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Biopharmaceutical studies on drug/conjugated-metabolite interactions. II. Effect of acetaminophen sulfate on pharmacokinetics of acetaminophen in rats

Taiji Sawamoto, Yuji Kurosaki, Kenji Sasaki, Toshikiro Kimura, Taiji Nakayama*

Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-naka 1-1-1, Okayama 700, Japan

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Abstract

The effect of conjugated-metabolite, acetaminophen sulfate (APAPS), on the pharmacokinetics of its parent drug, acetaimnophen (APAP), was examined in rats. Following the i.v. bolus administration of APAP with APAPS, the plasma elimination of APAP was delayed and the distribution volume of APAP was increased at the APAPS coadministration with 60 mg APAP equivalent per kg (eq/kg). The percentages of dose excreted in the urine and bile in 4 h as APAP and its conjugated metabolites, APAPS and acetaminophen glucuronide, were significantly decreased. On the other hand, following the i.v. bolus administration of APAP under the steady-state concentration of APAPS, the distribution volume and total body clearance of APAP were significantly increased. Competitive displacement in serum protein binding of APAP by APAPS was ascertained in vitro and in vivo. A part of the conflict between the bolus and infusion experiment may be explained by the changes in the distribution volume of APAP contributed to the APAPS concentration-dependent serum protein binding of APAP. It was speculated that the pharmacokinetics of APAP was partly interacted with APAPS by the displacement of serum protein binding. © 1997 Elsevier Science B.V.

Keywords: Acetaminophen; Acetaminophen sulfate; Pharmacokinetics; Conjugated metabolite; Drug/conjugated-metabolite interaction; Rat

1. Introduction

Multiple-drug therapy is common in current clinical practice. Considerable attention has been focused on drug-drug interaction in recent years (Stockley, 1994). The studies of drug interactions have become indispensable in order to enhance pharmacological effects or diminish toxicological ones.

* Corresponding author.

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Acetaminophen (APAP) is widely used as an oral nonprescription drug and is frequently used with other analgesic and antipyretic drugs as a multiple-drug therapy. Pharmacokinetics of APAP in rats has been reported in detail. Major biotransformation pathways of APAP in rats may be explained by the sulfation and glucuronidation (Galinsky and Levy, 1981; Watari et al., 1983; Tone et al., 1990). There are many reports on drug-drug interactions of APAP (Jaw and Jeffery, 1993; Monshouwer et al., 1994), however, such drug-drug metabolite(s) interactions as the effect on the fate of parent drug, APAP, of its two major metabolites, i.e. the sulfate (APAPS) and the glucuronide (APAPG) have not been investigated. As one of the drug-drug metabolite interactions, the displacement of in vivo rabbit serum protein bindings between sulfadimethoxine and its major metabolite, *N*-acetyl-sulfadimethoxine, was reported (Imamura et al., 1986; Otagiri et al., 1989). Nonlinear pharmacokinetics of prinomide tromethamine, a nonsteroidal antiinflammatory drug, caused by both saturable and competitive protein binding between prinomide and its *p*-hydroxy metabolite was also reported in man (Kochak et al., 1993). The decrease of protein binding of a parent drug may increase the pharmacological effect because of the changes in distribution, metabolism and excretion. And also dose-dependent bioavailability of propranolol could be caused by inhibition of metabolism of the parent drug by one or more of its accumulated metabolites (product inhibition) (Ghabrial et al., 1994).

From this information, the investigations of drug-drug metabolite interactions may be important for the precise clinical practice containing the effectiveness and safety of drugs, especially in the case of repeated administrations of multiple drugs, where the upward trend in drug interactions are respected.

In the present study, to clarify the drug-metabolite interaction(s) from the pharmacokinetic view points, pharmacokinetic aspects of APAP after intravenous coadministration by bolus injection and continuous infusion of APAPS were investigated in rats.

2. Materials and methods

2.1. Materials

APAP used was of JP grade. APAPS was synthesized as potassium salt in our laboratory (Nakayama et al., 1995). The amounts of APAPS and APAPG in this paper were expressed as the amount equivalent to APAP to facilitate balance study. All other chemicals and reagents were of analytical grade or better.

2.2. Animals

Male Wistar rats aged 10 weeks (Japan SLC, Hamamatsu, Japan) and weighing 250–300 g were used. The animals were maintained in conditions of constant temperature, humidity, lighting cycle and fed a standard diet (MF Diet, Oriental Weast, Japan) and water ad libitum.

