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Effect of Simultaneous Administration of Drugs on Absorption and Excretion. XI.¹⁾ Effect of Diphenhydramine on Sulfisomidine Absorption in Rabbits

HISASHI ICHIBAGASE,* YORISHIGE IMAMURA, and KAZUNORI SHIOZU

Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1,
Oe-honmachi, Kumamoto, 862, Japan

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The effect of diphenhydramine on the intestinal absorption of sulfisomidine was investigated in rabbits. Diphenhydramine significantly reduced the mean blood sulfisomidine concentrations at 0.5 and 1.0 hours. In addition, diphenhydramine altered the maximum blood concentration (C_{max}) and the time taken to reach the maximum blood concentration (T_{max}) of sulfisomidine from 103.6 ± 3.2 to 87.2 ± 7.5 $\mu\text{g/ml}$ and from 1.1 ± 0.2 to 2.1 ± 0.5 hour, respectively. However, diphenhydramine was found to have little effect on the area under the blood concentration-time curve ($AUC_{0-\infty}$) or on the elimination half-life ($t_{1/2}$) of sulfisomidine. Diphenhydramine also increased the amount of phenol-red remaining in the rabbit stomach from 26.8 ± 4.1 to $46.5 \pm 4.5\%$. These results indicate that diphenhydramine decreases the rate but not the extent of sulfisomidine absorption by delaying the gastric emptying.

Keywords—drug interaction; diphenhydramine; sulfisomidine; blood concentration; intestinal absorption; gastric emptying

Antihistamines are very often used in combination with other drugs in man. However, little is known about interactions between antihistamines and other drugs. Diphenhydramine is an effective antihistamine possessing anticholinergic, antitussive, antiemetic and sedative activities. Recently, diphenhydramine was found to delay the gastric emptying in rats.²⁾ In addition, our previous study in rabbits³⁾ demonstrated that diphenhydramine significantly decreases the rate of acetaminophen absorption by delaying the gastric emptying.

In the present study, the effect of diphenhydramine on the intestinal absorption of sulfisomidine in rabbits was examined to further elucidate the mechanism of interactions between diphenhydramine and other drugs.

Experimental

Materials—Sulfisomidine was obtained from Daiichi Pharm. Co., Tokyo. Diphenhydramine hydrochloride was kindly supplied by Kowa Co., Nagoya.

In Vivo Experiment—Male rabbits weighing 2.5–3.5 kg were fasted for 38–42 hours prior to the experiments, but water was allowed *ad libitum*. Food and water were withheld during the experiments.

a) Oral Administration: Sulfisomidine (100 mg/kg, 100 mesh powder) was suspended in 70 ml of distilled water and was administered orally.

b) Intravenous Administration: Sulfisomidine (25 mg/kg) was dissolved in 1–2 ml of saline solution by adding the same molar amount of NaOH and was administered intravenously. Diphenhydramine (50 mg/kg) was dissolved in 10 ml of distilled water and was administered orally 5 minutes before oral or intravenous administration of sulfisomidine. Blood samples (0.5 ml) were collected periodically from the ear vein. Assays of sulfisomidine were performed by the Bratton-Marshall method.⁴⁾ The area under the blood concentration-time curve was estimated according to the trapezoidal rule.⁵⁾ The elimination half-life was calculated from the regression slope of the log-linear portion of the blood concentration-time curve, assuming first-order kinetics.

Gastric Emptying Experiment—Gastric emptying experiments were performed by a slight modification of the method of Goto *et al.*⁶⁾ Male rabbits weighing 2.5–3.5 kg were fed soybean-crude refuse instead of the commercial solid diet for at least 5 days and were fasted for 24 hours before the experiments, though water was given freely. A vinyl stomach tube was inserted into the stomach and 50–100 ml of warm test solution (0.2% (w/v) NaCl adjusted to pH 1.2 with diluted HCl, at 37°) was injected. The fluid in the stomach

was rapidly withdrawn by suction with a syringe. This procedure was repeated until the fluid withdrawn contained hardly any solid material. Eighty ml of warm test solution containing phenol-red, a non-absorbable marker, with or without diphenhydramine (50 mg/kg) was injected into the stomach through the vinyl stomach tube. The gastric content was mixed by means of the syringe, then 1 ml of the gastric content was collected and the initial concentration of phenol-red (C_0) was determined. The initial volume of gastric content (V_0) was calculated from its phenol-red concentration. The vinyl stomach tube was then removed. After 0.5 hour, the vinyl stomach tube was inserted into the stomach again and the gastric content was withdrawn as completely as possible. The concentration of phenol-red in the gastric content (C_1) was determined and the volume of gastric content (V_1) was measured. In order to remove any residual gastric content, 80 ml of warm test solution was injected into the stomach through the vinyl stomach tube. The washing was recovered and the concentration of phenol-red in the washing (C_2) was determined. The volume of residual gastric content (V_2) was calculated as follows.

$$V_2 = \frac{80C_2}{C_1 - C_2}$$

The amount (%) of phenol-red remaining in the rabbit stomach 0.5 hour after injection (PR%) was calculated as follows.

$$\text{PR \%} = \frac{C_1(V_1 + V_2)}{C_0 V_0} \times 100$$

Assays of phenol-red were performed spectrophotometrically after alkalization by adding 1 N NaOH.

