Effect of Phenylbutazone on Serum Protein Binding and Pharmacokinetic Behavior of Sulfadimethoxine in Rabbits, Dogs and Rats

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The effect of phenylbutazone (PBZ) on the in vitro binding of sulfadimethoxine (SDM) to serum or albumin was compared among rabbits, dogs and rats. In rabbits, a major metabolite of SDM, N-acetyl-sulfadimethoxine (N-AcSDM), markedly reduced the in vitro binding of SDM, and PBZ significantly increased the serum concentration of N-AcSDM after SDM administration. PBZ did not affect the in vitro binding of SDM. These findings indicate that in rabbits, PBZ indirectly reduces the in vivo binding of SDM through the interaction of PBZ with N-AcSDM. In dogs, both PBZ and N-AcSDM caused the reduction in the in vitro binding of SDM. However, unlike rabbits, the contribution of N-AcSDM to the in vivo binding of SDM appeared to be negligible in dogs. In rats, PBZ or N-AcSDM had little effect on the in vitro binding of SDM. The co-administration of PBZ significantly increased the total body clearance and steady-state volume of distribution of SDM in rabbits. Such changes in pharmacokinetic behavior were not observed in dogs and rats.

Keywords — sulfadimethoxine; phenylbutazone; serum protein binding; pharmacokinetic behavior; species difference

Introduction

It has been reported that marked species differences exist in serum protein binding of drugs. However, information concerning differences in protein binding interaction between two drugs has been very limited. Our previous work showed that when sulfadimethoxine (SDM) was intravenously administered in combination with phenylbutazone (PBZ) to rabbits, dogs and rats, PBZ clearly reduced the in vivo binding of SDM to rabbit and dog sera, whereas it had little effect on the in vivo binding of SDM to rat serum. Furthermore, N-acetyl-sulfadimethoxine (N-AcSDM), a major metabolite of SDM, was found to contribute to the in vivo protein binding interaction of SDM with PBZ in rabbits. More recently, we have demonstrated that the effect of PBZ on the serum protein binding and pharmacokinetic behavior of SDM differs between humans and rabbits. The purpose of the present study is to examine further the mechanism of species differences in the in vivo protein binding interaction of SDM with PBZ, and to compare the effect of PBZ on the pharmacokinetic behavior of SDM in rabbits, dogs and rats.

Materials and Methods

Materials — SDM was purchased from Daiichi Seiyaku Co., Ltd. PBZ was kindly supplied by Ciba-Geigy Co., Ltd. N-AcSDM was synthesized from SDM by the method of Uno et al. Rabbit, dog and rat albumins (fraction V) were purchased from Sigma Chemical Co., Ltd. All other chemicals were of reagent grade.

Animal Experiments — Male rabbits (Japanese white, 2.4—3.0 kg), dogs (beagle, 10—12 kg) and rats (Wistar, 230—270 g) were fasted for 24—36 h prior to experiments, but drinking water was allowed ad libitum. SDM at a dose of 50 mg/kg or N-AcSDM at a dose of 25 mg/kg was administered intravenously as a bolus to rabbits, dogs and rats. PBZ at a dose of 10 mg/kg was administered intravenously as a bolus immediately after SDM or N-AcSDM administration. An interval of at least 2 weeks was taken to minimize the residual or cumulative effect of the preceding dose.

Protein Binding Experiments — The in vitro protein binding experiments were carried out by means of an ultrafiltration method described previously. SDM was added to serum or albumin solution with PBZ or N-AcSDM. The albumin concentration of each species was...
determined from the corresponding serum by the method of Pinnell and Northam. According to the results, the rabbit, dog and rat albumins were dissolved in 1/15 M phosphate buffer (pH 7.4) at concentrations of 4.2, 3.6 and 3.7 g/100 ml, respectively. The equation of Scatchard was used to estimate the nature of the displacement of SDM or $N^4$-AcSDM from its binding site on rabbit albumin by $N^4$-AcSDM or SDM: 

$$\frac{n}{D_i} = nK - rK$$

where $r$ is the number of moles of bound drug per mole of albumin, $n$ is the number of binding sites, $K$ is the binding constant and $D_i$ is the concentration of free drug.

