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Effects of Simultaneous Administration of Drugs on Absorption and Excretion. IV.[1] Effect of Salicylic Acid on Antibacterial Activity and Distribution of Sulfadimethoxine in Rabbits

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1. Plasma levels of sulfadimethoxine determined by bioassay, namely antibacterial activities, were markedly low in comparison with that of sulfadimethoxine determined by chemical assay in rabbits.

2. The antibacterial activities of sulfadimethoxine in rabbits following intravenous administration of sulfadimethoxine were significantly increased by the concomitant administration of salicylic acid.

3. On the other hand, plasma levels of sulfadimethoxine determined by chemical assay in rabbits following intravenous administration of sulfadimethoxine were decreased by the concomitant administration of salicylic acid.

4. These results suggest that the distribution of sulfadimethoxine from blood to tissues is enhanced by salicylic acid.

One of the important types of drug interactions is the displacement of highly protein-bound drugs from their binding sites.[3–8] For example, Anton[9,10] indicated that the antibacterial activity *in vitro* and the tissue levels of sulfonamides in rat could be significantly increased by displacing them from binding to protein with another drug. Furthermore, Scholtan[11] elucidated that a relationship existed between the degree of protein binding and tissue distribution of sulfonamides. However, there is not yet enough evidence to demonstrate the influence of another drug on the antibacterial activity and the distribution of sulfonamides.

In the previous paper,[1] the authors demonstrated that the antibacterial activity *in vitro* of sulfadimethoxine (SDM) was markedly reduced by the addition of bovine serum albumin, and that the antibacterial activity of SDM that decreased in the presence of bovine serum albumin was significantly recovered by the addition of displacing agents such as salicylic acid and phenylbutazone.

The purpose of the present investigation is to elucidate the influence of salicylic acid on the antibacterial activity *in vivo* and tissue distribution of SDM in rabbits.

Experimental

Materials——SDM (Daiichi Seiyaku Co., Ltd.), sodium salicylate (Nakarai Chemical Co., Ltd.).

Animals——Male rabbits weighing 2.8—3.2 kg were fasted about 24 hours prior to the experiments. However, drinking water allowed *ad libitum*.

1) Part III: Y. Imamura and H. Ichibagase, *Yakugaku Zasshi*, **93**, 1206 (1973).
2) Location: *5-1 Oe-honmachi, Kumamoto, 862, Japan.*
3) C.M. Kunin, *Clin. Pharmacol. Therap.*, **7**, 166 (1966).
4) P.M. Aggeler, R.A. O'Reilly, L. Leong and P.E. Kowitz, *New Engl. J. Med.*, **276**, 496 (1967).
5) Y. Ohmiya, *Nippon Yakurigaku Zasshi*, **67**, 193 (1968).
6) R.A. O'Reilly and G. Levy, *J. Pharm. Sci.*, **59**, 1258 (1970).
7) H.M. Solomon, J.J. Schrogie and D. Williams, *Biochem. Pharmacol.*, **17**, 143 (1968).
8) M.C. Meyer and D.E. Guttman, *J. Pharm. Sci.*, **57**, 895 (1968).
9) A.H. Anton, *J. Pharm. Exptl. Therap.*, **129**, 282 (1960).
10) A.H. Anton, *J. Pharm. Exptl. Therap.*, **134**, 291 (1961).
11) W. Scholtan, *Arzneimittel-Forsch.*, **11**, 707 (1961).

***In Vivo* Experimental Methods**——(a) Administration Method of Drugs: SDM (25 mg/kg body weight as sodium salt) with or without salicylic acid (100 mg/kg body weight as sodium salt) were administered intravenously to rabbits.

(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 hour.

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

(d) Collections of Plasma: A 4.0 ml of blood was collected from auricle vein of a rabbit using a heparinized syringe. After standing for about 60 minutes, the blood was centrifuged at 3000 rpm for 15 minutes and the supernatant fluid separated.

Determination Procedure of SDM by Chemical Assay——Total SDM (SDM+SDM N^4-acetate) in urine was analyzed after hydrolysis in hot 1/3 N-hydrochloric acid by the method of Bratton and Marshall.[12] Also, unchanged SDM in blood and plasma was determined by the same method without hydrolysis.

