EFFECT OF PHENYLBUTAZONE ON SERUM PROTEIN BINDING OF SULFADIMETHOXINE IN DIFFERENT ANIMAL SPECIES

YORISHIGE IMAMURA, HIDEKI NAKAMURA, AND MASAKI OTAGIRI

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

(Received May 13, 1986)

The effect of phenylbutazone (PBZ) on the *in vivo* serum protein binding of sulfadimethoxine (SDM) was examined in dogs, rabbits and rats. In dogs, PBZ itself was found to displace SDM from its protein binding sites. In rabbits, PBZ indirectly reduced the *in vivo* serum protein binding of SDM through the interaction of PBZ with *N*⁴-acetylsulfadimethoxine (*N*⁴-AcSDM), a major metabolite of SDM. In rats, however, PBZ had no effect on the *in vivo* serum protein binding of SDM. It is noteworthy that species differences were observed in the effect of PBZ on the *in vivo* serum protein binding of SDM.

Keywords — sulfadimethoxine; phenylbutazone; dog; rabbit; rat; protein binding displacement; species difference

INTRODUCTION

Species differences in serum protein binding of many drugs such as salicylate,¹⁾ camptothecin²⁾ and warfarin³⁾ have been reported. However, there is little information concerning species differences in the displacement of one drug from its protein binding sites by another. Variations in protein binding displacement of drugs among species may determine differences in drug-drug interaction. The purpose of this study is to elucidate the mechanism of species differences in the effect of phenylbutazone (PBZ) on serum protein binding of sulfadimethoxine (SDM).

MATERIALS AND METHODS

Materials — SDM was purchased from Daiichi Pharmaceutical Co., PBZ from Fujisawa Pharmaceutical Industry Co. and *N*⁴-acetylsulfadimethoxine (*N*⁴-AcSDM) was synthesized from SDM by the method of Uno *et al.*⁴⁾ Dog, rabbit and rat serum albumins (fraction V) were purchased from Sigma Chemical Co.

Animal Experiment — Male beagle dogs (10–12 kg), albino rabbits (2.5–3.0 kg) and Wistar rats (230–270 g) were used in a cross-over design. SDM at a dose of 50 mg/kg was injected intravenously as a bolus to dogs, rabbits and rats. PBZ at a dose of 10 mg/kg was injected intravenously as a bolus immediately after SDM injection.

Protein Binding Experiment — The *in vivo* and *in vitro* serum protein binding experiments were carried out by means of an ultrafiltration method described previously.⁵⁾

Analytical Method — SDM and *N*⁴-AcSDM concentrations in serum or ultrafiltrate were determined by high-performance liquid chromatography (HPLC). The detail will be described in our next publication.

RESULTS AND DISCUSSION

Figure 1 shows the effect of PBZ on the *in vivo* bindings of SDM to serum at 2 h after intravenous bolus injection of SDM to dogs, rabbits and rats. In dogs and rabbits, PBZ evidently reduced the *in vivo* serum protein binding of SDM. On the other hand, PBZ had no effect on the *in vivo* serum protein binding of SDM in rats. The fact that species differences were observed in the effect of PBZ on the *in vivo* serum protein binding of SDM is of interest.

In order to elucidate the mechanism of species differences in the *in vivo* protein binding interaction between SDM and PBZ, we examined the effect of PBZ on the *in vitro* bindings of SDM to serum or albumin of three animal species. In dogs, PBZ was found to reduce the *in vitro* bindings of SDM to serum and albumin. This implies that, in dogs, PBZ itself displaces SDM from its protein binding sites. In rabbits and rats, however, PBZ did not change the *in vitro* bindings of SDM to serum and albumin. Our previous paper⁶⁾ showed that in rabbits, *N*⁴-AcSDM, a major metabolite of SDM,⁷⁾ strongly displaces SDM from its protein binding sites. Thus, in rabbits, *N*⁴-AcSDM may contribute to the *in vivo* protein binding interaction between SDM and PBZ.

