[Chem. Pharm. Bull. 21(3) 668—671 (1973)]

UDC 615.276.015.2.076.9

Effects of Simultaneous Administration of Drugs on Absorption and Excretion. II.[1] Effects of Non-steroidal Anti-inflammatory Drugs on Absorption and Excretion of Sulfadimethoxine in Rabbits

Yorishige Imamura and Hisashi Ichibagase

Faculty of Pharmaceutical Sciences, Kumamoto University[2]

(Received September 25, 1972)

Non-steroidal anti-inflammatory drugs such as phenylbutazone and sulfinpyrazone have been known to bind strongly with plasma proteins and inhibit the binding of a sulfonamide by competing with the drug for the same binding sites on plasma proteins.[3,4] Furthermore, it has been shown that the liberation of a sulfonamide from its binding sites on plasma proteins by the non-steroidal anti-inflammatory drugs affects on the distribution and excretion of the sulfonamide in animals. For example, Anton[5] observed that sulfinpyrazone altered the concentration of sulfaethylthiadiazole between plasma and tissues in rats. Also, Arita, *et al.*[6] reported that sulfinpyrazone and oxphenbutazone inhibited the renal tubular secretion of sulfonamides in dogs. However, there is not yet enough evidence to demonstrate the effects of combinations of non-steroidal anti-inflammatory drugs and sulfonamides in view of protein binding.

In the previous paper,[1] the authors demonstrated that the binding of sodium cyclamate and rabbit serum albumin was competitively inhibited by phenylbutazone, consequently the urinary excretion of sodium cyclamate in rabbits was accelerated by simultaneous administration of phenylbutazone.

The purpose of this investigation is to demonstrate the effects of non-steroidal anti-inflammatory drugs on the absorption and excretion of sulfadimethoxine in rabbits.

Experimental

Materials——Sulfadimethoxine (Daiichi Seiyaku Co.), indomethacin (Banyu Seiyaku Co.), and benzydamine (Daiichi Seiyaku Co.) were obtained from commercial sources. Salicylic acid was of J.P. VII grade.

Animals——Male rabbits weighing 2.5—3.2 kg were fasted for approximately 20 hours prior to the experiments *in vivo* and *in situ*. However, drinking water was allowed *ad libitum*.

***In Vivo* Experimental Methods**——(a) Administration Method of Drugs: One hundred mg of sulfadimethoxine per kg body weight was administered to rabbits with or without non-steroidal anti-inflammatory drugs (100 mg/kg body weight). These drugs were administered orally by using Nelaton's catheter in the form of suspension in 100 ml of water.

(b) Collections of Urine: Urinary collections were made by using Nelaton's catheter during the following intervals after time zero: 0—1, 1—2, 2—3, 3—4, 4—5, 5—6, 6—8 hour.

(c) Collections of Blood: An 0.5 ml of blood was collected from auricle vein of a rabbit by using a 1.0 ml syringe containing 0.1 ml of 3.8% sodium citrate.

***In Situ* Intestinal Absorption Procedure**——*In situ* intestinal absorption experiment in rabbits was the same as described in the previous paper[1] except for the length (40 cm) of the small intestine used and the pH (7.38) of isotonic phosphate buffer solution.

1) Part I: H. Ichibagase, Y. Imamura, A. Kinoshita, and S. Kojima, *Chem. Pharm. Bull.* (Tokyo), **20**, 947 (1972).
2) Location: 5-1 *Oe-honmachi, Kumamoto.*
3) A.H. Anton, *J. Pharmacol. Exptl. Therap.*, **129**, 282 (1960).
4) P.M. Keen, *Brit. J. Pharmacol. Chemotherapy*, **26**, 704 (1966).
5) A.H. Anton, *J. Pharmacol. Exptl. Therap.*, **134**, 291 (1961).
6) T. Arita, R. Hori, and M. Takada, Proc. 3rd Symposium on Drug Metabolism and Action, ed. by H. Tsukamoto, 1972, pp. 174—193.

***In Situ* Gastric Absorption Procedure**——Experimental procedure for drug absorption from the rabbit stomach was carried out according to the method reported by Iguchi, *et al.*[7] A 1.0 ml aliquot was pipetted out at periodical intervals, and was then assayed for unabsorbed sulfadimethoxine according to the method described above.

Protein Binding Experiment *In Vivo*——Protein binding was determined by the method of equilibrium dialysis. The equilibrium dialysis experiment was carried out according to the method described in the previous paper.[1]

Analytical Method——Total sulfadimethoxine (sulfadimethoxine plus sulfadimethoxine N^4-acetate) in blood and urine was analyzed after hydrolysis in hot 1/3N-hydrochloric acid by the method of Bratton and Marshall.[8] Also, sulfadimethoxine in blood, *in situ* experiment and *in vitro* experiment was determined by the same method without hydrolysis.

