Effect of Simultaneous Administration of Drugs on Absorption and Excretion. XVII.[1] *In Vivo* Protein Binding Interaction between Sulfamethoxazole and Chloral Hydrate in Rabbits

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The effect of chloral hydrate (CH) on the *in vivo* and *in vitro* binding of sulfamethoxazole (SMX) to plasma proteins was investigated in rabbits. CH clearly reduced the *in vivo* binding of SMX to plasma proteins. On the other hand, CH had no effect on the *in vitro* binding of SMX to plasma proteins.

CH was found to indirectly reduce the *in vivo* plasma protein binding of SMX through the formation of trichloroacetic acid (TCA), which is a major metabolite of CH. In addition, CH was found to indirectly reduce the *in vivo* plasma protein binding of SMX by causing a significant increase in the plasma concentration of N^4-acetylsulfamethoxazole (N^4-AcSMX), which is the major metabolite of SMX and which markedly reduces the *in vitro* binding of SMX to plasma proteins. These results lead us to conclude that both TCA and N^4-AcSMX play an important role in the *in vivo* protein binding interaction betwen SMX and CH in rabbits.

Keywords——sulfamethoxazole; chloral hydrate; N^4-acetylsulfamethoxazole; trichloroacetic acid; *in vivo* protein binding interaction; plasma protein binding

Trichloroacetic acid (TCA) has been reported to be a major metabolite of chloral hydrate (CH), the oldest known hypnotic drug.[2] In addition, Sellers and Koch-Weser[3] demonstrated that TCA reduces the plasma protein binding of warfarin. Consequently, when CH is coadministered with another drug, it may indirectly reduce the *in vivo* plasma protein binding of the other drug through the formation of TCA.

Recently, we have pointed out that a metabolite is an important determinant affecting the *in vivo* binding of the parent drug to plasma proteins.[4] For example, probenecid indirectly reduces the *in vivo* plasma protein binding of sulfadimethoxine by causing a significant increase in the plasma concentration of N^4-acetylsulfadimethoxine, which is the major metabolite of sulfadimethoxine and which markedly reduces the *in vitro* binding of sulfadimethoxine to plasma proteins, despite the fact that probenecid itself cannot reduce the *in vitro* binding of sulfadimethoxine to plasma proteins.[4c]

The purpose of the present study was to elucidate the roles of TCA and N^4-acetylsulfamethoxazole (N^4-AcSMX) in the *in vivo* protein binding interaction between sulfamethoxazole (SMX) and CH in rabbits.

Experimental

Materials——Sulfamethoxazole (SMX) of JP grade was purchased from Shionogi Co., Ltd. N^4-AcSMX was synthesized from SMX by the method of Uno *et al.*[5] CH was obtained from Wako Pure Chemical Industries Ltd., and recrystallized from benzene. TCA and trichloroethanol (TCE) were purchased from Wako Pure Chemical Industries Ltd. and Nakarai Chemicals Ltd., respectively, and used without further purification. All other chemicals

were of reagent grade.

Animal Experiments——Male albino rabbits weighing 2.3—3.2 kg were fasted for 38—42 h prior to the experiments, but drinking water was allowed *ad libitum*.

a) Intravenous Bolus Injection of SMX and N^4-AcSMX: The SMX and N^4-AcSMX 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 SMX and N^4-AcSMX were 50 and 25 mg/kg, respectively.

b) Oral Administration of CH: CH dissolved in about 50 ml of distilled water was orally administered 1 h before intravenous bolus injection of SMX or N^4-AcSMX. The dose of CH was 300 mg/kg.

c) Plasma Sampling and Ultrafiltration: About 6 ml of blood was collected from the ear vein after intravenous bolus injection of SMX. After heparinization, the blood was immediately centrifuged and the plasma was separated. Plasma sampling was carried out 1 h after intravenous bolus injection of SMX, because the α phase of the log-plasma SMX concentration *versus* time curve was completed within 1 h.

d) Plasma Sampling for Determination of Elimination Half-Life of N^4-AcSMX: About 1 ml of blood was collected periodically from the ear vein after intravenous bolus injection of N^4-AcSMX. After heparinization, the blood was immediately centrifuged and the plasma was separated.

e) Plasma Sampling for Determination of TCA and TCE Concentrations: About 6 ml of blood were collected from the ear vein 2 h after oral administration of CH. After heparinization, the blood was immediately centrifuged and the plasma was separated.