**Analytical Method** — The concentrations of SDM and/or $N^4$-AcSDM in serum, ultrafilterate and urine were measured by high-performance liquid chromatography (HPLC). These samples (0.1 ml) were extracted with ethyl acetate (2.5 ml), after adjusting to pH 5.5 by the addition of McIlvaine buffer (pH 5.5, 1.0 ml).

After centrifugation at 2000 rpm for 10 min, the organic phase was separated and evaporated in vacuo. The residue was dissolved in 0.1 ml of acetonitrile and subjected to HPLC. HPLC was carried out using a Hitachi 655A-11 HPLC apparatus equipped with a LiChrosorb RP-18 column (250 × 4 mm i.d., Cica Merck) and a Hitachi 638-41 UV monitor (269 nm). Methanol-0.2% acetic acid (3:2 v/v) was employed as a mobile phase at a flow rate of 1.0 ml/min.

**Pharmacokinetic Analysis** — The SDM and $N^4$-AcSDM concentrations in serum were analyzed by statistical moment analysis to obtain values for the total body clearance ($Cl_{tot}$) of SDM or $N^4$-AcSDM, and the steady-state volume of distribution ($V_{dss}$) of SDM according to the following equation:

$$Cl_{tot} = \frac{D}{AUC}$$

$$V_{dss} = \frac{D \cdot MRT}{AUC}$$

where $D$ is the dose, $AUC$ is the area under the serum drug concentration-time curve from zero to infinite time and $MRT$ is the mean residence

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Fig. 1. Effect of PBZ on the in Vitro Binding of SDM to Rabbit, Dog, and Rat Serum

The in vivo binding of SDM was determined for the serum prepared by the addition of SDM (100 μg/ml) alone or in combination with PBZ. Each point represents the mean ± S.E. ($n = 5$).
Fig. 2. Effect of \(N^4\)-AcSDM on the in Vitro Binding of SDM to Rabbit, Dog, and Rat Serum

The in vitro binding of SDM was determined for the serum prepared by the addition of SDM (100 \(\mu\)g/ml) alone or in combination with \(N^4\)-AcSDM. Each point represents the mean ± S.E. (\(n = 5\)).

time. Moreover, the renal clearance (\(C_{lr}\)) and nonrenal clearance (\(C_{l_{nr}}\)) of \(N^4\)-AcSDM were estimated from the following equations:

\[
\begin{align*}
C_{lr} &= X / AUC \\
C_{l_{nr}} &= C_{l_{tot}} - C_{lr}
\end{align*}
\]

where \(X\) is the total urinary recovery of \(N^4\)-AcSDM 48 h. The values of \(AUC\) and MRT were calculated according to trapezoidal rule using a microcomputer (NEC, PC-9801).

**Statistical Analysis** — Statistical analysis was determined by Student’s paired or unpaired \(t\)-test.

### Results

**Effect of PBZ and \(N^4\)-AcSDM on the in Vitro Binding of SDM to Serum**

Figure 1 shows the effect of PBZ on the in vitro binding of SDM to rabbit, dog and rat serums. Although PBZ reduced the in vitro binding of SDM to dog serum, it had little effect

<table>
<thead>
<tr>
<th></th>
<th>SMD alone</th>
<th>With PBZ</th>
<th>With (N^4)-AcSDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>98.5 ± 0.1</td>
<td>96.0 ± 0.2</td>
<td>72.5 ± 0.8 (a)</td>
</tr>
<tr>
<td>Dog</td>
<td>78.4 ± 0.4</td>
<td>68.8 ± 0.9 (a)</td>
<td>63.9 ± 1.3 (a)</td>
</tr>
<tr>
<td>Rat</td>
<td>98.4 ± 0.2</td>
<td>97.9 ± 0.2</td>
<td>96.2 ± 0.2</td>
</tr>
</tbody>
</table>

The in vitro binding of SDM was determined for the albumin prepared by the addition of SDM (100 \(\mu\)g/ml); or in combination with PBZ (100 \(\mu\)g/ml) or \(N^4\)-AcSDM (100 \(\mu\)g/ml). Albumin concentration: rabbit (4.2 g/100 ml); dog (3.6 g/100 ml); rat (3.7 g/100 ml). Each value represents the mean ± S.E. (\(n = 5\)). \(a\) Significantly different from SDM alone \((p < 0.01)\).
on the \textit{in vitro} binding of SDM to rabbit and rat sera.