Determination Procedure of SDM by Bioassay——The determination of antibacterial activity of SDM in rabbit plasma was carried out according to the method describing in our previous paper.[1,13]

Result and Discussion

Comparison of Plasma Levels of SDM determined by Bioassay and by Chemical Assay in Rabbits

Plasma levels of SDM in rabbits following intravenous administration of SDM were determined by bioassay and by chemical assay, respectively. As shown in Fig. 1, plasma levels of SDM by bioassay, namely antibacterial activities, were markedly low in comparison with that of SDM by chemical assay. Previously, the authors revealed that the antibacterial activity *in vitro* of SDM was significantly decreased by the addition of bovine serum albumin, and that this decrease was dependent on the degree of binding of SDM with bovine serum albumin.[1] Accordingly, it is considered that the low antibacterial activities of SDM in rabbit plasma are attributed to the binding of SDM with rabbit plasma albumin.

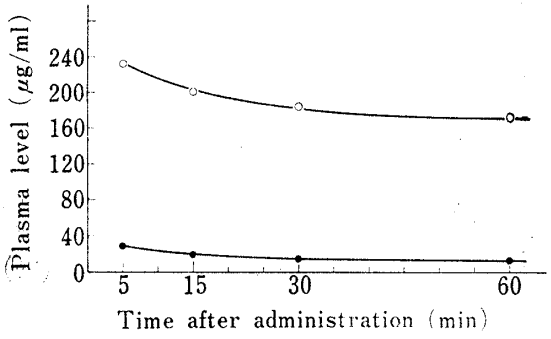

Fig. 1. Comparison of Plasma Levels of Sulfadimethoxine determined by Chemical Assay and by Bioassay

Each value is expressed as mean of 4 experiments.
—○—: plasma levels of unchanged SDM by chemical assay
—●—: plasma levels of SDM by bioassay

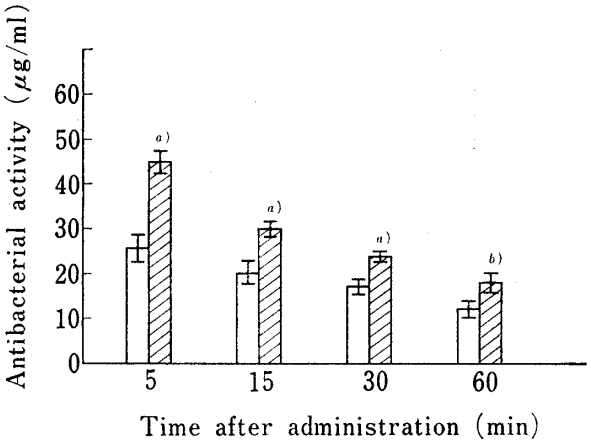

Fig. 2. Antibacterial Activities of Sulfadimethoxine in Rabbits following Intravenous Administration of Sulfadimethoxine with or without Salicylic Acid

The antibacterial activity in rabbit plasma following single administration of salicylic acid (100 mg/kg body weight) was not detected. Each value is expressed as mean±SD of 4 experiments. Mean values for concomitant administered and single administered rabbits are significantly different.
a) $p<0.01$, *b*) $p<0.05$, student's t test.
□: SDM alone (25 mg/kg body weight)
▨: SDM+salicylic acid (100 mg/kg body weight)

12) A.C. Bratton and E.K. Marshall, *J. Biol. Chem.*, **128**, 539 (1939).
13) M. Shibata, K. Shigemori and Y. Imamura, *Chemotherapy*, Submitted.

Effect of Salicylic Acid on Antibacterial Activity of SDM in Rabbits

In the preceding paper of this series,[1] we demonstrated that the antibacterial activity *in vitro* of SDM that decreased in the presence of bovine serum albumin was recovered by the addition of salicylic acid. In this paper, in order to examine further the influence of salicylic acid on the antibacterial activity *in vitro* of SDM, the antibacterial activities of SDM in rabbit plasma following intravenous administration of SDM with salicylic acid were compared with that in rabbit plasma following single administration of SDM. As shown in Fig. 2, the antibacterial activities of SDM in rabbit plasma were significantly increased by the concominant administration of salicylic acid. This result indicates that binding *in vivo* of SDM with rabbit plasma albumin also can be displaced by salicylic acid.

