Contribution of N4-Acetylsulfadimethoxine to the Interaction of Sulfadimethoxine with Ketoprofen in Rabbits

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The contribution of N4-acetylsulfadimethoxine (N4-AcSDM), a major metabolite of sulfadimethoxine (SDM), to the serum protein binding and pharmacokinetic interactions between SDM and ketoprofen (KPF) was investigated in rabbits. When SDM and KPF were intravenously co-administered, KPF indirectly reduced the serum protein binding of SDM through the interaction of KPF with N4-AcSDM, and significantly increased the total body clearance (Cltot) and steady-state volume of distribution (Vdss) of SDM. In addition, the co-administration of N4-AcSDM was found to increase Cltot and Vdss of SDM. These results indicate that N4-AcSDM contributes substantially to the serum protein binding and pharmacokinetic interactions between SDM and KPF in rabbits.

Keywords——sulfadimethoxine; ketoprofen; drug-metabolite interaction; N4-acetylsulfadimethoxine; serum protein binding; pharmacokinetic parameter; protein binding displacement

Several investigators have demonstrated that a metabolite can contribute to drug-drug interaction.1–3) For example, when warfarin and chloral hydrate were co-administered, a major metabolite of chloral hydrate, trichloroacetic acid, reduced the serum protein binding of warfarin and enhanced its anti-coagulant activity.1) In addition, probenecid indirectly reduced the serum protein binding of sulfadimethoxine (SDM) through the interaction of probenecid with N4-acetylsulfadimethoxine (N4-AcSDM),2) a major metabolite of SDM.4) However, the contribution of metabolites to drug–drug interaction has not yet been fully examined. The purpose of the present study was to elucidate the contribution of N4-AcSDM to the serum protein binding and pharmacokinetic interactions between SDM and ketoprofen (KPF) in rabbits.

Experimental

Materials——SDM was purchased from Daiichi Pharmaceutical Co., Tokyo. KPF was kindly supplied by Kaken Pharmaceutical Co., Tokyo. N4-AcSDM was synthesized from SDM by the method of Uno et al.5) All other chemicals were of reagent grade.

Animal Experiments——Male albino rabbits weighing 2.5–3.5 kg were used in a cross-over design. An interval of at least 10 d was taken to minimize the residual or cumulative effect of the preceding dose. SDM at a dose of 50 mg/kg was administered intravenously as a bolus to rabbits. KPF or N4-AcSDM at a dose of 25 mg/kg was administered intravenously as a bolus immediately after SDM administration. The injections of SDM, KPF and N4-AcSDM were prepared by dissolving the drugs in saline solution containing the same molar amount of NaOH. Blood samples were collected from the ear vein. The blood was centrifuged at 3000 rpm for 15 min and the serum or plasma was separated.

Protein Binding Experiments——In vivo and in vitro protein binding experiments were carried out by means of the ultrafiltration method described previously.6) The in vivo protein binding of SDM was determined for the serum obtained at 2 h after intravenous bolus administration of SDM alone or in combination with KPF. The in vitro protein binding of SDM was determined for the serum prepared by the addition of SDM with or without KPF or N4-AcSDM.

Pharmacokinetic Analysis——The plasma SDM concentration data were analyzed by statistical moment
analysis to obtain values for the total body clearance ($C_{ltot}$) and steady-state volume of distribution ($V_{dss}$) of SDM according to the following equation:

\begin{align}
C_{ltot} &= \frac{D}{AUC} \tag{1} \\
V_{dss} &= \frac{D \cdot MRT}{AUC} \tag{2}
\end{align}

where $D$ is the dose, $AUC$ is the area under the plasma SDM concentration–time curve from zero to infinite time and $MRT$ is the mean residence time. $AUC$ was determined by means of the trapezoidal rule until the last data point ($C_t$) with the area to infinity being calculated as $C_t/\beta$. The elimination rate constant ($\beta$) was obtained from the log-linear regression line of the SDM concentration time curve. The values of $AUC$ and $MRT$ were calculated by using a microcomputer.

**Analytical Methods** — SDM concentrations in serum, plasma and ultrafiltrate samples were measured by the method of Bratton and Marshall. Total SDM (SDM + metabolites) in serum was measured by the same method after hydrolysis (0.5 N HCl, 100 °C) for 1 h. $N^4$-AcSDM concentration in serum or plasma was estimated by subtracting SDM from total SDM concentration, since no metabolite other than $N^4$-AcSDM was detected in serum or plasma by the extraction method of Rieder.

**Statistical Analysis** — Statistical significance of differences between means was determined by the paired Student’s $t$-test. A $p$-value of 0.05 or less was considered to be significant.

## Results and Discussion

**Serum Protein Binding Interaction**

The effect of KPF on the in vivo and in vitro bindings of SDM to rabbit serum was examined. As shown in Fig. 1, KPF markedly reduced the in vivo binding of SDM to rabbit serum. On the other hand, KPF had little effect on the in vitro binding of SDM to rabbit serum (Fig. 2). It is noteworthy that KPF reduces only the in vivo binding of SDM to rabbit serum.

Our previous paper showed that $N^4$-AcSDM strongly displaces SDM from its protein binding sites. Since $N^4$-AcSDM is the major metabolite of SDM in rabbits, it may contribute to the in vivo protein binding interaction between SDM and KPF. As shown in Fig. 3, the co-administration of KPF was found to increase the serum concentration of $N^4$-AcSDM at 2 h after intravenous bolus administration of SDM. This finding implies that KPF indirectly reduces the in vivo serum protein binding of SDM, by causing an increase in the

![Fig. 1. Effect of KPF on the in Vivo Binding of SDM to Rabbit Serum](image)

The in vivo protein binding of SDM was determined for the serum obtained at 2 h after intravenous bolus administration of SDM alone (○) or in combination with KPF (●).