2.3. In vivo experiment

The rats were fasted overnight before use, but with free access to water. Polyethylene tubes were cannulated into both the left femoral artery and the bile duct for blood and bile samplings, respectively. Urine collection was performed by the attachment of urine collecting tips.

Rats were settled in a Bollman cage (Bollman, 1948). For each operation, the animals were slightly anesthetized with ether at suitable intervals as necessary during surgery. All experiments were started after the rats had recovered from ether anesthesia.

2.3.1. Bolus injection study

APAP (30 mg/kg) and APAPS (10, 30, 60 and 120 mg APAP equivalent per kg (eq/kg) dissolved in saline solution were administered via the right femoral vein. The drug solution was administered in a volume of 1 ml/kg. Blood, urine and bile samples were collected through the fastened cannula at appropriate times, respectively. Normal saline solution (1.5 ml aliquot) was orally administered every 2 h in order to maintain the bile and urine flows.

2.3.2. Continuous infusion study

APAPS (27.9 APAP equivalent mg/kg) dissolved in saline was administered via the right femoral vein as an initial dose, followed by a continuous infusion (118 mg APAP eq/h/kg) to reach a target steady-state concentration of 200 μg APAP eq/ml via the left femoral vein throughout the experiment. APAP (30 mg/kg) was administered under the steady-state of APAPS at 70 min after the start of APAPS or saline infusion. Blood samples were collected through the fastened cannula at appropriate times, respectively. Inulin (20 mg/kg), extracellular space marker, dissolved in saline was administered via the right femoral vein as an initial dose, followed by continuous infusion (65 mg/h/kg) via the left femoral vein throughout the experiment. Blood and tissue samples were collected through the fastened cannula at 70 min after the start of inulin infusion.

To examine the tissue to plasma concentration ratios (kp), blood and various tissue samples were collected at 5 min after i.v. administration of APAP (30 mg/kg) under the continuous infusion of APAPS or saline. The continuous infusion of APAPS was performed by using the pharmacokinetic parameters calculated from the preliminary study. An initial dose of APAPS was calculated as the infusion rate divided by the slope of the log-linear terminal phase of plasma elimination.

2.4. In vitro metabolism experiment

Cytosol and microsome fractions were isolated using standard centrifugation techniques with minor modification (Mizuma et al., 1984; Bolanowska and Gessner, 1978). Briefly, the animals were killed by decapitation and the liver was immediately removed. Freshly excised livers were homogenized with 4 volumes of 1.19% KCl in a Teflon-glass homogenizer. After centrifuging liver homogenate at 4°C with a refrigerated centrifuge (Hitachi 2OPR-5, Hitachi Koki, Tokyo, Japan) at $9000 \times g$, its resultant supernatant fraction was further centrifuged at $100\,000 \times g$ for 60 min. The final supernatant fraction was used as the cytosol fraction and the microsomal pellet was suspended in 3 volumes of 1.19% KCl in ice cold conditions. The resultant suspension was used as the microsomal fraction.

2.4.1. APAP sulfation in the presence of APAPS

After 0.5 ml of the cytosol fraction, equivalent to 0.25 g tissue/ml, diluted with 5-fold 0.4 M Tris–HCl buffer (pH 7.4) was incubated at 37°C for 2 min, 1/4 ml of the mixed solution of 0.25 mM APAP and 0.75 mM APAPS, containing 2.6 mM KH_2PO_4 and 5 mM EDTA, and 0.25 ml of 200 μM 3-phosphoadenosine-5-phosphosulfate (PAPS) were added to the fraction and the mixture was incubated at 37°C for 15 min. The reaction was terminated by adding 0.5 ml of the incubation mixture to 0.5 ml of acetonitrile to denaturalize the cytosol protein in ice cold. APAPS formation was calculated from the remaining of APAP in the incubation mixture.

2.4.2. APAP glucuronidation in the presence of APAPS

A 0.7 ml of microsomal fraction, equivalent to 0.13 g tissue/ml in incubation mixture, containing 14 mM $MgCl_2$ and 0.15 M phosphate buffer (pH 7.4) was preincubated at 37°C for 2 min. A 0.1 ml of the mixed solution of 0.2 mM APAP and 0.4 mM APAPS and 0.2 ml of 20 mM uridine-5-diphosphoglucuronic acid (UDPGA) were added to the fraction and the mixture was incubated at 37°C for 15 min. The reaction was terminated by flash freezing and solvent extraction. Determination of APAPG was performed after removal of APAP from incubation mixture. Briefly, the incubation mixture was saturated with NaCl and extracted two times with 5 ml of diethyl ether in order to remove APAP from the incubation mixture. The tubes were shaken for 10 min and centrifuged at 3000 rpm for 10 min, the organic layer was removed by aspiration. The hydrophilic APAPG in aqueous layer was determined by HPLC after hydrolysis (see analytical method). Each incubation time (15 min) of this conjugation study was decided by the preliminary experiment.