Recently, we have demonstrated that \( N^4\)-AcSDM, a major metabolite of SDM,\(^2\) markedly reduces the \textit{in vitro} binding of SDM to rabbit serum.\(^9\) Thus the effects of \( N^4\)-AcSDM on the \textit{in vitro} serum protein binding of SDM were compared among rabbits, dogs, and rats (Fig. 2). In rabbits and dogs, \( N^4\)-AcSDM clearly reduced the \textit{in vitro} binding of SDM. On the other hand, \( N^4\)-AcSDM had little effect on the \textit{in vitro} binding in rats.

\textbf{Effect of PBZ and \( N^4\)-AcSDM on the \textit{in Vitro} Binding of SDM to Albumin}

The effects of PBZ and \( N^4\)-AcSDM on the \textit{in vitro} binding of SDM to rabbit, dog, and rat albumins are summarized in Table I. The results were in good agreement with those obtained from the corresponding serum (see Figs. 1 and 2). Figure 3A illustrates Scatchard plots for the binding of SDM to rabbit albumin in the absence and presence of \( N^4\)-AcSDM. The plots showed a linear relationship. The values of \( n \) and \( K \) were, respectively, 0.97 and \( 4.39 \times 10^5 \) M\(^{-1}\) in the absence of \( N^4\)-AcSDM and, respectively, 1.01 and \( 3.1 \times 10^6 \) M\(^{-1}\) in the presence of \( N^4\)-AcSDM. A similar phenomenon was observed in Scatchard plots for the binding of \( N^4\)-AcSDM to rabbit albumin in the absence and presence of SDM (Fig. 3B). The values of \( n \) and \( K \) were, re-

\begin{table}
\centering
\caption{Serum Concentration of \( N^4\)-AcSDM at 2 h after Intravenous Bolus Administration of SDM Alone or in Combination with PBZ to Rabbits, Dogs and Rats}
\begin{tabular}{lcc}
\hline
\multicolumn{3}{c}{Serum concn. of \( N^4\)-AcSDM (\( \mu g/ml \))} \\
\hline
& SDM alone & With PBZ \\
Rabbit & 56.7±9.2 & 86.9±6.3 \(^{a)}\) \\
Dog & n.d. \(^{b)}\) & n.d. \\
Rat & 4.5±0.5 & 6.2±0.3 \\
\hline
\end{tabular}
\end{table}

Each value represents the mean ± S.E. (\( n = 5 \)). Dose, SDM; 50 mg/kg, PBZ; 10 mg/kg. \(^{a)}\) Significantly different from SDM alone (\( p < 0.05 \)). \(^{b)}\) Not detected.
TABLE III. Effect of PBZ on Pharmacokinetic Parameter of SDM in Rabbits, Dogs and Rats

<table>
<thead>
<tr>
<th></th>
<th>SDM alone</th>
<th>With PBZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{t}_{\text{ot}}}$ (ml/h/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>34.2 ± 2.1</td>
<td>53.7 ± 4.9 a)</td>
</tr>
<tr>
<td>Dog</td>
<td>13.8 ± 1.3</td>
<td>15.3 ± 1.3</td>
</tr>
<tr>
<td>Rat</td>
<td>4.7 ± 0.3</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>$V_{d_{\text{ss}}}$ (ml/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>374 ± 23</td>
<td>509 ± 34 a)</td>
</tr>
<tr>
<td>Dog</td>
<td>342 ± 12</td>
<td>509 ± 11</td>
</tr>
<tr>
<td>Rat</td>
<td>196 ± 2</td>
<td>195 ± 2</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. ($n = 5$). a) Significantly different from SDM alone ($p < 0.05$).

findings imply that SDM and $N^4$-AcSDM compete strongly for the same binding site on rabbit albumin.

