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Effect of Simultaneous Administration of Drugs on Absorption and Excretion. XV.[1] Effect of Probenecid on Plasma Protein Binding of Sulfadimethoxine in Rabbits: The Role of N^4-Acetylsulfadimethoxine, the Major Metabolite of Sulfadimethoxine

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The effect of probenecid on the plasma protein binding of sulfadimethoxine (SDM) was investigated in rabbits. Probenecid markedly reduced the *in vivo* binding of SDM to rabbit plasma proteins. On the other hand, probenecid had no effect on the *in vitro* binding of SDM to rabbit plasma proteins. Probenecid significantly increased N^4-acetylsulfadimethoxine (N^4-AcSDM) concentration in plasma after intravenous bolus injection of SDM, and prolonged the elimination half-life of N^4-AcSDM after intravenous bolus injection of N^4-AcSDM. In addition, N^4-AcSDM reduced the *in vitro* binding of SDM to rabbit plasma proteins. These results indicate that probenecid indirectly reduces the *in vivo* binding of SDM to rabbit plasma proteins, by causing a significant increase of N^4-AcSDM concentration in plasma.

Keywords——sulfadimethoxine; N^4-acetylsulfadimethoxine; probenecid; *in vivo* protein binding experiment; *in vitro* protein binding experiment; protein binding displacement; elimination half-life; rabbit

Several investigators have demonstrated that a metabolite is more strongly bound to plasma proteins than the parent drug. For example, Shams-Eldeen *et al.* revealed that the *in vitro* binding of sulindac sulfide, an active metabolite of sulindac, to human serum albumin is considerably stronger than that of the parent drug.[2] Thus, if the plasma concentration of a metabolite which is strongly bound to plasma proteins is sufficiently high, the displacement of the parent drug from its plasma protein binding sites will occur. Recently, Souich *et al.* reported that N^4-acetylsulfadiazine displaces sulfadiazine from its plasma protein binding sites.[3] We also reported that N^4-acetylcarbutamide displaces carbutamide from its plasma protein binding sites.[4]

In the present paper, we describe the role of N^4-acetylsulfadimethoxine (N^4-AcSDM), the major metabolite of sulfadimethoxine (SDM), in the *in vivo* protein binding interaction between SDM and probenecid.

Experimental

Materials——Probenecid was kindly supplied by Merck-Banyu Co., Ltd. N^4-Acetylsulfadimethoxine (N^4-AcSDM) was synthesized from SDM by the method of Uno *et al.*[5] Sulfadimethoxine (SDM) and other chemicals were obtained commercially.

Animal Experiments——Male albino rabbits weighing 2.3—3.0 kg were fasted for 38—42 h prior to the experiments, but drinking water was allowed *ad libitum*.

a) Intravenous Bolus Injection: The SDM, N^4-AcSDM and probenecid solutions for injection were prepared by dissolving the compounds in 1—3 ml of saline solution containing the same molar amount of NaOH. The doses of SDM, N^4-AcSDM and probenecid were 50, 25 and 25 mg/kg, respectively.

b) Plasma Sampling for Ultrafiltration: About 6 ml of blood was collected from the ear vein 2 h after SDM or probenecid injection. After heparinization, the blood was immediately centrifuged and the plasma was separated.

c) Plasma Sampling for Elimination Half-life Determination: About 1 ml of blood was collected periodically from the ear vein after N^4-AcSDM injection. After heparinization, the blood was immediately centrif-

uged and the plasma was separated.

Protein Binding Experiments——*In vivo* and *in vitro* protein binding experiments were carried out according to the ultrafiltration method described previously.[4]

a) *In Vivo* Protein Binding Experiment: *In vivo* binding of SDM was determined for rabbit plasma obtained 2 h after SDM injection.

b) *In Vitro* Protein Binding Experiment: *In vitro* binding of SDM or N^4-AcSDM was determined for rabbit plasma prepared by adding SDM or N^4-AcSDM.