2.5. Serum protein binding experiment

Serum protein binding measurements were made by ultrafiltration (Amicon Centrifree Micropartition System). The preliminary studies were performed with solutions of APAP and APAPS in pH 7.4 isotonic phosphate buffer, to

examine the possibilities of membrane binding. The membrane binding of APAP and APAPS was below 5%. Approximately 1.0 ml of buffer solution (preliminary studies) or serum was placed in the ultrafiltration system which was mounted in plastic centrifuge tube. The tubes were centrifuged at 2500 rpm for 15 min at 37°C, and only the mimmum amount (0.1–0.15 ml) of ultrafiltrate for analysis was collected. Such a limited centrifugation prevents appreciable concentration of protein in the cone.

2.6. Analytical method

APAP and APAPS in plasma, urine and bile were determined by the reversed-phase HPLC method described in our previous report (Nakayama et al., 1995). In in vitro conjugation study, APAP in the incubation mixture was determined with minor modification according to a reversed-phase HPLC method (Cohn and Sirois, 1987). Briefly, a high performance liquid chromatograph (LC-3A, Shimadzu, Kyoto) connected with a Inertsil ODS column (average particle size of 5 μm, 4.6 mm i.d. × 250 mm, GL Sciences, Tokyo) and with a UV detector (SPD-2A, Shimadzu) operated at 254 nm was used. A mixture of 50 mM phosphate buffer solution (pH 7.4) and methanol (90:10 by volume) was used as the mobile phase. The flow rate was 0.9 ml/min. APAPG was determined as APAP after hydrolyzing the sample (after the removal of APAP in microsomal incubation mixture) by the incubation with β-glucuronidase solution (1000 U dissolved in 100 mM acetate buffer solution, pH 5.0) at 37°C for 20 h. Then, 150 μl of methanol was added to 100 μl of all plasma, urine and bile samples to denaturalize the plasma protein. After the precipitation of protein by centrifugation (10 000 rpm × 1 min), 50 μl of supernatant filtered through a 0.45 μm pore-size membrane filter (Nihon Millipore Kogyo, Yonezawa, Japan) was injected into HPLC. The APAP and APAPS in all samples were detected separately as symmetrical peaks.

In in vitro sulfation study, 0.5 ml of incubation mixture was added to 0.5 ml of acetonitrile. After the precipitation of protein by centrifugation (10 000 rpm × 1 min), 150 μl of the supernatant filtered as described above was injected to HPLC. In glucuronidation study, 0.5 ml of APAP-extracted incubation mixture was added to 1 ml of acetonitrile to precipitate proteins. Of the clear supernatant, 1 ml was then transferred to a conical glass tube and evaporated at 40°C under vacuum with a vortex evaporator. Each residue obtained was dissolved in 200 μl of the mobile phase and 150 μl of the solution was then injected to HPLC. Protein concentration was determined by Folin phenol reagent using bovine serum albumm as a standard.

2.7. Pharmacokinetic analysis

Plasma elimination profiles of APAP and APAPS after i.v. bolus coadminstration were analyzed by a two-compartment model with successive generation of APAPS via the Michaelis-Menten type kinetics shown in Chart 1 (Sawamoto et al., 1996) using a nonlinear least-squares analysis program, MULTI(RUNGE) (Yamaoka and Nakagawa, 1983). The pharmacokinetic parameters of APAPS after i.v. admimstration were fixed to the values reported in our previous paper (Nakayama et al., 1995). In estimating pharmacokinetic parameters, the damping Gauss-Newton method was used and the reciprocal of each plasma concentration value was selected as the weight of the data. The slope of the log-linear terminal phase of plasma elimination (β) by using the last three plasma concentrations was calculated by the linear least-squares regression. The area under the plasma concentration-time curve (AUC) was estimated by the trapezoidal rule extrapolated to infinity. The total body clearance (CL_{total}) was calculated as the dose divided by AUC. The volume of distribution at steady-state (Vd_{ss}) was calculated as $(1 + k_{A12}/k_{A21}) \times Vd_{A1}$. These parameters will be explained later in the text.