Protein Binding Experiments——The *in vivo* and *in vitro* protein binding experiments were carried out according to the ultrafiltration method described previously.[4d] Plasma was centrifuged at 2800 rpm for 40 min. Under these experimental conditions, the volume of ultrafiltrate obtained was about 15% of the initial volume of plasma.

a) *In Vivo* Protein Binding Experiment: The *in vivo* binding of SMX to plasma proteins was determined with plasma obtained 1 h after intravenous bolus injection of SMX.

b) *In Vitro* Protein Binding Experiment: The *in vitro* binding of SMX to plasma proteins was determined with plasma to which SMX had been added.

Analytical Methods——Unchanged SMX concentrations in plasma and its ultrafiltrate were measured by the Bratton–Marshall method.[6] Total (unchanged + metabolites) SMX concentration in plasma was measured after hydrolysis (0.5 N HCl, at 100 °C for 1 h) by the Bratton–Marshall method.[6] N^4-AcSMX concentration in plasma was calculated from the difference between unchanged and total SMX concentrations in plasma, because metabolites other than N^4-AcSMX were not detected in plasma by the separation method of Okamoto.[7] TCA and TCE concentrations in plasma were measured by the colorimetric method of Friedman *et al.*[8]

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

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

Results and Discussion

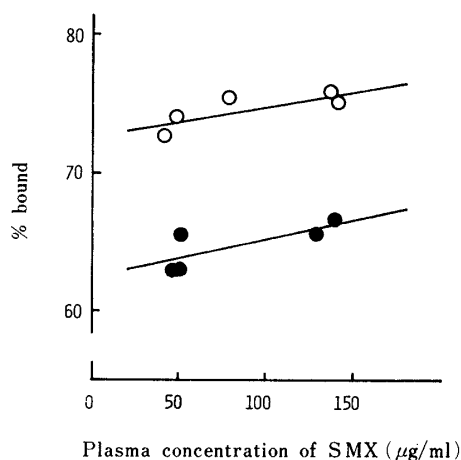The effect of CH on the *in vivo* and *in vitro* binding of SMX to plasma proteins was

Fig. 1. Effect of CH on the *in Vivo* Binding of SMX to Plasma Proteins

O, SMX alone; ●, with CH.
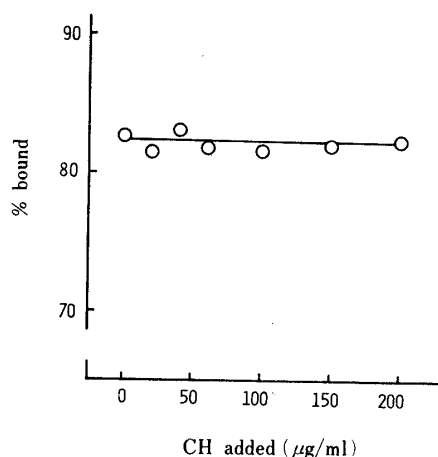Five rabbits were used in a crossover design.

Fig. 2. Effect of CH on the *in Vitro* Binding of SMX to Plasma Proteins

SMX added: 100 μg/ml.

