EFFECT OF CHLORPROMAZINE ON INTESTINAL ABSORPTION OF SULFAMETHOXAZOLE IN RATS*

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The effect of chlorpromazine (CPZ) on the intestinal absorption of sulfamethoxazole (SMZ) was studied in isolated perfused rat small intestine by comparing two determinants, *i.e.* the epithelial permeability and the intestinal blood flow. The appearance rate of SMZ in blood in the presence of CPZ decreased to one-half of the control without CPZ. The pH of perfusion solution was significantly decreased by CPZ after 10 min perfusion. According to the Winne's absorption model, CPZ did not change the apparent epithelial permeability of SMZ, but decreased the epithelial permeability of unionized SMZ due to the decrease in the pH of perfusion solution by CPZ. It was also suggested that CPZ decreased the fraction of the total blood flow rate in the subepithelial capillaries to less than one-half.

Keywords — chlorpromazine; intestinal absorption; sulfamethoxazole; Winne's model; epithelial permeability; intestinal blood flow

INTRODUCTION

Chlorpromazine (CPZ), a phenothiazine derivative, is a major tranquilizer that can be administered orally. It has been reported that long oral administration of CPZ affects gastrointestinal function and inhibits the absorption of acetaminophen by delaying the gastric emptying rate.^{1,2)} Recently, Sundaresan *et al.*^{3,4)} reported that CPZ inhibited the L-methionine transport in rats but did not the D-xylose transport. L-Methionine is known to be transported actively *via* the neutral amino acid pathway, while D-xylose is transported *via* another carrier mediated system. They suggested that CPZ inhibited the metabolism of cells and/or inhibited the Na⁺, K⁺-ATPase in the epithelial membrane.

The effects of CPZ on transport systems other than the small intestine have been extensively studied. Several investigators reported that CPZ changed the permeability of the

plasma membranes of liver and brain.^{5–9)} Other experiments showed that CPZ inhibits the oxidative phosphorylation in tissues, suppressed the glycolytic enzyme, and inhibited the Na⁺, K⁺-ATPase activity.^{10–13)}

Sulfamethoxazole (SMZ), a sulfonamide derivative, was chosen as a model drug for intestinal absorption, since SMZ has both molecular forms, *i.e.* unionized and ionized forms, at physiological pH and has been suggested to be absorbed against its concentration gradient in the rat everted intestine¹⁴⁾ and, also, can be used clinically as an antibacterial drug by simultaneous oral administration with CPZ.

In this study we examined the effects of CPZ on the intestinal absorption of SMZ in rats, in an attempt to elucidate the mechanisms of inhibition of CPZ by comparing the two determinants, *i.e.* the epithelial permeability and the intestinal blood flow.

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MATERIALS AND METHODS

Materials — Chlorpromazine hydrochloride (CPZ) was supplied by the Yoshitomi Pharm. Ind. Co. Ltd., Osaka, Japan. Sulfamethoxazole (SMZ) was supplied by Shionogi Co. Ltd., Osaka, Japan. All other reagents were commercial products and of analytical grade.

Perfusion Study — Male Wistar rats, weighing 220–250 g, were fasted for 16–20 h prior to the experiments, but water was allowed *ad libitum*. The rats were anesthetized with ethyl-carbamate (25 w/v %) by an intraperitoneal injection of 4.5 ml/kg. After an abdominal incision, an intestinal segment of about 10 cm long, situated in the proximal jejunal region at 26 cm distal to the duodenojejunal flexure was chosen. Both ends of the intestinal loop were cannulated with polyethylene tubings. The intestinal loop was rinsed with 0.154 M NaCl solution and was perfused with 40 ml of the solution, containing 0.154 M NaCl, 2 mM SMZ and 2 mM CPZ adjusted to pH 6.50 by 1N NaOH and/or 1N HCl at a flow rate of 6 ml/min; in the reference experiments (control) CPZ was omitted. Heparin (100 IU/100 g) was injected *via* a femoral artery of the rat. Fifty μ l of the perfusion solution was sampled and the concentrations of CPZ and SMZ were determined as described below. Ten min after the initiation of perfusion, the intestinal veins supplying the selected intestinal segment were cut and the draining blood was soaked up by absorbent cotton. The cotton was changed every 30 s for 5 min. The blood sampling was performed from 10 to 15 min after the initiation of perfusion. The blood flow rate was calculated by the weight of the soaked blood multiplied by the density of the blood ($d = 1.069$ g/ml) using the linear portion (from the 2-nd to the 8-th interval) of the total blood flow rate of the intestinal segment (V_B) vs. time curve. The pH and the concentrations of protein and phospholipid in the perfusion solution were determined before and after perfusion. At the end of the experiments, the jejunum was excised from the rat, and its length was determined.