2.8. Statistical analysis

Statistical analysis of pharmacokinetic parameters of APAP at the simultaneous administration of APAPS was performed by Tukey's multiple comparison procedure (ANOVA) and Student's

t-test. In all statistical testings, a p value of < 0.05 was considered to be significantly different.

3. Results and discussion

3.1. Plasma profile of APAP after i.v. bolus coadministration of APAP with APAPS

Fig. 1 shows the plasma profiles of APAP and APAPS after i.v. bolus administration of APAP (30 mg/kg) with APAPS (0, 10, 30 and 60 mg APAP eq/kg). The plasma concentration of APAP at 5 min after administration of APAP with APAPS (60 mg APAP eq/kg) was decreased from 57.6 to 46.4 μg/ml and plasma elimination profiles of APAP were prolonged in proportion to the coadmimstration dose of APAPS. The plasma

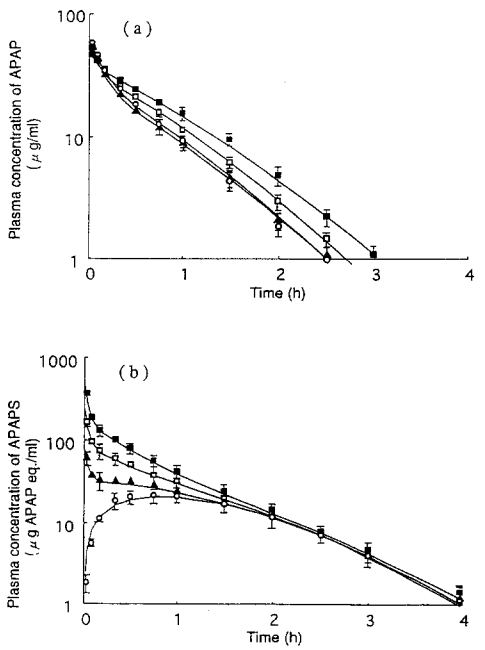

Fig. 1. Plasma concentration profiles of APAP (panel a) and APAPS (panel b) after intravenous administration of APAP with various doses of APAPS in rats. The doses of APAP were 30 mg/kg. The simultaneous doses of APAPS were 0 (○), 10 (▲), 30 (□) and 60 (■) mg APAP eq/kg. Results are expressed as the mean ± S.E. of 3–4 rats. Each line represents the simulation curve calculated according to the model shown in chart 1 by using the recalculated parameters of the mean plasma concentration of APAP and APAPS.

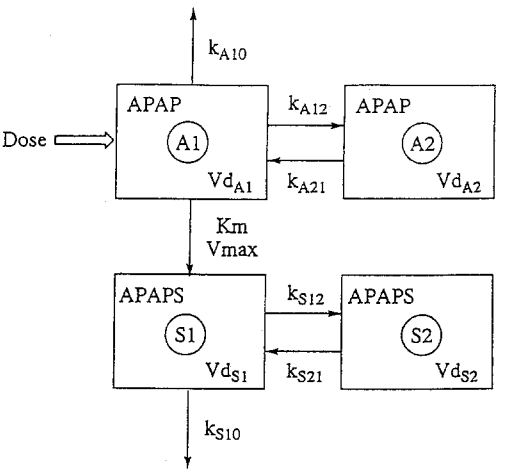

Fig. 2. Pharmacokinetic model describing dose-dependent. Plasma elimination of intravenously administered APAP with APAPS in rats. Pharmacokinetic parameters describing the distribution and the elimination of APAPS were fixed in the regression according to our previous report (Nakayama et al., 1995): $k_{S12} = 14.2$ h^{-1}; $k_{S21} = 11.1$ h^{-1}; k_{S10} 4.87 h^{-1}; $Vd_{S2} =$ 156.3 ml/kg.

profiles of APAP and APAPS after the coadministration of APAP with APAPS were analyzed by a simplified model (Fig. 2), solving the differential equations by the Runge-Kutta-Gill method, shown in the following equations which consists of Michaelis-Menten type kinetics for APAPS formation and the total first-order elimination kinetics for APAP, including APAPG and unknown metabolites formation.