Result and Discussion

In order to examine the effects of non-steroidal anti-inflammatory drugs on the blood level and urinary excretion of sulfadimethoxine and its metabilotes, sulfadimethoxine was administered orally to rabbits with or without the non-steroidal anti-inflammatory drugs such as salicylic acid, indomethacin and benzydamine. As shown in Fig. 1 and 2, the blood levels of unchanged and total sulfadimethoxine were markedly reduced by simultaneous administration of salicylic acid. Moreover, as seen in Fig. 3, the urinary excretion of total sulfadimethoxine was also decreased by simultaneous administration of salicylic acid. From these results, it is presumed that the gastrointestinal absorption of sulfadimethoxine is inhibited by salicylic acid.

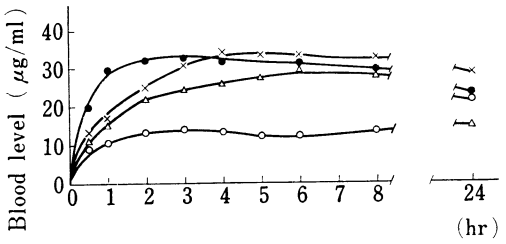

Fig. 1. Mean Blood Level Curves for Unchanged Sulfadimethoxine following Oral Administration of Sulfadimethoxine with Non-steroidal Anti-inflammatory Drugs

—●—: sulfadimethoxine alone (100 mg/kg)
—○—: sulfadimethoxine+salicylic acid (100 mg/kg)
—△—: sulfadimethoxine+indomethacin (100 mg/kg)
—×—: sulfadimethoxine+benzydamine (100 mg/kg)
Each value is expressed as the mean of 4 experiments.

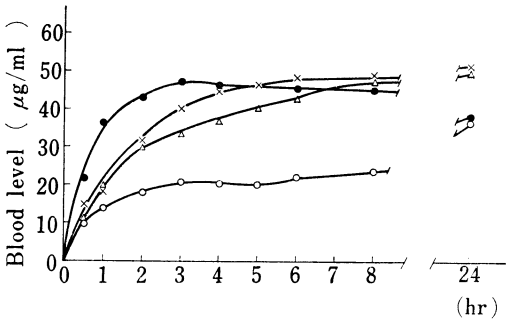

Fig. 2. Mean Blood Level Curves for Total Sulfadimethoxine following Oral Administration of Sulfadimethoxine with Non-steroidal Anti-inflammatory Drugs

—●—: sulfadimethoxine alone (100 mg/kg)
—○—: sulfadimethoxine+salicylic acid (100 mg/kg)
—△—: sulfadimethoxide+indomethacin (100 mg/kg)
—×—: sulfadimethoxine+benzydamine (100 mg/kg)
Each value is expressed as the mean of 4 experiments.

In order to clarify further the effect of salicylic acid on the blood level of sulfadimethoxine *in vivo*, the *in situ* gastrointestinal absorption of sulfadimethoxine administered together with salicylic acid was compared with that of single administration in rabbits. As shown in Table I, the rate constant of absorption of sulfadimethoxine from the small intestine was significantly decreased by the concomitant administration of salicylic acid. Whereas, as seen in Table II, there was no evident difference between single and concomitant administration in the absorp-

7) S. Goto, R. Takamatsu, M. Shibao, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 332 (1968).
8) A.C. Bratton and E.K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

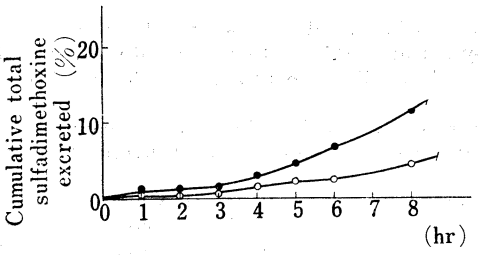

Fig. 3. Mean Cumulative Excretion Curves for Total Sulfadimethoxine following Oral Administration of Sulfadimethoxine with Salicylic Acid

—●—: sulfadimethoxine alone (100 mg/kg)
—○—: sulfadimethoxine+salicylic acid (100 mg/kg)
Each value is expressed as the mean of 4 experiments.

tion of sulfadimethoxine from the stomach *in situ*. Also, the rate constant of absorption of sulfadimethoxine from the stomach was a small number as compared with that of absorption of the drug from the small intestine (see Tables I and II). Accordingly, from these results described above, it is considered that the inhibition of intestinal absorption of sulfadimethoxine by salicylic acid is an important factor that reduces markedly the blood level of sulfadimethoxine in rabbits.

