus, the effect of gastrointestinal mucin on the absorption of QAC remains unclear.

Moreover, if the binding to mucin is based on

electrostatic binding with acid components of mucin, as reported by Levine *et al.*,^{3a)} it is possible that tertiary amines such as chlorpromazine, imipramine and diphenhydramine, which are mostly ionized over the pH range in the gastrointestinal tract, also bind gastrointestinal mucin. There are few reports concerning the binding between these tertiary amines and gastrointestinal mucin or about the effects of gastrointestinal mucin on the absorption of these tertiary amines.

In this report, we studied the binding between some QAC and gastric mucin to clarify the effect of mucin on the absorption of QAC. Moreover, the binding property of several tertiary amines to gastric mucin was also studied and compared with those of QAC.

MATERIALS AND METHODS

Materials — Methylbenactyzium bromide and butylscopolamine bromide (Yamanouchi Pharmaceutical Co., Tokyo, Japan), and mepenzolate bromide (Fujisawa Pharmaceutical Co., Osaka, Japan) were kindly supplied by the respective companies. Propantheline bromide, chlorpromazine hydrochloride, promethazine hydrochloride, imipramine hydrochloride and ergotamine tartrate were obtained from Sigma Chemical Co., St. Louis, MO., U.S.A. Methylene blue trihydrate, sodium dodecyl sulfate, neostigmine bromide and pig gastric mucin were pur-

FIG. 1. Structures of Tertiary Amines and Quaternary Ammonium Compounds Tested

chased from Wako Pure Chemical Ind., Osaka Japan. Pig gastric mucin used in this study was ocher powder, containing about 57% protein by weight. Dibucaine hydrochloride was obtained from Teikoku Kagaku Sangyo Co., (Osaka, Japan) and diphenhydramine hydrochloride from Nakarai Chemicals (Kyoto, Japan). All the chemicals were of reagent grade and used without further purification. The structures of QAC and tertiary amines tested are presented in Fig. 1.

Binding to Gastric Mucin — The drugs were dissolved in modified Ringer solution⁵⁾ (pH 6.5) and gastric mucin, a readily available model mucus material was suspended in the same modified Ringer solution.

a) **Dialysis Method:** A dialysis method was used to examine the time course of binding. The method was a modification of that described by Cavallito and O'Dell.⁶⁾ The dialysis membrane was Spectrapor 2 (M.W. cut off 12 000–14 000, 2 ml/cm, Spectrummedical Ind., Los Angeles, Cal., U.S.A.). Five milliliters of drug solution (250 μ M) with or without 0.5% gastric mucin were added to the dialysis sac (8 cm) and the sac was gently stirred in 100 ml of modified Ringer solution (pH 6.5) at room temperature. At the time intervals indicated, 2 ml aliquots were removed from the outer medium for analysis.

b) **Centrifugation:** A centrifugation method was used for the experiment on the effect of the concentration of mucin on drug binding, since this method was simple and not time-consuming. The procedure was based on that described by Mayer *et al.*⁷⁾ Two milliliters of drug solution were mixed with 2 ml of mucin suspension and the mixture was incubated for 15 min at 37 °C. Then, the mixture was centrifuged at 29000 $\times g$ for 30 min at 20 °C and 2 ml of supernatant fluid were used for analysis.

Procedure of Absorption Experiment

— Wistar male rats (180–250 g) were anesthetized with ether and sodium pentobarbital (2 mg/100 g). The absorption experiment was carried out at pH 6.5 using an *in situ* loop technique by Levine and Perikan.⁸⁾ Three loops (each 10 cm) were prepared in each rat. The proximal ligature of the first loop (duodenum loop) was placed about 2 cm from pylorus and that of the second loop (jejunum loop) was placed about 20 cm from the pylorus and that of the third loop (ileum loop) was placed about 15 cm above the ileo-cecal junction. The bile duct was ligated in

all experiments. After washing the loop gently with 10 ml of drug-free modified Ringer solution (pH 6.5), 1 ml of drug solution with or without gastric mucin was injected into the loop using a syringe. At a selected time, before the animal was sacrificed, the loop was removed and the contents were emptied into a 5 ml volumetric flask. The mucosal side of the loop was rinsed with the modified Ringer solution to give a volume of 5 ml and 2 ml were removed for analysis.

Analytical Method — QAC and tertiary amines were assayed by high-performance liquid chromatography (HPLC).

QAC: one half milliliter of 2 mM sodium dodecyl sulfate (SDS) and 5 ml of chloroform were added to all sample solutions. The mixture was shaken for 15 min and centrifuged at 1000 $\times g$ for 5 min. Three milliliters of the chloroform layer were taken and dried *in vacuo*. The residue was dissolved in 200–500 μ l of internal standard methanol solution and a sample (10–15 μ l) of the solution was injected into HPLC.

