[Chem. Pharm. Bull. 31(5)1768—1771(1983)]

Effect of Simultaneous Administration of Drugs on Absorption and Excretion. XVI.[1] Effect of Probenecid on Plasma Protein Binding of Sulfadimethoxine in Fast and Slow Acetylator Rabbits

Yorishige Imamura,* Hiroyuki Mori, and Hisashi Ichibagase

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

(Received September 24, 1982)

Two groups of rabbits categorized on the basis of their acetylation capacity were used in this study as fast and slow acetylator rabbits. The ratio of the unbound fraction of sulfadimethoxine (SDM) in plasma at 2 h after intravenous bolus injection of SDM in combination with probenecid to the unbound fraction of SDM in plasma at 2 h after intravenous bolus injection of SDM alone was determined in fast and slow acetylator rabbits. A significant difference in the ratio of unbound fraction of SDM was observed between fast and slow acetylator rabbits. This indicates that the effect of probenecid on the *in vivo* binding of SDM to plasma proteins is dependent on the acetylation capacity for SDM in rabbits. In addition, probenecid was found to cause a significant decrease in the plasma concentration of SDM in fast acetylator rabbits, but caused no significant change in the plasma concentration of SDM in slow acetylator rabbits.

Keywords——sulfadimethoxine; probenecid; fast acetylator rabbit; slow acetylator rabbit; protein binding displacement; unbound fraction; acetylation capacity; plasma concentration

The genetic polymorphism in the acetylation of many drugs such as isoniazid,[2] procainamide,[3] hydralazine[4] and sulfonamides[5] are well known to affect the therapeutic and toxic responses of an individual to these drugs. To study the genetic polymorphism in acetylation, the closest animal model to man is the rabbit, because this animal shows a bimodal distribution of acetylation capacity.[6,7]

In the preceding paper,[1] we have demonstrated that when sulfadimethoxine (SDM) is injected intravenously in combination with probenecid into rabbits, probenecid indirectly reduces the *in vivo* binding of SDM to plasma proteins by causing a significant increase in the plasma concentration of N^4-acetylsulfadimethoxine (N^4-AcSDM), which strongly displaces SDM from its plasma protein binding sites, despite the fact that probenecid itself cannot displace SDM from its plasma protein binding sites. This suggests that the acetylation capacity may be an important factor determining the effect of probenecid on the *in vivo* binding of SDM to plasma proteins in rabbits. The present study was undertaken to elucidate the effect of probenecid on the *in vivo* binding of SDM to plasma proteins in fast and slow acetylator rabbits.

Experimental

Materials——Probenecid was kindly supplied by Merck-Banyu Co., Ltd. SDM and other chemicals were obtained commercially.

Animal Experiments——Male albino rabbits weighing 2.5—3.2 kg were fasted for 38—42 h prior to the experiments, but drinking water was allowed *ad libitum*.

a) Intravenous Bolus Injection: The SDM and Probenecid solutions for injection were prepared by dissolving a suitable amount of drug in 1—3 ml of saline solution containing the same molar amount of NaOH. These solutions were injected intravenously into the ear vein. The doses of SDM and probenecid were 50 and 25 mg/kg, respectively.

b) Plasma Sampling: About 6 ml of blood was collected from the ear vein. After heparinization, the blood was immediately centrifuged and the plasma was separated.

c) Sampling Time: Plasma sampling was carried out at 2 h after intravenous bolus injection of SDM alone or in combination with probenecid, because the α phase of plasma SDM concentration *versus* time curve was recognized to be completed within 2 h.

***In Vivo* Protein Binding**——The *in vivo* protein binding experiment was carried out by means of the ultrafiltration method described previously.[8]

Analytical Methods——Unchanged SDM concentrations in rabbit plasma and its ultrafiltrate were measured by the Bratton–Marshall method.[9] Total SDM concentration in rabbit plasma was measured after hydrolysis (0.5 N HCl, at 100°C for 1 h) by the Bratton–Marshall method.[9]

Percentage of N^4-Acetylated SDM——The percentage of N^4-acetylated SDM was calculated from the difference between unchanged and total SDM concentrations in rabbit plasma. In this calculation, metabolites other than N^4-AcSDM were neglected, because none was detected in rabbit plasma by the separation method of Okamoto.[10] More recently, a high-performance liquid chromatographic method (HPLC method) and the Bratton–Marshall method were reported to give similar results with respect to the percentage of N^4-acetylated form.[11]

Statistical Analysis——Statistical analysis was performed by means of the paired Student t-test or the non-paired Student t-test. The difference between means was considered to be significant when $p<0.05$.