Analytical Methods——The SDM concentrations in rabbit plasma and its ultrafiltrate were measured by the Bratton-Marshal method.[6] The N^4-AcSDM concentrations in rabbit plasma and its ultrafiltrate were measured after acid hydrolysis (0.5 N HCl, at 100°C for 1 h) by the Bratton-Marshall method.[6] The N^4-AcSDM concentration in rabbit plasma after intravenous bolus injection of SDM was calculated from the difference between unchanged and total (unchanged+metabolites) SDM concentrations measured before and after acid hydrolysis. Since over 90% of SDM in 24 h urine after oral administration of SDM to rabbits has been reported to be in the N^4-acetylated form,[7] the presence of metabolites other than N^4-AcSDM was neglected in the above calculation.

Pharmacokinetic Analysis——The elimination half-life of N^4-AcSDM was calculated by linear regression analysis of the log-plasma concentration *versus* time during β phase.

Statistical Analysis——Statistical analysis was performed by means of the paired Student *t*-test. Differences between means were considered to be significant when $p<0.05$.

Results and Discussion

The effect of probenecid on the plasma protein binding of SDM was investigated in rabbits. The plasma protein binding was determined by the ultrafiltration method.[4] Figure 1 shows the effect of probenecid on the *in vivo* binding of SDM to rabbit plasma proteins. It is evident that probenecid markedly reduces the *in vivo* binding of SDM to rabbit plasma proteins. This effect was confirmed by comparing the intercepts of the two regression lines obtained. On the other hand, as shown in Fig. 2, probenecid had no effect on the *in vitro* binding of SDM to rabbit plasma proteins.

To elucidate the reason why probenecid can reduce only the *in vivo* binding of SDM to rabbit plasma proteins, the *in vitro* binding of SDM to rabbit plasma proteins was compared before and after intravenous bolus injection of probenecid. As shown in Table I, no significant difference in the *in vitro* binding of SDM to rabbit plasma proteins was observed before and after intravenous bolus injection of probenecid. This finding implies that metabolites of

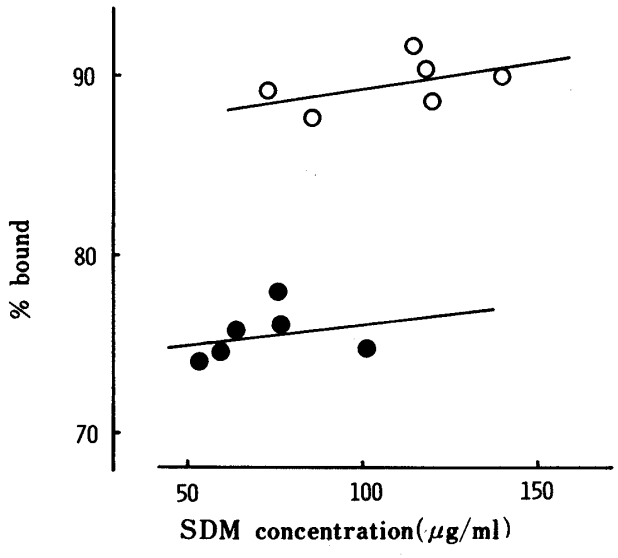

Fig. 1. Effect of Probenecid on the *in Vivo* Binding of SDM to Rabbit Plasma Proteins

○, SDM alone ($Y=0.026X+86.6$);
●, with probenecid ($Y=0.020X+74.0$).

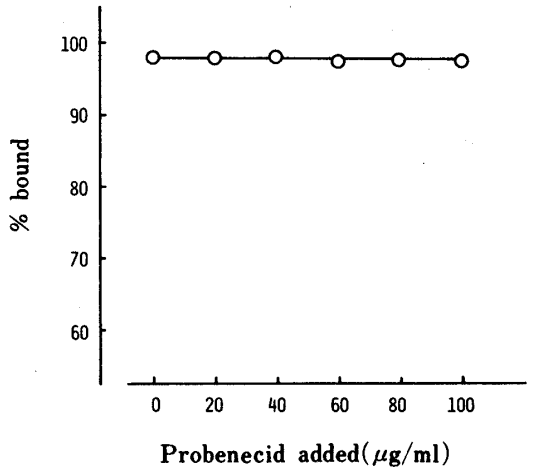

Fig. 2. Effect of Probenecid on the *in Vitro* Binding of SDM to Rabbit Plasma Proteins

SDM added: 100 μg/ml.