Effect of PBZ on Serum Concentration of $N^4$-AcSDM after SDM Administration

The serum concentration of $N^4$-AcSDM at 2 h after intravenous bolus administration of SDM alone or in combination with PBZ to rabbits, dogs and rats are shown in Table II. In rabbits, the intravenously administered PBZ signifi-
Species Difference of Drug Interaction

![Graph](image)

Fig. 6. Serum Concentration-Time Curve of N\(^{4}\)-AcSDM after Intravenous Bolus Administration of N\(^{4}\)-AcSDM Alone (○) or in Combination with PBZ (●) to Rabbits

Each point represents the mean ± S.E. (n = 3). Dose, N\(^{4}\)-AcSDM; 25 mg/kg, PBZ; 10 mg/kg.

Significantly increased the serum concentration of N\(^{4}\)-AcSDM from 56.7 ± 9.2 to 86.9 ± 6.3 μg/ml, while in rats, it caused no significant increase in the serum concentration of N\(^{4}\)-AcSDM. In dogs, there was no detectable amount of N\(^{4}\)-AcSDM in serum at 2 h after SDM administration. Furthermore, in the case of rabbits, the time course of the serum concentration of N\(^{4}\)-AcSDM after intravenous bolus administration of SDM alone or in combination with PBZ was examined. As shown in Fig. 4, the PBZ markedly increased and maintained the serum concentration of N\(^{4}\)-AcSDM over the 6 h period.

**Effect of PBZ on Pharmacokinetic Behavior of SDM**

Figure 5 shows the serum concentration-time profiles of SDM after intravenous bolus administration of SDM alone or in combination with PBZ to rabbits, dogs and rats, and Table III summarizes the pharmacokinetic parameters. In rabbits, the co-administration of PBZ caused a significant increase in the Cl\(_{tot}\) and V\(_{dss}\) of SDM. In dogs and rats, however, no significant difference in these pharmacokinetic parameters was observed between the control and PBZ-treated groups.

**Effect of PBZ on Pharmacokinetic Behavior of N\(^{4}\)-AcSDM**

In order to elucidate the mechanism of the pharmacokinetic interaction between N\(^{4}\)-AcSDM and PBZ in rabbits, we examined the effect of PBZ on the serum concentration and urinary excretion of N\(^{4}\)-AcSDM after intravenous bolus administration of N\(^{4}\)-AcSDM. Figures 6 and 7 show the results, and Table IV summarizes the pharmacokinetic parameters derived from the data of the serum concentration and urinary excretion. The co-administration of PBZ significantly decreased the Cl\(_{tot}\) and Cl\(_{r}\) of N\(^{4}\)-AcSDM, but had no significant effect on the Cl\(_{nr}\) of N\(^{4}\)-AcSDM, indicating that the PBZ reduces the renal excretion of N\(^{4}\)-AcSDM in rabbits.

**Table IV. Effect of PBZ on Pharmacokinetic Parameter of N\(^{4}\)-AsCDM in Rabbits**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N(^{4})-AcSDM alone</th>
<th>With PBZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl(_{tot}) (ml/h/kg)</td>
<td>19.1 ± 0.6</td>
<td>14.1 ± 1.6</td>
</tr>
<tr>
<td>Cl(_{r}) (ml/h/kg)</td>
<td>15.5 ± 0.4</td>
<td>10.9 ± 1.6</td>
</tr>
<tr>
<td>Cl(_{nr}) (ml/h/kg)</td>
<td>3.6 ± 0.3</td>
<td>3.2 ± 0.2</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. (n = 3). a) Significantly different from N\(^{4}\)-AcSDM alone (p < 0.05).
Discussion

The co-administration of PBZ markedly reduced the in vivo serum protein binding of SDM in rabbits, as reported previously.\(^1\) However, the results of the present study clearly show that PBZ does not possess the ability to displace SDM from its binding sites on rabbit albumin, suggesting that rabbit albumin has different binding sites for SDM and PBZ.