Results and Discussion

The plots of percentage of N^4-acetylated SDM in plasma at 2 h after intravenous bolus injection of SDM into 28 rabbits are shown in Fig. 1. There was no evidence of a clear bimodal distribution of acetylation capacity for SDM in 28 rabbits. However, two groups of 5 rabbits (shown in Fig. 1) were used in the subsequent experiments and designated for convenience as fast and slow acetylators.

Table I shows the *in vivo* binding of SDM to plasma proteins at 2 h after intravenous bolus injection of SDM alone or in combination with probenecid to fast and slow acetylator rabbits. In both fast and slow acetylator rabbits, probenecid significantly reduced the *in vivo* binding of SDM to plasma proteins. However, the effect of probenecid in fast acetylator rabbits appeared to be considerably greater than that in slow acetylator rabbits. Thus, the ratio of the unbound fraction of SDM in plasma at 2 h after intravenous bolus injection of SDM in combination with probenecid to the unbound fraction of SDM in plasma at 2 h after intravenous bolus injection of SDM alone was determined in fast and slow acetylator rabbits (Table II). As was expected, a significant difference in the ratio of unbound fraction of SDM was observed between fast and slow acetylator rabbits. This finding indicates that the effect of probenecid on the *in vivo* binding of SDM to plasma proteins is dependent on the acetylation capacity for SDM in rabbits.

Our preceding paper[1] showed that N^4-AcSDM, the major metabolite of SDM, strongly displaces SDM from its plasma protein binding sites, and that probenecid indirectly reduces the *in vivo* binding of SDM to plasma proteins in rabbits, by causing a significant increase in the plasma concentration of N^4-AcSDM. Therefore, it may be concluded that the magnitude of the increase in plasma concentration of N^4-AcSDM caused by probenecid

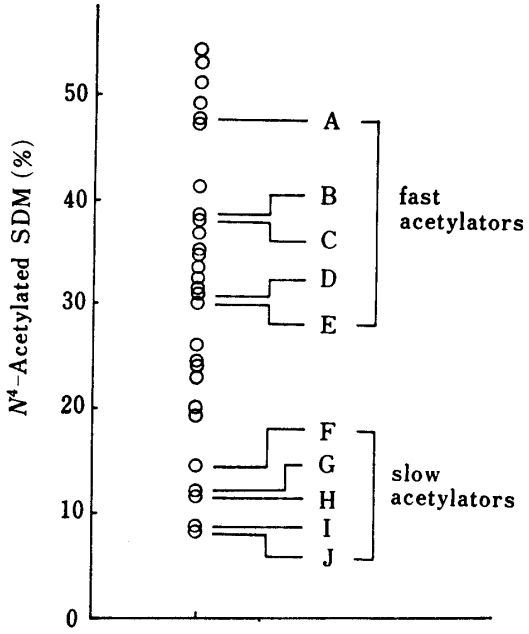

Fig. 1. Percentages of N^4-Acetylated Sulfadimethoxine in Plasma at 2 h after Intravenous Bolus Injection of Sulfadimethoxine into 28 Rabbits

Rabbits A—E and F—J were used in the subsequent experiments as fast and slow acetylators, respectively.

TABLE I. *In Vivo* Binding of SDM to Plasma Proteins at 2 h after Intravenous Bolus Injection of SDM alone or in Combination with Probenecid (PBC) into Fast and Slow Acetylator Rabbits

Rabbit	% bound	
	SDM alone	With PBC
Fast acetylators		
A	89.0	74.0
B	90.3	77.8
C	91.6	74.3
D	89.7	75.8
E	88.8	76.0
Mean	89.9	75.6[a]
S.D.	1.1	1.5
Slow acetylators		
F	89.3	86.0
G	87.0	79.6
H	90.1	84.0
I	86.7	78.7
J	83.7	79.7
Mean	87.4	81.6[b]
S.D.	2.5	3.2

a) Significantly different from SDM alone in fast acetylators, $p<0.001$.
b) Significantly different from SDM alone in slow acetylators, $p<0.005$.