TABLE I. *In Vitro* Binding of Sulfadimethoxine to Rabbit Plasma Proteins determined before and after Intravenous Bolus Injection of Probenecid

Rabbit	% bound	
	Before	After
A	98.4	98.8
B	98.9	99.0
C	99.0	98.9
D	98.8	98.8
E	98.9	99.0
F	99.0	98.9
Mean	98.8	98.9
S.D.	0.2	0.1

Sulfadimethoxine was added to rabbit plasma obtained before and after intravenous bolus injection of probenecid at a 25 mg/kg dose.
Sulfadimethoxine added: 100 μg/ml.

probenecid[8] or endogenous substances accumulated by probenecid cannot affect the *in vivo* binding of SDM to rabbit plasma proteins.

The major metabolite of SDM in rabbits is well known to be N^4-AcSDM.[7] As shown in Table II, N^4-AcSDM was more strongly bound to rabbit plasma proteins than SDM. Moreover, N^4-AcSDM markedly reduced the *in vitro* binding of SDM to rabbit plasma proteins (Fig. 3). From these results, it is suggested that N^4-AcSDM may play an important role in the *in vivo* binding of SDM to rabbit plasma proteins.[9]

Figure 4 shows N^4-AcSDM concentration in plasma after intravenous bolus injection of SDM alone or in combination with probenecid to rabbits. Probenecid was found to significantly increase N^4-AcSDM concentration in plasma. Therefore, it is concluded that probenecid indirectly reduces the *in vivo* binding of SDM to rabbit plasma proteins, by causing a significant increase of N^4-AcSDM concentration in plasma.

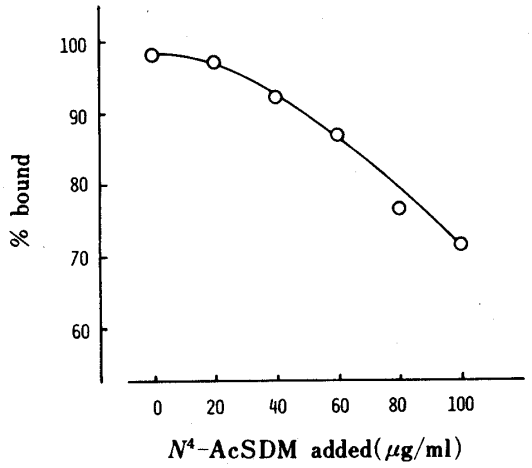

Fig. 3. Effect of N^4-AcSDM on the *in Vitro* Binding of SDM to Rabbit Plasma Proteins

SDM added; 100 μg/ml.

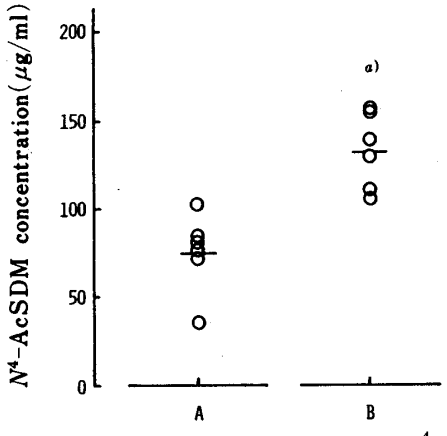

Fig. 4. Effect of Probenecid on N^4-AcSDM Concentration in Plasma 2 h after Intravenous Bolus Injection of SDM to Rabbits

A, SDM alone; B, with probenecid.
The horizontal bars indicate the mean value.
a) Significantly different from SDM alone, $p<0.001$.

TABLE II. *In Vitro* Binding of SDM and N^4-AcSDM to Rabbit Plasma Proteins

Drug concn. (μg/ml)	% bound	
	SDM	N^4-AcSDM
75	98.6	99.1
100	98.6	99.2
150	91.2	95.9
200	82.1	86.4

TABLE III. Effect of Probenecid on the Elimination Half-life of N^4-AcSDM after Intravenous Bolus Injection of N^4-AcSDM

Rabbit	Elimination half-life (h)	
	N^4-AcSDM alone	With probenecid
A	5.08	8.61
B	4.72	8.87
C	4.08	6.73
D	4.35	7.25
E	4.00	5.81
F	4.18	6.10
Mean	4.40	7.23[a)]
S.D.	0.42	1.28

a) Significantly different from N^4-AcSDM alone, $p<0.001$.