Tertiary Amines: Dialysis samples of all tertiary amines and ergotamine were directly injected into HPLC after the addition of an internal standard. The procedure for assay of the centrifugation samples was as follows. Except for diphenhydramine, 2 ml of 1 N NaOH and 5 ml of ethyl acetate were added to sample solutions. In the case of diphenhydramine, 2 ml of 1 N NaOH and 5 ml of benzene were added. The mixture was shaken for 15 min and then centrifuged at 1000 $\times g$ for 5 min. Three milliliters of the organic layer were taken and dried *in vacuo*. The residue was dissolved in 500 μ l of internal standard methanol solution and a sample (10–15 μ l) of the solution was injected into the HPLC.

Dialysis samples of methylene blue were determined by spectrophotometry (Hitachi 124) at 665 nm.

The conditions of HPLC were as follows; A liquid chromatograph (Hitachi 635A) equipped with variable wavelength ultraviolet (UV) monitor (Hitachi 638-41) was used. The column, 25 cm $\times$ 4 mm i.d., of stainless steel, was packed with Hitachi gel 3053 (ODS, 5 μ m, Hitachi Ltd., Tokyo, Japan) and warmed at 55 °C using a water bath circulator. The mobile phase for assay of tertiary amines consisted of 0.05 M KH_2PO_4 (pH 2.5) and acetonitrile (2:3)

and for assay of QAC, 1 mM SDS containing 1% acetic acid (pH 4) and acetonitrile (3:7). The flow rate was 0.6–0.8 ml/min. The wavelengths used were 230 nm (QAC, diphenhydramine, ergotamine) and 250 nm (chlorpromazine, promethazine, imipramine, dibucaine). The internal standard were Nocrac NS-6 (Ouchi Shinko Ind., Tokyo, Japan) for QAC, butamben for chlorpromazine and chlorpromazine for other tertiary amines.

RESULTS

Binding of Tertiary Amines and QAC to Gastric Mucin

Figure 2 shows the dialysis profiles of tertiary amines (chlorpromazine and imipramine) in the presence or absence of gastric mucin. The diffusion of chlorpromazine was significantly inhibited in the presence of gastric mucin. In the case of imipramine, although the degree of inhibition

by gastric mucin was not as great as chlorpromazine, the diffusion was nevertheless inhibited. The degree of inhibition of the diffusion of all tertiary amines tested by gastric mucin is shown in Fig. 3. It was found that tertiary amines bound gastric mucin to varying degrees.

Methylene blue and ergotamine, which were previously shown to bind isolated intestinal mucin markedly,^{2c,e)} also bound gastric mucin as strong as chlorpromazine (data not shown).

The effect of concentration of gastric mucin on the binding of tertiary amines was investigated by using the centrifugation method. As shown in Fig. 4, the concentrations of all tertiary amines in the supernatant fluid were markedly decreased following increase in gastric mucin concentration. There were large differences in the binding behaviors of tertiary amines.

In contrast to tertiary amines, the dialysis of all QAC tested, which are summarized in Fig. 5,

FIG. 2. Effects of Gastric Mucin on the Dialysis of Chlorpromazine (A) and Imipramine (B). Drug concn.: 250 μ M, mucin concn.: 0.5%. $\circ$, control; $\bullet$, with mucin. Results are expressed as the mean of 4 experiments with S.D. a) $p < 0.001$. b) $p < 0.05$.

FIG. 3. Effects of Gastric Mucin on the Dialysis of Five Tertiary Amines

Drug concn.: $250\ \mu\text{M}$, mucin concn.: 0.5%.

Results are expressed as the mean of 4 experiments with S.D. a) $p < 0.001$. b) $p < 0.005$. c) $p < 0.01$. d) $p < 0.05$.

FIG. 4. Effects of the Concentration of Gastric Mucin on the Binding of Four Tertiary Amines

▲, diphenhydramine; △, imipramine; ●, promethazine; ○, chlorpromazine.

Experiments were performed by the centrifugation method. Drug concn.: $250\ \mu\text{M}$. Results are expressed as the mean of 4 experiments with S.D.

were not affected by the presence of gastric mucin. This indicated that these QAC did not bind gastric mucin. Moreover, the binding of methylbenactyzium, butylscopolamine and mepenzolate was independent of the concentration of gastric mucin. Although the effect of mucin concentration was observed in the case of

FIG. 5. Effects of Gastric Mucin on the Dialysis of Five Quaternary Ammonium Compounds

Drug concn.: $250\ \mu\text{M}$. Results are expressed as the mean of 4 experiments with S.D.