Ordinarily, it has been known that a very large number of drugs are bound to some degree to plasma proteins and drugs bound to plasma proteins cannot transfer across the biological membranes. Thus, it is considered that the transfer of a drug from plasma to tissues is dependent on the concentration of the free drug in plasma. As seen in Fig. 4, the inhibitory effect of salicylic acid to the binding of sulfadimethoxine with bovine serum albumin was much greater than that of benzydamine or indomethacin. From these results, it is

Table I. Effect of Salicylic Acid on the Absorption of Sulfadimethoxine from Rabbit Small Intestine, *in Situ*

Exp. No.	Absorption rate constant (hr^{-1})	
	Single administration[a]	Simultaneous administration[b]
1	0.288	0.173
2	0.297	0.198
3	0.322	0.227
4	0.322	0.231
5	0.335	
Mean ± SD[c]	0.313 ± 0.018	0.207 ± 0.025[d]

a) initial concentration of drug sulfadimethoxine: 200 μg/ml
b) initial concentration of drugs sulfadimethoxine: 200 μg/ml+salicylic acid: 200 μg/ml
c) standard deviation
d) significant difference to single administration, $P < 0.01$

Table II. Effect of Salicylic Acid on the Absorption of Sulfadimethoxine from Rabbit Stomach, *in Situ*

Exp. No.	Absorption rate constant (hr^{-1})	
	Single administration[a]	Simultaneous administration[b]
1	0.030	0.025
2	0.040	0.040
3	0.049	
Mean ± SD[c]	0.039 ± 0.008	0.033 ± 0.008

a) initial concentration of drug sulfadimethoxine: 100 μg/ml
b) initial concentration of drugs sulfadimethoxine: 100 μg/ml+salicylic acid: 100 μg/ml
c) standard deviation

apparent that the concentration of free sulfadimethoxine in plasma is increased by salicylic acid. Consequently, it is presumed that the increase of transfer of sulfadimethoxine from plasma to tissues, in cooperation with the inhibition of intestinal absorption of sulfadimethoxine by salicylic acid described above, results in the decrease in the blood levels of unchanged and total sulfadimethoxine by salicylic acid in rabbits. Further works on these problem are now under way and the details will be reported in near future.

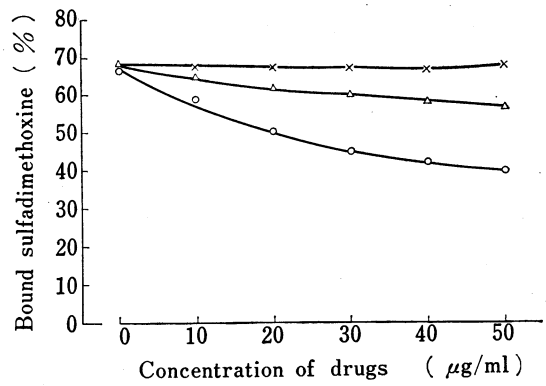

Fig. 4. Inhibitory Effect of Non-steroidal Anti-inflammatory Drugs on the Binding of Sulfadimethoxine and Bovine Serum Albumin

—○—: salicylic acid
—△—: indomethacin
—×—: benzydamine
initial concentration of sulfadimethoxine: 30 μg/ml
albumin concentration: 1.0 (w/v)%

Furthermore, the attainment of the maximum blood levels of unchanged and total sulfadimethoxine was delayed by simultaneous administration of indomethacin or benzydamine as shown in Fig. 1 and 2. Recently, Kato, *et al.*[9] reported that indomethacin or benzydamine evidently decreased the gastric emptying rate of phenol red in rats. As seen in Tables I and II, it is apparent that the absorption rate of sulfadimethoxine is much greater in the small intestine than the stomach, and that the primary site for the absorption of the drug is the small intestine. Accordingly, it may be considered that the simultaneous administration of indomethacin or benzydamine alters the absorption of sulfadimethoxine by delaying the gastric emptying and hence increasing the time for sulfadimethoxine to reach its primary absorption site.

9) R. Kato, A. Takanaka, K. Onoda, and Y. Omcri, *Nippon Yakurigaku Zasshi*, **67**, 134 (1971).

[Chem. Pharm. Bull. 21(3) 671—672 (1973)]

UDC 547.56'292.04 : 542.98

Microbial Transformation of 2,4-D and Its Analogues

Shoichi Nakajima,[1a] Nakato Naito, and Toshikazu Tani[1b]

Hoshi College of Pharmacy[1a] and Faculty of Agriculture, Kagawa University[1b]

(Received October 21, 1972)

2,4-Dichlorophenoxyacetic acid(2,4-D) and the analogous phenoxyacetic acids have been known to possess not only the growth regulating action on plants but also the antimicrobial activities against bacteria and moulds. It was during 1951 and 1958 that Naito, Tani, Kishi and Kojima examined antifungal activities of 2,4-D and its analogues, *i.e.* 2-chloro-, 2-methyl-, 2,4-dichloro-, 2-methyl-4-chloro-, and 2,4,5-trichloro-phenoxyacetic acids, against

1) Location: *a) 2-Chome, Ebara, Shinagawa-ku, Tokyo; b) Miki-machi, Kida-gun, Kagawa-ken.*