TABLE II. Ratio of the Unbound Fraction of SDM in Plasma at 2 h after Intravenous Bolus Injection of SDM in Combination with PBC to the Unbound Fraction of SDM in Plasma at 2 h after Intravenous Bolus Injection of SDM alone

Rabbit	Unbound fraction ratio	Rabbit	Unbound fraction ratio
Fast acetylators		Slow acetylators	
A	2.36	F	1.31
B	2.29	G	1.57
C	3.06	H	1.62
D	2.34	I	1.60
E	2.14	J	1.25
Mean	2.44	Mean	1.47
S.D.	0.36	S.D.	0.18
		$p<0.001$	

contributes to the difference between fast and slow acetylator rabbits in the effect of probenecid on the *in vivo* binding of SDM to plasma proteins.

Recently, many investigators have concluded that the acetylation capacity for sulfonamides in rabbits is under genetic control.[6,7] However, little is known about the effect of genetic factors on the *in vivo* binding of sulfonamides to plasma proteins. In this paper, we present evidence that a genetic factor, namely the genetic polymorphism in the acetylation of SDM, plays an important role in the effect of probenecid on the *in vivo* binding of SDM to plasma proteins in rabbits.

Table III shows the plasma concentration of SDM at 2 h after intravenous bolus injection of SDM alone or in combination with probenecid into fast and slow acetylator rabbits. Probenecid caused a significant decrease in the plasma concentration of SDM in fast acetylator rabbits, but caused no significant change in the plasma concentration of SDM in slow acetylator

rabbits. This finding suggests that a difference in the effect of probenecid on the pharmacokinetic behavior of SDM may be observed between fast and slow acetylator rabbits. Further studies on this are in progress.

TABLE III. Plasma Concentration of SDM at 2 h after Intravenous Bolus Injection of SDM alone or in Combination with PBC to Fast and Slow Acetylator Rabbits

Rabbit	Plasma concentration (μg/ml)	
	SDM alone	With PBC
Fast acetylators		
A	73.4	54.1
B	119.1	76.2
C	116.0	59.2
D	141.1	116.2
E	121.9	77.9
Mean	114.3	76.7[a]
S.D.	24.9	24.4
Slow acetylators		
F	189.2	176.1
G	176.1	213.1
H	159.1	153.4
I	207.4	167.6
J	208.8	190.9
Mean	188.1	180.2
S.D.	21.1	22.9

a) Significantly different from SDM alone in fast acetylators, $p<0.005$.

References and Notes

1) Part XV: Y. Imamura, H. Mori and H. Ichibagase, *Chem. Pharm. Bull.*, **31**, 274 (1983).
2) D.A.P. Evans, K.A. Manley, and V.A. Mckusick, *Brit. Med. J.*, **2**, 485 (1960).
3) M.M. Reidenberg, D. Drayer, M. Levy, and H. Warner, *Clin. Pharmacol. Ther.*, **17**, 722 (1975).
4) A.J. Jounela, M. Pasanen, and M.J. Mattila, *Acta Med. Scand.*, **197**, 303 (1975).
5) T.A. White and D.A.P. Evans, *Clin. Pharmacol. Ther.*, **9**, 80 (1968).
6) *a*) J.W. Frymoyer and R.F. Jacox, *J. Lab. Clin. Med.*, **62**, 891 (1963); *b*) *Idem*, *ibid.*, **62**, 905 (1963).
7) G.R. Gordon, A.G. Shafizadeh, and J.H. Peters, *Xenobiotica*, **3**, 133 (1973).
8) Y. Imamura, M. Sonoda, K. Arimori, and H. Ichibagase, *Chem. Pharm. Bull.*, **27**, 463 (1979).
9) A.C. Bratton and E.K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).
10) S. Okamoto, *Saishin Igaku*, **15**, 142 (1960).
11) H. Olsen, *Acta Pharmacol. et Toxicol.*, **50**, 75 (1982).