Table III shows the elimination half-life of N^4-AcSDM after intravenous bolus injection of N^4-AcSDM alone or in combination with probenecid to rabbits. Probenecid significantly prolonged the elimination half-life of N^4-AcSDM. This indicates that when SDM is injected intravenously in combination with probenecid to rabbits, the increased N^4-AcSDM concentration in plasma results from the prolonged elimination half-life of N^4-AcSDM.

It is generally recognized that urinary excretion of drugs involves any or all of glomerular filtration, active tubular secretion and passive tubular reabsorption. Recently, Arita *et al.* have pointed out that although SDM is not actively secreted by the tubules, N^4-AcSDM is.[10] In addition, probenecid has been reported to competitively inhibit the active tubular secretion of many drugs such as penicillins and furosemide.[11,12] Thus, probenecid appears to prolong the elimination half-life of N^4-AcSDM by inhibiting the active tubular secretion.

All the above results are summarized in Fig. 5. It is evident from this figure that when SDM is injected intravenously in combination with probenecid into rabbits, N^4-AcSDM plays an important role in the *in vivo* binding of SDM to rabbit plasma proteins.

The *in vivo* binding of SDM to rabbit plasma proteins decreased slightly with decrease of SDM concentration in plasma (see Fig. 1). This phenomenon may be related to N^4-AcSDM concentration in plasma, because the N^4-AcSDM concentration in plasma was observed to increase with decrease of SDM concentration in plasma (Fig. 6).

In this study, we have obtained evidence that probenecid markedly reduces the *in vivo* binding of SDM to rabbit plasma proteins, despite the fact that probenecid itself cannot displace SDM from its plasma protein binding sites. The displacement of one drug from its plasma protein binding sites by another drug is well known, but most information has been derived from *in vitro* protein binding experiments. Evidence obtained in this study suggests that *in vitro* protein binding experiments alone are inadequate for evaluating protein binding displacement in the living body.

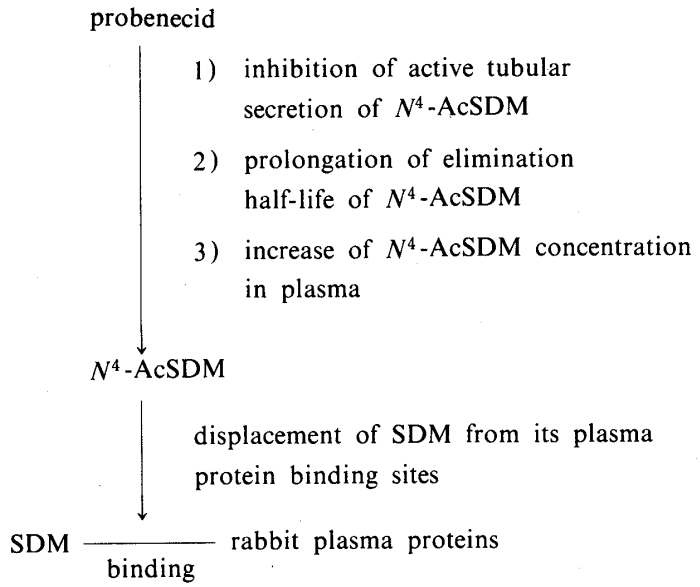

Fig. 5. The Role of N^4-AcSDM in the *in Vivo* Protein Binding Interaction between SDM and Probenecid

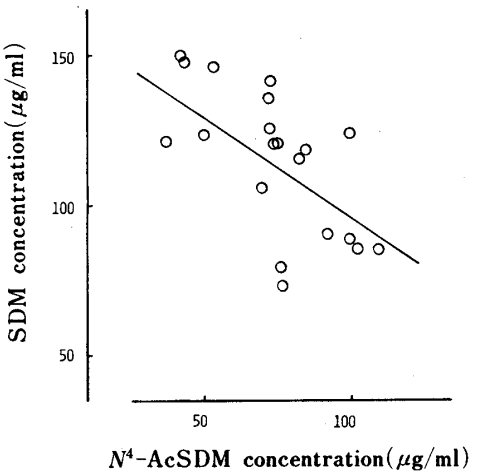

Fig. 6. Correlation of SDM and N^4-AcSDM Concentrations in Plasma 2 h after Intravenous Bolus Injection of SDM to Rabbits

$n=20$; $r=-0.645$; $p<0.005$.

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