FIG. 1. *In Vivo* Serum Protein Binding of SDM at 2 h after Intravenous Bolus Injection of SDM Alone (○) or in Combination with PBZ (●) to Dogs, Rabbits and Rats

FIG. 2. *In Vitro* Serum Protein Binding of SDM in the Presence of N⁴-AcSDM at the Same Concentration as 2 h after Intravenous Bolus Injection of SDM alone (△) or in Combination with PBZ (▲) to Rabbits

FIG. 3. *Proposed Mechanism of the in Vivo Protein Binding Interaction between SDM and PBZ in Dogs and Rabbits*

In rabbits, PBZ markedly increased the serum concentration of N^4 -AcSDM from 39.6 ± 6.5 to 79.8 ± 4.4 $\mu\text{g/ml}$ ($p < 0.01$) at 2 h after intravenous bolus injection of SDM. In addition, as shown in Fig. 2, the *in vitro* serum protein binding of SDM in the presence of N^4 -AcSDM was very similar to the *in vivo* serum protein binding of SDM in rabbits shown in Fig. 1. Therefore, it was concluded that in rabbits, PBZ indirectly reduced the *in vivo* serum protein binding of SDM through the interaction of PBZ with N^4 -AcSDM.

In dogs and rats, N^4 -AcSDM was found to displace SDM from its protein binding sites. However, in dogs and rats the serum concentrations of N^4 -AcSDM at 2 h after intravenous bolus injection of SDM alone, or in combination with PBZ, were very low. For this reason, it appears that in dogs and rats, N^4 -AcSDM does not contribute to the *in vivo* protein binding interaction between SDM and PBZ.

PBZ has been reported to depress the renal excretion of drugs or metabolites which are actively secreted by the tubules.⁸⁾ Since N^4 -AcSDM is actively secreted by the tubules,⁹⁾ PBZ may cause an increase in the serum concentration of N^4 -AcSDM in rabbits, by depressing its renal excretion.

Figure 3 shows the proposed mechanism of the *in vivo* protein binding interaction between SDM and PBZ in dogs and rabbits. These may provide an approach to animal extrapolation in the *in vivo* protein binding interaction between two drugs.

REFERENCES

- 1) J. A. Sturman and M. J. H. Smith: The binding of salicylate to plasma proteins in different species, *J. Pharm. Pharmacol.*, **19**, 621–622 (1967).
- 2) A. M. Guarino, J. B. Anderson, D. K. Starkweather and C. F. Chignell: Pharmacologic studies of camptothecin (NSC-100880): Distribution, plasma protein binding, and biliary excretion, *Cancer Chemotherapy Reports, Part I*, **57**, 125–140 (1973).
- 3) E. M. Sellar, M. L. Lang-Sellers and J. Koch-Weser: Comparative warfarin binding to albumin from various species, *Biochem. Pharmacol.*, **26**, 2445–2447 (1977).
- 4) T. Uno and M. Ueda: Studies on the metabolism of sulfathiazole. I. On the excreted substance in the human urine after administration of sulfathiazole, *Yakugaku Zasshi*, **80**, 1785–1788 (1960).
- 5) Y. Imamura, M. Sonoda, K. Arimori and H. Ichibagase: Effect of simultaneous administration of drugs on absorption and excretion. X. Plasma protein binding of carbutamide in rabbits, *Chem. Pharm. Bull.*, **27**, 463–467 (1979).
- 6) Y. Imamura, H. Mori and H. Ichibagase: Effect of simultaneous administration of drugs on absorption and excretion. XV. Effect of probenecid on plasma protein binding of sulfadimethoxine in rabbits: The role of N^4 -acetylsulfadimethoxine, the major metabolite of sulfadimethoxine, *Chem. Pharm. Bull.*, **31**, 274–278 (1983).
- 7) J. W. Bridges, M. R. Kibby, S. R. Walker and R. T. Williams: Species difference in the metabolism of sulfadimethoxine, *Biochem. J.*, **109**, 851–856 (1968).
- 8) J. P. Griffin and P. F. D'Arcy: "A Manual of Adverse Drug Interactions," third edition, Wright, Bristol, 1984, pp. 46–51.
- 9) T. Arita, R. Hori, M. Takada, S. Akuzu and A. Misawa: Transformation and excretion of drugs in biological system. VII. Effect of biotransformation on renal excretion of sulfonamides, *Chem. Pharm. Bull.*, **20**, 570–580 (1972).