Correction for the Water Absorbed to the Intesti-

nal Segment — The intestine was perfused for 10 min with a solution containing polyethylene-glycol 4000 (PEG 4000, 80 mg/ml), which is not absorbed. Then the intestine was placed in a 20 ml beaker containing 10 ml of normal saline, and the increase of the weight was estimated to yield the apparent wet weight of the intestine. The 50 μ l portion of the solution in the beaker was sampled and the PEG concentration that was released from the adsorbed water was determined by equation

$$\text{absorbed water (ml)} = \frac{\text{PEG concentration in beaker (mg/ml)} \times 10 \text{ ml}}{80 \text{ (mg/ml)}} \quad (\text{Eq.1})$$

The true wet weight of the intestine was calculated by subtracting the weight of the adsorbed water from the apparent wet weight.

Analytical Methods — Fifty μ l of the perfusion solution was removed and then mixed with 0.5 ml of water and 0.5 ml of 5% trichloroacetic acid (TCA). CPZ was determined by the following method. After centrifugation at 3000 rpm for 10 min, 0.8 ml of supernatant was removed and then mixed with 2.5 ml of water. The absorbance was measured at 255 nm in a Hitachi 124 spectrophotometer (Hitachi, Tokyo, Japan). To determine SMZ, 0.8 ml of supernatant was removed. One ml of ethanol, 3 ml of 5% TCA, 0.25 ml of 0.1% sodium nitrite, 0.3 ml of 12.5% urea, and 0.2 ml of Tsuda reagent¹⁶⁾ were added in this order. The absorbance was measured at 550 nm in a Hitachi 124 spectrophotometer. The concentration of PEG 4000 was determined by measuring the turbidity at 300 nm after the addition of TCA in a Hitachi 124 spectrophotometer. Protein concentration was determined by the Lowry's method.¹⁷⁾ Phospholipid concentration was determined by the Fiske-Subbarow's method.¹⁸⁾ The concentration of SMZ in blood was determined as follows. Five times volume of distilled water was added to each blood sample collected for 30 s and was stirred vigorously on the Vortex mixer. An aliquot (0.1 ml) of this solution was mixed with 0.5 ml of distilled water and 0.5 ml of 10% TCA solution. After centrifugation at 3000 rpm for 15 min, 0.8 ml of the su-

pernatant was removed and was analyzed as described above in a Hitachi 356 double wave length double beam spectrophotometer ($\lambda_1=620$ nm, $\lambda_2=550$ nm).

Calculation—The two parameters, *i.e.* the epithelial permeability (KA_L) and the effective blood flow ratio ($a\alpha$) were calculated according to the absorption model proposed by Winne *et al.*¹⁵⁾ In this model, the net flux of the drug from the lumen to the blood through the interstitial space ($\bar{\Phi}_n$) is given by

$$\bar{\Phi}_n = \frac{1}{\frac{1}{K_1 A_L} + \frac{K_2 A_L}{K_1 A_L a \alpha V_B}} C_L - \frac{1}{\frac{1}{K_1 A_L} + \frac{1}{a \alpha V_B}} C_P \quad (\text{Eq. 2})$$

where K_1 is the permeability coefficient from the lumen to the interstitial space, A_L is the mucosal area, C_L is the drug concentration in the lumen, K_2 is the permeability coefficient from the interstitial space to the lumen, C_P is the concentration in plasma, " a " is the ratio of the concentration in the blood to that in plasma, α is the fraction of the total blood flow rate that is attributable to the subepithelial capillaries, and V_B is the total blood flow rate in the intestinal segment.