PBZ is well known to be biotransformed to two metabolites in humans.\(^10\) Thus, if the serum concentrations of the metabolites are sufficiently high in rabbits and if the metabolites displace SDM from its binding site on the albumin, the co-administration of PBZ will reduce the in vivo serum protein binding of SDM. However, no significant difference in the in vitro bindings of SDM to rabbit serum obtained before and after intravenous bolus administration of PBZ alone was observed (before; 98.2 ± 0.2%, after; 98.0 ± 0.2%, \(n = 5\)). SDM (100 \(\mu\)g/ml) was added to rabbit serum obtained before and at 2 h after intravenous bolus administration of PBZ at a dose of 10 mg/kg, and the in vitro bindings of SDM were determined. The results indicate that in rabbits, the metabolites of PBZ are not involved in the in vivo protein binding interaction between SDM and PBZ.

Recently, several investigators have demonstrated that a metabolite can reduce the binding of its parent drug to serum (plasma) or albumin.\(^{11-13}\) In this study, \(N^4\)-AcSDM, a major metabolite of SDM, displaced competitively and strongly the parent drug from its binding site on rabbit albumin. The intravenously administered PBZ caused a marked increase in the serum concentration of \(N^4\)-AcSDM for a long time after SDM administration to rabbits, by reducing the renal excretion of \(N^4\)-AcSDM. Therefore, it is concluded that in rabbits, PBZ indirectly reduces the in vivo binding of SDM to the serum through the interaction of PBZ with \(N^4\)-AcSDM.

In dogs, both PBZ and \(N^4\)-AcSDM were found to displace SDM from its binding site on the albumin. However, unlike rabbits, it is suggested that the contribution of \(N^4\)-AcSDM to the in vivo protein binding interaction between SDM and PBZ is negligible in dogs, since there was no detectable amount of \(N^4\)-AcSDM in dog serum after SDM administration.

In rats, PBZ or \(N^4\)-AcSDM had little effect on the in vitro binding of SDM to the serum or albumin. Recently, Dirr and Schabort have reported that rat albumin has some specific sites for drug binding.\(^{14}\) Thus the SDM-binding site on rat albumin may differ from that of PBZ or \(N^4\)-AcSDM, although additional studies including the binding capacity to rat albumin of PBZ and \(N^4\)-AcSDM are necessary.

Evidence has been presented that the displacement of one drug from its binding site on albumin by another produces their pharmacokinetic interaction. For example, Arimori et al.\(^{15}\) have demonstrated that penicillins reduce the in vivo serum protein binding of phenytoin, and significantly increase the \(Cl_{tot}\) and \(V_{dss}\) of the drug. As expected from the results of protein binding experiments, the co-administration of PBZ significantly increased the \(Cl_{tot}\) and \(V_{dss}\) of SDM in rabbits. On the other hand, the PBZ did not induce the change in pharmacokinetic behavior of SDM in dogs. This may be because the in vivo serum protein binding of SDM in dogs is much smaller than that in rabbits.\(^1\) Intravenously administered PBZ was found to increase the serum concentration of free SDM at 2 h after SDM administration to rabbits (SDM alone; 12.4 ± 1.0 \(\mu\)g/ml, with PBZ; 19.8 ± 1.7 \(\mu\)g/ml, \(p < 0.01\), \(n = 5\)), but no significant increase in the serum concentration of free SDM was observed in dogs.

In conclusion, this study demonstrates that species differences exist in the in vivo protein binding interaction between SDM and PBZ and in their pharmacokinetic interaction. Furthermore, this study provides an approach for elucidating the mechanism of difference of drug interaction in animal species.

References

2) J. W. Bridges, N. R. Kibby, S. R. Walker and R. T. Williams: Species difference in the metabolism of sulfadi-
Species Difference of Drug Interaction