$$dX_{A1}/dt = \{k_{A12} + k_{A10} + (V_{max}/Vd_{A1})/(K_m + X_{A1}/Vd_{A1})\}X_{A1} + k_{A21}X_{A2} \quad (1)$$

$$dX_{A2}/dt = k_{A12}X_{A1} - k_{A21}X_{A2} \quad (2)$$

$$dX_{S1}/dt = \{(V_{max}/Vd_{A1})/(K_m + X_{A1}/Vd_{A1})\}X_{A1} - (k_{S12} + k_{S10})X_{S1} + k_{S21}X_{S2} \quad (3)$$

$$dX_{S2}/dt = k_{S12}X_{S1} - k_{S21}X_{S2} \quad (4)$$

where X, Vd and k mean the amount, the distribution volume and the transfer rate constant, respectively, with the subscripts of A and S for parameters of APAP and APAPS, respectively, with the subscripts of 1 and 2 for the central and

Table 1
Pharmacokinetic parameters of APAP after intravenous coadministration of APAP with various doses of APAPS in rats

Parameters	Control	Dose of APAPS (mg APAP eq/kg)		
		10 ($n=3$)	30 ($n=3$)	60 ($n=4$)
k_{A12} (1/h)	2.45 ± 0.01	2.51 ± 0.05	2.46 ± 0.14	2.84 ± 0.12
k_{A21} (1/h)	4.36 ± 0.18	4.23 ± 0.69	5.77 ± 0.28	5.28 ± 0.23
k_{A10} (1/h)	0.51 ± 0.03	0.36 ± 0.04	0.37 ± 0.04	0.35 ± 0.02
Km (μM)	67.5 ± 6.7	83.3 ± 6.2	127.0 ± 23.1	160.1 ± 12.2
V_{max} (μmol/min/kg)	2.79 ± 0.06	3.02 ± 0.16	3.12 ± 0.28	3.20 ± 0.07
V_{max}/Km (ml/min/kg)	41.3 ± 4.3	36.2 ± 2.6	24.6 ± 4.2	20.0 ± 3.6
Vd_{A1} (ml/kg)	526.7 ± 11.0	559.1 ± 6.0	567.5 ± 5.2	594.8 ± 8.3
Vd_{A2} (ml/kg)	293.1 ± 5.2	366.4 ± 57.8	243.8 ± 15.2	321.7 ± 18.4
Vd_{ss} (ml/kg)	819.8 ± 12.2	925.5 ± 58.1	811.3 ± 16.1	916.5 ± 20.2
$AUC_{0-\infty}$ (μg·h/ml)	29.0 ± 1.2	27.9 ± 1.5	32.9 ± 2.1	39.4 ± 1.5
CL_{total} (ml/min/kg)	17.2 ± 0.7	17.9 ± 1.0	15.2 ± 1.0	12.7 ± 0.5

The dose of APAP was 30 mg/kg.
Each value represents the mean ± S.E. of 3–4 rats.
Underlined means are not significantly different to each other at p value of 0.05 by Tukey's multiple comparison procedure (ANOVA).

the peripheral compartment, respectively; k_{12} and k_{21} mean transfer rate constants from the central to the peripheral compartment and the vice versa, respectively; V_{max} and K_m are the maximum velocity and the Michaelis constant for the sulfation, respectively; and k_{A10} and k_{S10} are the apparent first-order elimination rate constant for APAP and APAPS, respectively. Initial value of S1 compartment at zero time is defined as the coadministration dose of APAPS.

Pharmacokinetic parameters of APAP after i.v. bolus administration of APAP (30 mg/kg) with APAPS (10, 30 and 60 mg APAP eq/kg) to rats were summarized in Table 1. V_{max} and K_m values for APAPS formation after iv. administration of APAP (30 mg/kg) alone were 2.79 μmol/min/kg and 67.5 μM, respectively. However, following the coadministration of APAPS at 10, 30 and 60 mg APAP eq/kg, the K_m value was increased, although the V_{max} value was not significantly affected. The V_{max}/K_m value, which means hepatic intrinsic clearance, was decreased and also the total first-order elimination rate constant for APAP (k_{A10}) was decreased by the coadministration of APAPS. The CL_{total} was significantly decreased by the coadministration of APAPS with 60 mg APAP eq/kg, while the distribution volume of the central compartment (Vd_{A1}) was increased. Accordingly, it was suggested that the decrease of CL_{total} may contribute to the conjugative metabolism of APAP.

On the other hand, it can be assumed that the plasma profiles of intravenously administered and generated APAPS profile were well explained by linear pharmacokinetics described by a two-compartment model (Nakayama et al., 1995; Sawamoto et al., 1996).