When a membrane does not show selectivity in the direction of permeation between the lumen and the blood, and C_P is much smaller than C_L , equation 2 becomes

$$\bar{\Phi}_n = \frac{1}{\frac{1}{KA_L} + \frac{1}{a \alpha V_B}} C_L \quad (\text{Eq. 3})$$

where $K = K_1 = K_2$

Rearrangement of equation 3 yields

$$\frac{C_L}{C_B} = \frac{1}{KA_L} V_B + \frac{1}{a \alpha} \quad (\text{Eq. 4})$$

where C_B is $\bar{\Phi}_n / V_B$. In equation 4, C_B , C_L and V_B can be determined experimentally. Therefore, KA_L and $a\alpha$ can be obtained from the reciprocals of the slope and the intercept on the plot of V_B versus C_L / C_B , respectively.

Statistical Analysis—All means are presented with their standard errors. Student's *t*-test was utilized to determine the significance of the difference between the control and the CPZ-treated groups, with $p=0.05$ as the minimal level of significance.

RESULTS

The time course of CPZ in the perfusion solution is shown in Fig. 1. The concentration of CPZ

FIG. 1. Time Course of CPZ Concentration in the Intestinal Lumen

Each point and vertical bar represent the mean $\pm$ S.E. of eight experiments (See text).

TABLE I. Effect of CPZ on the Appearance Rate of SMZ ($\bar{\Phi}$) and on the Blood Flow Rate (V_B)^{a)}

	$\bar{\Phi}$ ($\mu\text{g}/\text{min}/\text{g}$)	V_B ($\text{ml}/\text{min}/\text{g}$)
Control	205 ± 12	0.42 ± 0.03
+CPZ	117 ± 5	0.29 ± 0.02
	$p < 0.01$	$p < 0.01$

^{a)} Results are given as the mean $\pm$ S.E. of seven rats (see text).

from 10 to 20 min after the initiation of perfusion decreased exponentially obeying the first order kinetics.

Table I shows the effect of CPZ on the appearance rate of SMZ into the venous blood ($\bar{\phi}$) and the blood flow rate (V_B). The values of $\bar{\phi}$ and V_B (the mean $\pm$ S.E.) were calculated from each mean values of five data points of one rat, respectively. In the presence of CPZ, both the $\bar{\phi}$ and V_B decreased significantly ($p < 0.01$) to 57% and 69% of those of the control without CPZ, respectively.

A representative plot of the data from one rat in the presence and absence of CPZ according to

FIG. 2. Effect of CPZ on the Relationship between the Lumen to Blood Concentration Ratio of SMZ (C_L/C_B) and the Intestinal Blood Flow Rate (V_B)

A representative data from each one rat was plotted according to equation 4 in the Text.

Key: (●) control, and (○) with CPZ.

equation 4 is shown in Fig. 2. The plot is well approximated by a straight line, and the slope of the line and the intercept of the ordinate give the values of $1/K_A L$ and $1/a\alpha$, respectively.

The epithelial permeability ($K_A L$) and the effective blood flow coefficient ($a\alpha$) are summarized in Table II. CPZ had no significant effect on $K_A L$, but decreased $a\alpha$ to less than one-half.

The pH of the perfusion solution before and after the perfusion for 10 min are shown in Table III. CPZ decreased significantly the pH after perfusion, resulting in the change of the molar ratio between unionized and ionized forms of SMZ.

After 10 min perfusion, the concentrations of protein and phospholipid, that were recovered from the perfusion solution, increased significantly in the presence of CPZ as shown in Table IV.

DISCUSSION

In the present work, the effects of CPZ on the intestinal transport of SMZ were studied by comparing epithelial permeability and blood flow.

Twenty min after the initiation of perfusion, the concentration of CPZ decreased to 0.6 mM, but these concentrations seem to be enough to affect the absorption of SMZ in view of the duration of the effects of CPZ.