In Situ Intestinal Absorption Experiment—*In situ* intestinal absorption experiments were carried out as described previously.⁷⁾

Statistical Analysis—Statistical analyses were performed by the paired Student *t*-test. A *p*-value of 0.05 or less was considered significant.

Results and Discussion

Figure 1 shows the time course of blood sulfisomidine concentration after oral administration of sulfisomidine alone or in combination with diphenhydramine. Diphenhydramine significantly reduced the blood sulfisomidine concentrations at 0.5 and 1.0 hours. In addition, diphenhydramine significantly altered the maximum blood concentration (C_{max}) and the time taken to reach the maximum blood concentration (T_{max}) of sulfisomidine from 103.6 ± 3.2

Fig. 1. Time Course of Blood Sulfisomidine Concentration in Rabbits after Oral Administration of Sulfisomidine alone or in Combination with Diphenhydramine

Each point represents the mean $\pm$ S.E. of 5 rabbits.
 —○—, control; —●—, diphenhydramine; * $p < 0.05$.
 ** $p < 0.005$.

to 87.2 ± 7.5 µg/ml and from 1.1 ± 0.2 to 2.1 ± 0.5 hour, respectively (Table I). However, diphenhydramine did not significantly affect the area under the blood concentration-time curve ($AUC_{0-\infty}$) of sulfisomidine (Table I). These experimental results suggest that diphenhydramine may decrease the rate but not the extent of sulfisomidine absorption.

The effect of diphenhydramine on the elimination half-life of sulfisomidine is shown in Table II. Diphenhydramine was found to have little effect on the elimination half-life ($t_{1/2}$) of sulfisomidine after intravenous administration. This finding indicates that diphenhydramine does not affect the sulfisomidine elimination.

Diphenhydramine is chemically similar to SKF 525-A, which is a typical inhibitor of oxidative drug metabolism. Consequently, diphenhydramine may affect the oxidative metabolism of other drugs. Hindmarsh *et al.*⁸⁾ have pointed out that diphenhydramine mark-

TABLE I. Effect of Diphenhydramine on the Maximum Blood Concentration ($C_{\max}$), the Time Taken to Reach the Maximum Blood Concentration ($T_{\max}$) and the Area under the Blood Concentration–Time Curve ($AUC_{0-\infty}$) of Sulfisomidine in Rabbits

Rabbit	$C_{\max}$ ($\mu\text{g/ml}$)		$T_{\max}$ (hr)		$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{hr/ml}$) $\times 10^{-2}$	
	Control	Diphenhydramine	Control	Diphenhydramine	Control	Diphenhydramine
A	108.2	100.8	1.0	2.0	21.7	22.1
B	113.5	108.8	1.0	3.0	14.6	17.1
C	100.1	78.8	2.0	3.0	8.8	9.4
D	96.0	78.9	1.0	2.0	10.8	11.2
E	100.2	68.7	0.5	0.5	6.6	5.5
Mean	103.6	87.2 ^{a)}	1.1	2.1 ^{a)}	12.5	13.1
S.E.	3.2	7.5	0.2	0.5	2.6	2.9

a) Significantly different from control, $p < 0.05$.

TABLE II. Effect of Diphenhydramine on the Elimination Half-Life ($t_{1/2}$) of Sulfisomidine after Intravenous Administration in Rabbits

Rabbit	$t_{1/2}$ (hr)	
	Control	Diphenhydramine
F	12.8	12.2
G	11.0	15.6
H	9.5	10.3
I	8.5	7.1
J	8.2	7.1
Mean	10.0	10.5
S.E.	0.9	1.6

edly inhibits the oxidative metabolism of methaqualone. However, it would appear that diphenhydramine has little effect on the metabolism of sulfisomidine, because the major metabolic pathway of sulfonamides in rabbits is well known to be acetylation at the 4-amino position.⁹⁾

According to the pH-partition hypothesis, acidic drugs should be absorbed more readily from the stomach than from the small intestine.^{10,11)} However, many investigators have demonstrated that even acidic drugs such as aspirin and warfarin are absorbed much more slowly from the stomach than from the small intestine, presumably because of the relatively large surface area of the latter.^{12,13)} Delayed gastric emptying is therefore likely to cause a marked decrease in the rate of drug absorption. Recently, Feldman and Putcha²⁾ have reported that diphenhydramine delays gastric emptying in rats. In addition, diphenhydramine was found to decrease the rate of *p*-aminosalicylate absorption in rats by delaying the gastric emptying.¹⁴⁾ These results suggest that diphenhydramine may decrease the rate of sulfisomidine absorption by delaying the gastric emptying.