Effect of Salicylic Acid on Distribution of SDM in Rabbits

In contrast with the antibacterial activity, plasma levels of SDM determined by chemical assay were significantly decreased by the concomitant administration of salicylic acid. Fig. 3 shows this effect. It is presumed that this decrease in plasma levels of SDM may be due to the following biopharmaceutical modification: (1) Gastrointestinal absorption of SDM is depressed by salicylic acid. (2) Distribution of SDM into tissues is increased by salicylic acid. (3) Metabolism of SDM is induced by salicylic acid. (4) Urinary excretion of SDM is enhanced by salicylic acid. As can be seen from Fig. 4, however, there was no significant difference between urinary excretion of total SDM in rabbit following simultaneous administration of SDM with salicylic acid and that in rabbit following single administration of SDM. Also, the gastrointestinal absorption can be disregarded since both drugs were administered intravenously to rabbits. Further, as discussed by McQueen, *et al.*,[14] it is difficult to explain the rapid onset of the effect of salicylic acid on a metabolic or an excretory basis. Consequently, it can be assumed that the tissue distribution plays an important part in the decrease in plasma levels of SDM following simultaneous administration of SDM with salicylic acid.

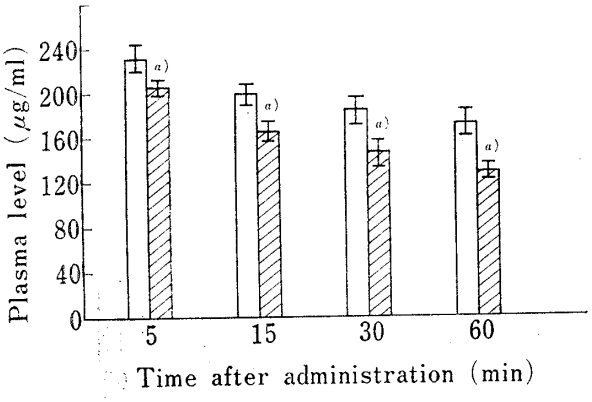

Fig. 3. Plasma Levels of Unchanged Sulfadimethoxine determined by Chemical Assay in Rabbits following Intravenous Administration of Sulfadimethoxine with or without Salicylic Acid

Each value is expressed as mean±SD of 4 experiments. Mean values for concomitant administered and single administered rabbits are significantly different,
a) $p<0.05$, student's t test.
□: SDM alone (25 mg/kgbody weight)
▨: SDM+salicylic acid (100 mg/kg body weight)

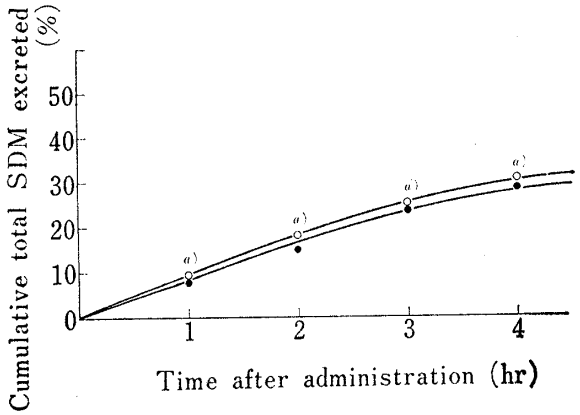

Fig. 4. Urinary Excretion Curves for Total Sulfadimethoxine in Rabbit following Intravenous Administration of Sulfadimethoxine with or without Salicylic Acid

Each value is expressed as mean of 5 experiments. Mean values for concomitant administered and single aaministered rabbits are not significantly different,
a) $p<0.05$, student's t test.
—○—: SDM alone (25 mg/kg body weight)
—●—: SDM+salicylic acid (100 mg/kg body weight)

In order to clarify the influence of salicylic acid on the distribution of SDM in rabbit, the blood level-time relationships for SDM were determined in rabbit after intravenous

14) E.G. McQueen and W.M. Wardell, *Brit. J. Pharmacol.*, **43**, 312 (1971).