SMZ is a weak acid with a pK_a of 5.81. The decrease in pH of the perfusion solution caused by CPZ seemed to be an important factor in the absorption of SMZ. It is well known that non-

TABLE II. Effect of CPZ on the Epithelial Cell Permeability ($K_A L$) and the Effective Blood Flow Rate Coefficient ($a\alpha$)^{a)}

	$K_A L$ (ml/min/g)	$a\alpha$
Control	0.251 ± 0.026	0.854 ± 0.052
+CPZ	0.214 ± 0.025	0.421 ± 0.044
	N.S. ^{b)}	$p < 0.01$

a) Results are given as the mean $\pm$ S.E. of eight rats.

b) Not significant from the control.

ionized molecules permeate the membrane faster than ionized molecules.¹⁹⁾ The decrease in pH by CPZ from 6.34 to 5.87 may cause the increase in the fraction of the non-ionized SMZ from 0.23 to 0.47. The appearance rate of SMZ in blood was decreased by CPZ, while the apparent permeability of SMZ was not changed by CPZ. Assuming that only unionized SMZ can permeate the membrane, CPZ decreased the epithelial permeability of the unionized SMZ to 41.7% of that of the control as listed in Table V. This pH partition theory of SMZ was also suggested by Nogami *et al.*¹⁴⁾ to explain the ac-

cumulation of SMZ against its concentration gradient rather than the carrier-mediated transport. The reason why CPZ decreased the pH of the perfusion solution has not been well elucidated. Sheetz *et al.*²⁰⁾ suggested that CPZ releases a proton on the outer side of the cell membrane and then permeates rapidly as a neutral form, and that on the inner side of the cell membrane CPZ gets a proton and has a positive charge. The outside of the cell membrane would, then, be acidic after the absorption of CPZ.

CPZ may damage the ion transport mechanisms of the epithelial cells, because CPZ has been

TABLE III. Effect of CPZ on the pH in the Lumen after 10 min Perfusion^{a)}

	pH _{initial}	pH _{10 min}	
Control	6.50	6.34 ± 0.01	N.S. ^{b)}
+CPZ	6.50	5.87 ± 0.02	<i>p</i> < 0.01

a) Results are given as the mean ± S.E. of eight rats.

b) Not significant from the initial pH.

TABLE IV. Effect of CPZ on Protein and Phospholipid released into Perfusion Solution^{a)}

	Protein (μg/g)	Phospholipid (μg/g)
Control	680 ± 109	357 ± 82
+CPZ	1246 ± 184	940 ± 89
	<i>p</i> < 0.01	<i>p</i> < 0.01

a) Results are given as the mean ± S.E. of eight rats.

TABLE V. Effect of CPZ on the Permeability of Epithelial Cells (KA_L) for Total and Unionized SMZ

	Unionized fraction of SMZ ^{b)}	KA_L (ml/min/g) ^{a)}	
		Total SMZ	Unionized SMZ
Control	0.23	0.251 ± 0.026	1.091 ± 0.114
+CPZ	0.47	0.214 ± 0.025	0.455 ± 0.053
		N.S. ^{c)}	<i>p</i> < 0.01

a) Obtained from the literature.^{21,22)}

b) Results are given as the mean ± S.E. of seven rats.

c) Not significant from the control.

reported to inhibit Na^+ , K^+ -ATPase.^{3,4)} The release of protein and phospholipid, which are the main components of the cell membrane, indicates that the microvilli and the cells may be damaged by CPZ. The value of α was decreased by CPZ (Table II). Both CPZ and SMZ bind extensively to plasma protein, and the binding ratios of CPZ and SMZ reported are 89²¹⁾ and 69%,²²⁾ respectively. So if SMZ is replaced by CPZ at the binding site, the ratio of the concentration of SMZ in blood to that in plasma (α) should increase. Because the ratio (α) could not be decreased, CPZ should decrease the fraction of the total blood flow rate in subepithelial capillaries (α). CPZ is known to have a strong adrenergic and weaker cholinergic blocking activity in autonomic nervous system, resulting in the periferal vasodilation and the reduction of the blood pressure.²³⁾ Thus, the decrease in α might result from pharmacological effects of CPZ.

In conclusion, CPZ may inhibit intestinal absorption by decreasing both epithelial permeability of the non-ionized SMZ and the fraction of the total blood flow rate in the subepithelial capillaries. CPZ also may affect the absorption of SMZ by decreasing the pH in the intestinal lumen.

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