TABLE I. *In Vitro* Binding of SMX to Plasma Proteins Determined
before and after Oral Administration of CH to Rabbits

Rabbit	% bound	
	Before	After
A	82.1	73.3
B	83.2	79.0
C	87.4	82.9
D	84.7	82.0
E	79.5	71.0
Mean	83.4	77.6[a]
S.D.	2.9	5.3

SMX was added to plasma obtained before and after oral administration of CH. SMX added: 100 μg/ml.
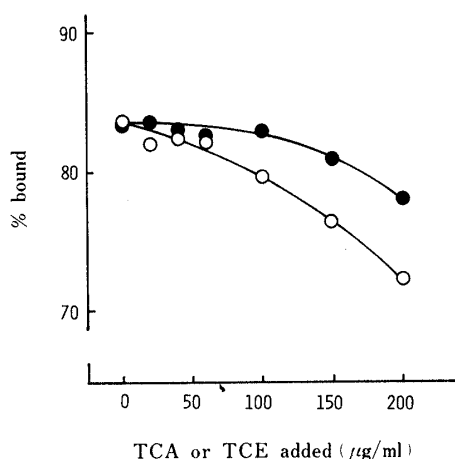
a) Significantly different from "Before", $p < 0.01$.

TCA or TCE added (μg/ml)

Fig. 3. Effect of TCA or TCE on the *in Vitro* Binding of SMX to Plasma Proteins
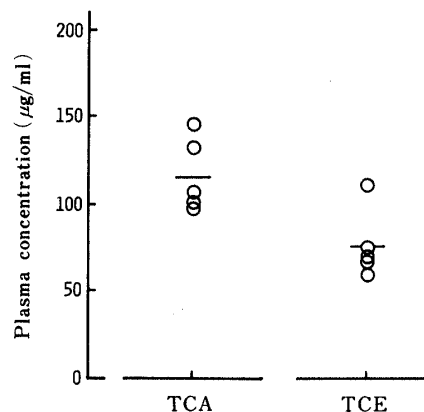
SMX added: 100 μg/ml. ○, TCA; ●, TCE.

Fig. 4. Plasma Concentration of TCA or TCE 2 h after Oral Administration of CH to Rabbits

The horizontal bars indicate the mean values.

investigated in rabbits. As shown in Fig. 1, CH evidently reduced the *in vivo* binding of SMX to plasma proteins. On the other hand, as shown in Fig. 2, CH had no effect on the *in vitro* binding of SMX to plasma proteins.

To elucidate the reason why CH can reduce only the *in vivo* binding of SMX to plasma proteins, the *in vitro* binding of SMX to plasma proteins was compared before and after oral administration of CH (Table I). As expected, a significant difference in the *in vitro* binding of SMX to plasma proteins was observed before and after oral administration of CH. This observation suggests that a metabolite of CH may reduce the *in vivo* binding of SMX to plasma proteins.

CH is well known to be mainly biotransformed to TCA and TCE as shown in Chart 1. Thus, if TCA or TCE reduces the *in vitro* binding of SMX to plasma proteins, and if the plasma concentration of TCA or TCE is sufficiently high, CH will indirectly reduce the *in vivo* plasma protein binding of SMX through the formation of TCA or TCE. As shown in Figs. 3 and 4, TCA reduced the *in vitro* binding of SMX to plasma proteins, and the plasma concentration of TCA was sufficiently high to produce the reducing effect. These findings indicate that TCA plays an important role in the *in vivo* protein binding interaction between SMX and CH. On the other hand, as shown in Figs. 3 and 4, although TCE slightly reduced
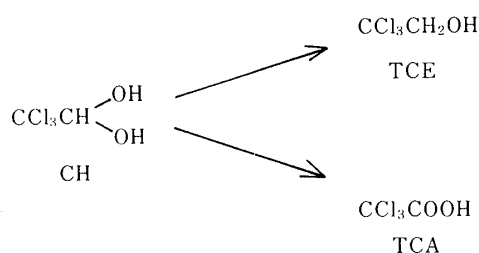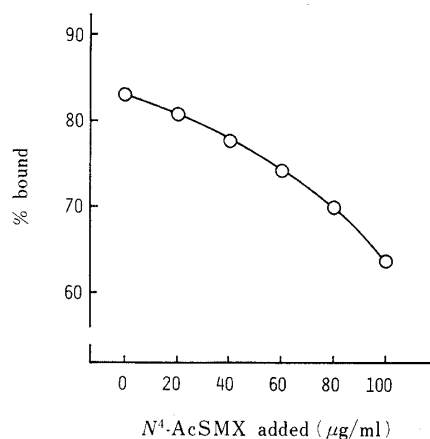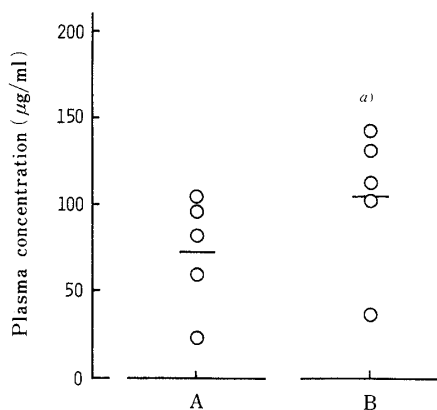
CCl$_3$CH$\diagdown$OH$\diagup$OH → CCl$_3$CH$_2$OH
TCE