FIG. 6. Effects of Gastric Mucin on the Disappearance of Chlorpromazine from Rat Intestinal Loops

Drug concn.: $250\ \mu\text{M}$, mucin concn.: 0.5%.

□, control; ▨, with mucin.

Experimental time: 15 min. Results are expressed as the mean of 4 experiments with S.D. a) $p < 0.01$.

propantheline, the degree of binding was much smaller than that of tertiary amines.

Effect of Gastric Mucin on the Absorption

In order to investigate the effect of gastric mucin on intestinal absorption, absorption behaviors were studied in the presence or absence of gastric mucin by using an *in situ* loop technique. Figure 6 shows the results of such an experiment with chlorpromazine. The disappearance of

FIG. 7. Effects of Gastric Mucin on the Disappearance of Propantheline from Rat Intestinal Loops
Drug concn.: $100\ \mu\text{M}$, mucin concn.: 0.5%.

□, control; ▨, with mucin.

Experimental time: 60 min. Results are expressed as the mean of 4 experiments with S.D.

chlorpromazine from the intestine was significantly inhibited in the presence of gastric mucin. On the other hand, as shown in Fig. 7, the disappearance of propantheline was not affected by gastric mucin. These inhibitory effect of gastric mucin on absorption was in agreement with the results observed in *in vitro* experiments.

DISCUSSION

Several observations concerning interactions between drugs and gastrointestinal mucin have been reported. In such experiments, isolated intestinal mucin or commercially available gastric mucin was used. In this study, we used gastric mucin without analyzing its composition in detail, since the gross composition of mucin isolated from various organs, has been established.^{7,9} Differences in composition and structure have been indicated.¹ Generally, for example, gastric mucin has a lower content of sialic acid than other intestinal mucins.^{9d,10} Accordingly, any differences between the effect of the gastric mucin used in this study and the native intestinal mucin should be taken into consideration. However, methylene blue and ergotamine, which were reported to bind isolated intestinal mucin strongly,^{2c,e} also showed high binding property to gastric mucin in this study. Therefore, it was assumed that gastric mucin was an appropriate model compound for the examination of drug

binding to gastrointestinal mucin.

The concentration of mucin (0.5%) used in this study was based on previous reports,² and it was confirmed, as shown in Fig. 4, that binding became nearly constant at 0.5% mucin concentration.

There is little information on the binding property of tertiary amines, such as chlorpromazine, promethazine and imipramine, for gastrointestinal mucin. In the present study, as shown in Fig. 3, all tertiary amines tested bound gastric mucin to varying extents and the binding of chlorpromazine was especially strong. These tertiary amines tested, except dibucaine, have the same tertiary structure for the N atom and chlorpromazine, promethazine and imipramine have similar pK_a values as reported by Green.¹¹ Therefore, differences of binding cannot be explained by the degree of ionization. It is thought that differences of structures of the hydrophobic moiety possibly contribute to the differences of binding to gastric mucin. Moreover, the intestinal absorption of chlorpromazine was seriously affected in the presence of gastric mucin. It has been indicated that these tertiary amines have low bioavailability after oral administration¹² due possibly to incomplete absorption,¹³ as well as first-pass metabolism¹⁴ and biotransformation in the gastrointestinal wall.¹⁵ In this study, gastric mucin had a significant effect on tertiary amine absorption. More details concerning the effect of mucin, including the change of mucin concentrations, on absorption should be obtained. If the binding is based on the electrostatic binding with acid components of mucin, the effect of native intestinal mucin which is more abundant in sialic acid than gastric mucin might be greater. Currently, we are examining these points in great detail including the relationship between the binding property and structure of tertiary amine.

In contrast to tertiary amines, the oral QAC tested in this study did not show strong binding to gastric mucin. This result was obviously inconsistent with that of Levine *et al.*^{3a} who used benzomethamine. As shown in Fig. 7, the disappearance of propantheline, in the absence or presence of gastric mucin, was slight even after 60 min and there was no change in the disappearance in both cases. Levine *et al.* suggested that this lower absorption of QAC was due to the binding to intestinal mucin. However, even con-

sidering the difference between gastric mucin and intestinal mucin, the small disappearance of propantheline cannot be explained fully only by the binding to mucin. Therefore, binding property of QAC to gastric mucin observed in this study suggested that the results of Levine *et al.*^{3a)} might not be applicable to all QAC. Further investigation is required using additional QAC and isolated intestinal mucin.

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