![Fig. 2. Effect of KPF on the in Vitro Binding of SDM to Rabbit Serum](image)

The in vitro protein binding of SDM was determined for the serum prepared by the addition of SDM (100 µg/ml) with or without KPF.
serum concentration of $N^4$-AcSDM.

To elucidate further the mechanism of the \textit{in vivo} protein binding interaction between SDM and KPF, we examined the \textit{in vitro} binding of SDM to rabbit serum in the presence of $N^4$-AcSDM at the same concentration as that found at 2 h after intravenous bolus administration of SDM alone or in combination with KPF. As shown in Fig. 4, the \textit{in vitro} serum protein binding of SDM in the presence of $N^4$-AcSDM was closely similar to the \textit{in vivo} serum protein binding of SDM shown in Fig. 1. Therefore, it is concluded that $N^4$-AcSDM plays an important role in the \textit{in vivo} protein binding interaction between SDM and KPF in rabbits. A similar mechanism has been observed in the \textit{in vivo} protein binding interaction between SDM and phenylbutazone in rabbits.\(^{10}\)

It is well-known that nonsteroidal anti-inflammatory drugs such as KPF and bucolome depress the renal excretion of drugs or metabolites which are actively secreted by the tubules.\(^{11,12}\) Since $N^4$-AcSDM is actively secreted by the tubules\(^{13}\) unlike SDM, KPF may cause the increase in the serum concentration of $N^4$-AcSDM by depressing its renal excretion.

**Pharmacokinetic Interaction**

Figure 5 shows the time course of the plasma concentrations of SDM and $N^4$-AcSDM after intravenous bolus administration of SDM alone or in combination with KPF. The co-administration of KPF markedly decreased the plasma concentration of SDM, while the co-administration of KPF markedly increased the plasma concentration of $N^4$-AcSDM. In addition, the pharmacokinetic parameters were derived from the SDM plasma concentration data. As shown in Table I, the co-administration of KPF significantly increased $C_{tot}$ and $V_{dss}$ of SDM. Recently, the displacement of one drug from its protein binding sites by another has been reported to induce increases in $C_{tot}$ and/or $V_{dss}$ of the drug.\(^{14,15}\) For example, Arimori \textit{et al.} reported that penicillins displace phenytoin from its protein binding sites, and significantly increase $C_{tot}$ and $V_{dss}$ of phenytoin.\(^{15}\) Thus, the increases in $C_{tot}$ and $V_{dss}$ of SDM induced by KPF may be explained on the basis of the displacement of SDM from its protein binding sites by $N^4$-AcSDM.

To confirm the contribution of $N^4$-AcSDM to the pharmacokinetic interaction between
SDM and KPF, we examined whether the co-administration of N\textsuperscript{4}-AcSDM decreases the plasma concentration of SDM after intravenous bolus administration. As expected, the co-administration of N\textsuperscript{4}-AcSDM markedly decreased the plasma concentration of SDM (Fig. 6), resulting in significant increases in $C_{\text{tot}}$ and $V_{\text{dss}}$ of SDM (Table II). Interestingly, the co-administration of N\textsuperscript{4}-AcSDM, in contrast to that of KPF, caused a marked decrease in the plasma concentration of SDM at the early stage (distribution phase) after intravenous bolus administration. These observations indicate that N\textsuperscript{4}-AcSDM contributes to the pharmacokinetic interaction between SDM and KPF in rabbits.

SDM and KPF, we examined whether the co-administration of N\textsuperscript{4}-AcSDM decreases the plasma concentration of SDM after intravenous bolus administration. As expected, the co-administration of N\textsuperscript{4}-AcSDM markedly decreased the plasma concentration of SDM (Fig. 6), resulting in significant increases in $C_{\text{tot}}$ and $V_{\text{dss}}$ of SDM (Table II). Interestingly, the co-administration of N\textsuperscript{4}-AcSDM, in contrast to that of KPF, caused a marked decrease in the plasma concentration of SDM at the early stage (distribution phase) after intravenous bolus administration. These observations indicate that N\textsuperscript{4}-AcSDM contributes to the pharmacokinetic interaction between SDM and KPF in rabbits.

**Table I. Pharmacokinetic Parameters of SDM after Intravenous Bolus Administration of SDM Alone or in Combination with KPF to Rabbits**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SDM alone</th>
<th>With KPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{tot}}$ (ml/h/kg)</td>
<td>35.4 ± 3.2</td>
<td>52.1 ± 6.2*</td>
</tr>
<tr>
<td>$V_{\text{dss}}$ (ml/kg)</td>
<td>358 ± 13</td>
<td>526 ± 43*</td>
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</tbody>
</table>

Each value represents the mean ± S.E. of 5 rabbits.

*Significantly different from SDM alone (p < 0.05).

**Table II. Pharmacokinetic Parameters of SDM after Intravenous Bolus Administration of SDM Alone or in Combination with N\textsuperscript{4}-AcSDM to Rabbits**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SDM alone</th>
<th>With N\textsuperscript{4}-AcSDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{tot}}$ (ml/h/kg)</td>
<td>35.9 ± 1.6</td>
<td>51.9 ± 4.4*</td>
</tr>
<tr>
<td>$V_{\text{dss}}$ (ml/kg)</td>
<td>384 ± 8</td>
<td>620 ± 11*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. of 5 rabbits.

*Significantly different from SDM alone (p < 0.01).

*Significantly different from SDM alone (p < 0.001).
References