CH

CCl$_3$COOH
TCA

Chart 1. Biotransformation of CH

Fig. 5. Effect of N^4-AcSMX on the *in Vitro* Binding of SMX to Plasma Proteins

SMX added: 100 μg/ml.

Fig. 6. Effect of CH on the Plasma Concentration of N^4-AcSMX 1 h after Intravenous Bolus Injection of SMX to Rabbits

A, SMX alone; B, with CH.
Five rabbits were used in a crossover design.
CH was orally administered 1 h before intravenous bolus injection of SMX. The horizontal bars indicate the mean values.
a) Significantly different from SMX alone, $p < 0.005$.

Plasma concentration of N^4-AcSMX (μg/ml)
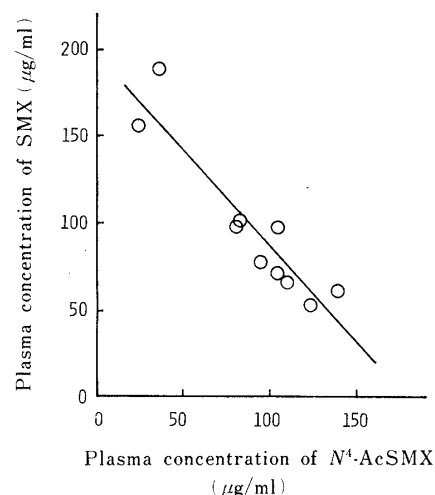
Fig. 7. Correlation between Plasma Concentrations of SMX and N^4-AcSMX 1 h after Intravenous Bolus Injection of SMX to Rabbits

$n = 10$; $r = -0.927$.

the *in vitro* binding of SMX to plasma proteins, the plasma concentration of TCE was not sufficiently high to account for the reducing effect.

Recently, it has been reported that a metabolite can reduce the plasma protein binding of the parent drug.[4,9] For example, Selley *et al.*[9a] reported that a major metabolite of tolmetin, 1-methyl-5-(4-carboxybenzoyl)-1*H*-pyrrole-2-acetic acid, reduces the plasma protein binding of tolmetin. We also reported that a major metabolite of carbutamide, N^4-acetylcarbutamide, reduces the plasma protein binding of carbutamide.[4b] It is well known that N^4-AcSMX is a major metabolite of SMX.[10] Thus, the effect of N^4-AcSMX on the *in vitro* binding of SMX to plasma proteins was determined. As shown in Fig. 5, N^4-AcSMX markedly reduced the *in vitro* binding of SMX to plasma proteins. In addition, CH caused a significant increase in the plasma concentration of N^4-AcSMX after intravenous bolus injection of SMX (Fig. 6). These findings indicate that N^4-AcSMX also plays an important role in the *in vivo* protein binding interaction between SMX and CH. That is, CH indirectly reduces the *in vivo* plasma protein binding of SMX by causing a significant increase in the plasma concentration of N^4-AcSMX.