administration of SDM with or without salicylic acid (Fig. 5), and these results were fitted to a two-compartment open model describing by Riegelman, *et al.*[15] The model which includes peripheral compartment is shown in Fig. 6. In this model, k_{12} is the first-order rate constant controlling diffusion of the drug from central compartment to peripheral compartment, k_{21} is the first-order rate constant controlling return of the drug from peripheral compartment to central compartment, and k_{el} is the sum of the first-order rate constant for the simultaneous processes of metabolism and excretion. The above two-compartment open model results in the following equation (Eq. 1) describing the blood level-time curve after intravenous administration:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

where C_p is the blood level of the drug. Estimates of the values of A, B, α and β for SDM in rabbits were obtained by graphical analysis of semilogarithmic plots of blood levels *versus* time.[16] These values are given in Table I. Also, the values of the individual rate constant for SDM in this model, k_{12}, k_{21} and k_{el}, were calculated from the values of A, B, α and β by the method of Riegelman, *et al.*[15] As shown in Table II, the value of k_{12} was evidently increased by the concomitant administration of salicylic acid, whereas the increase in the values of k_{21} and k_{el} was small. These results suggest that the distribution of SDM from blood to tissues is enhanced by salicylic acid.

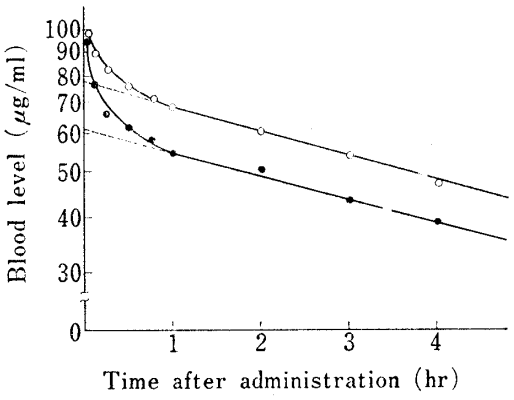

Fig. 5. Blood Level Curves for Unchanged Sulfadimethoxine in Rabbits following Intravenous Administration of Sulfadimethoxine with or without Salicylic Acid

Each value is expressed as mean of 4 experiments.
—○—: SDM alone (25 mg/kg body weight)
—●—: SDM+salicylic acid (100 mg/kg body weight)

Recently, Anton[17] revealed that the distribution of sulfamethoxypyridazine was markedly altered in both the mother rat and its fetus by interfering with the binding of this drug

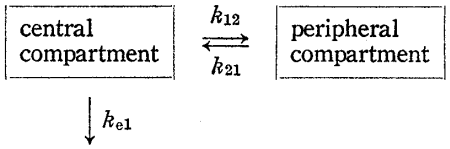

Fig. 6. Two-compartment Pharmacokinetic Model

to plasma protein in the mother, and that this effect was due to binding displacement, since the displacing agent had little or no effect on the distribution of sulfanilamide with very low binding to plasma protein. Furthermore, Wardell, *et al.*[14,18] showed that intravenous admini-

TABLE I. Values of Parameters for $C_p = Ae^{-\alpha t} + Be^{-\beta t}$ for Sulfadimethoxine in Rabbits following Intravenous Administration of Sulfadimethoxine with or without Salicylic Acid

	A (μg/ml)	α (hr^{-1})	B (μg/ml)	β (hr^{-1})
Single administration	16.5	2.89	77.0	0.12
Concomitant administration	23.5	3.65	61.5	0.11

These values were calculated from the data given in Fig. 5.

15) S. Riegelman, J.C.K. Loo and M. Rowland, *J. Pharm. Sci.*, **57**, 117 (1968).
16) S. Kojima, *Chem. Pharm. Bull.* (Tokyo), **21**, 2432 (1973).
17) A.H. Anton and R.W. Rodriguez, *Science*, **180**, 974 (1973).
18) W.M. Wardell, *Brit. J. Pharmacol.*, **43**, 325 (1971).

Table II. Values of Individual Rate Constants for Sulfadimethoxine in Rabbits

	k_{12} (hr^{-1})	k_{21} (hr^{-1})	k_{el} (hr^{-1})
Single administration	0.46	2.40	0.14
Concomitant administration	0.93	2.68	0.15

stration of phenylbutazone caused a rapid fall in the concentration of total sulfadoxine together with a simultaneous rise in the concentration of free sulfadoxine in plasma, and concluded that this effect of phenylbutazone was solely to cause redistribution of the sulfadoxine. These findings support our suggestion that salicylic acid increases not only the antibacterial activity of SDM, but also the transfer of SDM into tissues in rabbits. However, further experimentation is necessary to explain fully this interesting observation.