In order to elucidate the effect of diphenhydramine on gastric emptying in rabbits, the warm test solution containing phenol-red with or without diphenhydramine was injected into the stomach through a vinyl stomach tube and the amount of phenol-red remaining in the stomach 0.5 hour after injection was determined. As shown in Table III, diphenhydramine significantly increased the amount of phenol-red remaining in the rabbit stomach from 26.8 ± 4.1 to $46.5 \pm 4.5\%$. It is evident from this experimental result that diphenhydramine delays gastric emptying in rabbits. Furthermore, diphenhydramine was observed to have little effect on the *in situ* intestinal absorption of sulfisomidine in rabbits (Fig. 2). Therefore,

Fig. 2. Effect of Diphenhydramine on the *in Situ* Intestinal Absorption of Sulfisomidine in Rabbits

Semilogarithmic plots for the amount (%) of sulfisomidine remaining in the small intestine (SIM%) versus time are shown. Diphenhydramine was added to the perfusate 60 minutes after the beginning of the experiment. Dose of diphenhydramine: 25 mg/kg.

Fig. 3. Relationship between the Amount (%) of Phenol-Red Remaining in the Stomach (PR%) and the Time Taken to Reach the Maximum Blood Concentration (T_{max}) of Sulfisomidine in Rabbits

$n=5$ (rabbits A-E), $r=0.755$.

TABLE III. Effect of Diphenhydramine on the Amount (%) of Phenol-Red Remaining in the Stomach (PR%)

Rabbit	Phenol-Red remaining (PR%)	
	Control	Diphenhydramine
K	18.8	33.8
L	41.2	61.2
M	21.0	47.0
N	22.2	41.5
O	30.7	49.1
Mean	26.8	46.5 ^{a)}
S.E.	4.1	4.5

a) Significantly different from control, $p < 0.001$.

it is concluded that diphenhydramine decreases the rate of sulfisomidine absorption by delaying the gastric emptying.

A good correlation was observed between the amount of phenol-red remaining in the stomach 0.5 hour after injection (PR%) and the time taken to reach the maximum blood concentration (T_{max}) of sulfisomidine (Fig. 3). This finding implies that gastric emptying is an important factor affecting the rate of sulfisomidine absorption, and supports the conclusion that when sulfisomidine is administered orally in combination with diphenhydramine, delayed gastric emptying causes the decrease in the rate of sulfisomidine absorption.

In this study, we have obtained evidence that diphenhydramine decreases the rate of sulfisomidine absorption by delaying the gastric emptying. A similar interaction was observed when atropine, a typical anticholinergic drug, was used instead of diphenhydramine.¹⁵⁾ This indicates that the effect is probably due to the anticholinergic activity of diphenhydramine. Many drugs, such as antihistamines, anti-parkinsonism drugs and tricyclic antidepressants, possess anticholinergic activity and might therefore influence drug absorption by delaying the gastric emptying.

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Some Structure-Activity Relationships for Bactobolin Analogs in the Treatment of Mouse Leukemia P388¹⁾

TOMOHIKO MUNAKATA and TAKEKI OKUMOTO*

Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd.,
Nishigahara 1-26-1, Kita-ku, Tokyo 114, Japan

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Bactobolin, (3*S*,4*R*,4*aR*,5*R*,6*R*)-4-(*L*-alanyl-amino)-3-(dichloromethyl)-3,4,4*a*,5,6,7-hexahydro-5,6,8-trihydroxy-3-methyl-1*H*-2-oxa-1-naphthalenone, is an antitumor antibiotic produced by *Pseudomonas*. In the present studies, 14 bactobolin analogs with or without substituents on the amino group attached to the 4 position of the bactobolin nucleus were synthesized and evaluated for activity against mouse leukemia P388. These compounds were given intraperitoneally to mice bearing ascitic leukemia P388. Compounds with the *L*-seryl group and some *L*-alanine derivatives on the amino group were found to possess antileukemic activity, but at the dose levels tested, they were neither more effective nor more potent than bactobolin which increased the lifespan of leukemic mice by 110% over the control at an optimal dose of 2.5 mg/kg/day. The other analogs examined were found to be ineffective and nonlethal for mice at doses of 10 mg/kg/day or less. The structure-activity relationships for bactobolin analogs are discussed.

Keywords—bactobolin; bactobolin analogs; antitumor antibiotic; antileukemic activity; mouse leukemia P388; structure-activity relationship

Bactobolin is an antibiotic produced by *Pseudomonas*.²⁻⁴⁾ Its chemical structure is 3-dichloromethylactinobolin, (3*S*, 4*R*, 4*aR*, 5*R*, 6*R*)-4-(*L*-alanyl-amino)-3-(dichloromethyl)-3,4,4*a*,5,6,7-hexahydro-5,6,8-trihydroxy-3-methyl-1*H*-2-oxa-1-naphthalenone.^{2,5)}

This chlorine-containing antibiotic has been shown to possess antitumor activity against mouse leukemias L1210 and P388, and several lines of rat hepatomas at low doses, *e.g.*, 1 mg/