Table II. Effect of CH on the Elimination Half-Life of N^4-AcSMX
after Intravenous Bolus Injection of N^4-AcSMX to Rabbits

Rabbit	Elimination half-life (h)	
	N^4-AcSMX alone	With CH
F	1.37	3.49
G	1.37	2.89
H	1.25	2.47
I	1.22	4.00
J	1.27	1.78
Mean	1.30	2.93[a]
S.D.	0.07	0.87

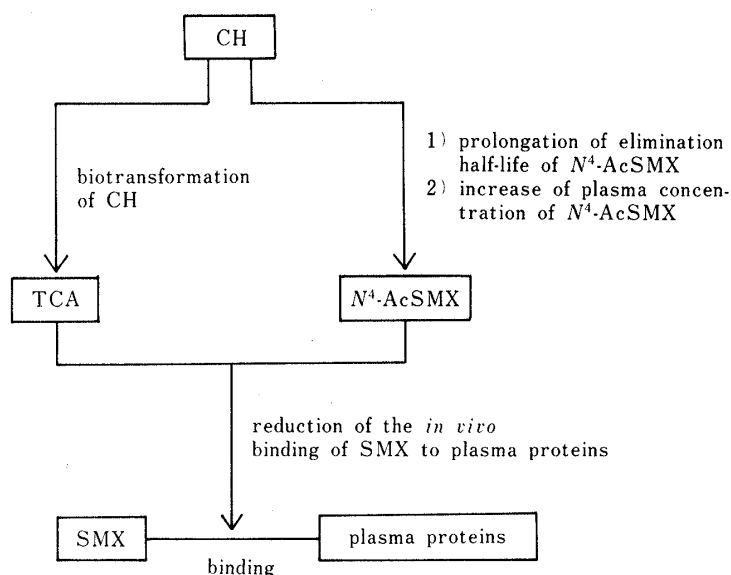a) Significantly different from N^4-AcSMX alone, $p < 0.05$.

Fig. 8. The Roles of TCA and N^4-AcSMX in the *in Vivo* Protein Binding
Interaction between SMX and CH in Rabbits

Table II shows the effect of CH on the elimination half-life of N^4-AcSMX after intravenous bolus injection of N^4-AcSMX to rabbits. CH significantly prolonged the elimination half-life of N^4-AcSMX. This finding implies that the increased plasma concentration of N^4-AcSMX, shown in Fig. 6, results from the prolonged elimination half-life of N^4-AcSMX. Recently, Arita *et al.*[11] have reported that N^4-AcSMX is actively secreted by the tubules, and that some nonsteroidal anti-inflammatory drugs such as bucolome and sulfinpyrazone prolong the elimination half-life of N^4-AcSMX by inhibiting competitively the active tubular secretion. Furthermore, we found that CH markedly decreases the renal excretion of phenol red, which is actively secreted by the tubules (unpublished data). Consequently, CH appears to prolong the elimination half-life of N^4-AcSMX by competitively inhibiting the active tubular secretion. However, further studies are necessary to elucidate the mechanism of the renal excretion interaction between N^4-AcSMX and CH.

The *in vivo* binding of a drug to plasma proteins has been reported to decrease with increase in the plasma concentration of the drug.[12] However, the *in vivo* binding of SMX to plasma proteins slightly decreased with a decrease in the plasma concentration of SMX (see Fig. 1). This may be due to the increase in the plasma concentration of N^4-AcSMX, since a

negative correlation was observed between the plasma concentrations of SMX and N^4-AcSMX, as shown in Fig. 7; N^4-AcSMX markedly reduces the *in vitro* binding of SMX to plasma proteins (see Fig. 5).

On the basis of all the above results, the roles of TCA and N^4-AcSMX in the *in vivo* protein binding interaction between SMX and CH are summarized in Fig. 8. It is evident that these two metabolites are important determinants affecting the *in vivo* binding of SMX to plasma proteins. Our previous papers showed the complexity of the *in vivo* protein binding interaction between sulfadimethoxine and probenecid.[1,4c] In the present paper, we present evidence that the *in vivo* protein binding interaction between SMX and CH is even more complex.

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