3.2. Urinary and biliary excretion of APAP and its conjugated metabolites

As shown in our previous paper (Nakayama et al., 1995), approximately 80% of intravenously administered APAPS was excreted as the unchanged form in the urine over 4 h, while biliary excretion was only a few percent of the dose. Both urinary and biliary excretions of APAPS were linear in the dose range from 10 to 120 mg APAP eq/kg. Thus, the corrected percentage of dose excreted as APAPS in urine in 4 h was calculated from the value subtracted from the total amount excreted as APAPS at the simultaneous administration to the amount excreted as APAPS after i.v. administration of APAPS at each simulta-

Table 2
Urinary and biliary excretion of APAP, APAPS and APAPG in 4 h after intravenous administration of APAP with various doses of APAPS

Form in excreta	APAP alone	APAPS (mg APAP eg/kg)		
		10	30	60
Urinary excretion				
APAP	1.3 ± 0.1[a]	1.2 ± 0.5[a]	1.6 ± 0.2[a]	1.9 ± 0.4
APAPS	76.5 ± 1.6[a]	74.8 ± 3.3[a]	67.2 ± 3.8[a]	50.4 ± 2.2
APAPG	7.8 ± 0.2[a]	7.1 ± 1.7[a]	5.0 ± 1.2[a]	3.6 ± 0.9
Total	85.5 ± 2.0[a]	83.1 ± 3.5[a]	73.8 ± 4.4	55.9 ± 2.1
Biliary excretion (%)				
APAP	0.6 ± 0.1[a]	0.5 ± 0.1[a]	0.4 ± 0.0[a]	0.3 ± 0.1
APAPS	5.3 ± 0.7[a]	5.4 ± 0.4[a]	2.9 ± 0.2[a]	1.5 ± 0.5
APAPG	6.9 ± 0.3[a]	5.6 ± 0.6[a]	4.0 ± 0.2[a]	3.1 ± 0.4
Total	12.8 ± 0.5[a]	11.5 ± 0.7[a]	7.2 ± 0.5	4.9 ± 0.6

Values represent the mean ± S.E. of 3–4 rats.
[a]Means not significantly different from each other at p value of 0.05 by Tukey's multiple comparison procedure (ANOVA)

neous dose. The corrected percentage of dose excreted in the urine and bile in 4 h as APAP and its conjugated metabolites, APAPS and APAPG, were summarized in Table 2. The percentages of dose excreted in the urine and bile were significantly decreased according to the increase of simultaneous dose of APAPS. The corrected percentage of dose excreted in the urine as APAPS was also decreased. Similarly, the urinary excretion of APAPG was decreased. Biliary excretions of APAPS and APAPG were also decreased. These results were consistent with the delayed plasma elimination profile of APAP following the i.v. administration with APAPS.

3.3. Plasma elimination profile of APAP under the steady-state concentration of APAPS

In order to clarify the pharmacokinetic interactions between APAP and APAPS in more detail, the plasma profiles of APAP were investigated under the steady-state concentration of APAPS adjusted to be high level of 200 μg APAP eq/ml to uniform the effect of APAPS on the pharmacokinetics of APAP such as serum protein binding and metabolism. The plasma profiles of APAP are shown in Fig. 3 and pharmacokinetic parameters of APAP under the steady-state concentration of APAPS were summarized in Table 3. Although both K_m and V_{max} values of APAP under steady-state concentration of APAPS were slightly increased in comparison to control, V_{max}/K_m values were not altered. The terminal elimination phase

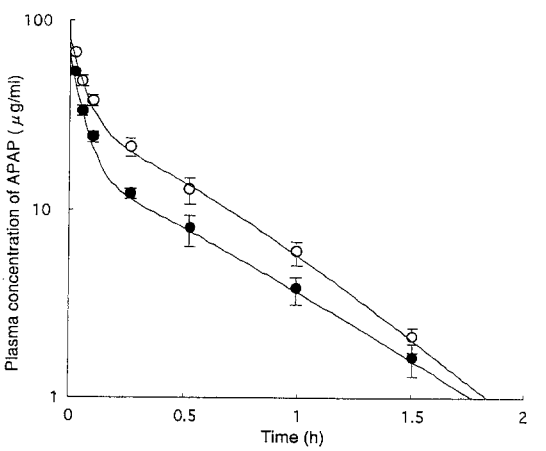

Fig. 3. Plasma profiles of APAP after intravenous administration of APAP under the continuous infusion of APAPS in rats. The doses of APAP was 30 mg/kg. Steady-state plasma concentration of APAPS was kept to 200 μg APAP eq/ml in rat plasma. Saline infusion (○); APAPS infusion (●). Results are expressed as the mean ± S.E. of 3–4 rats. Each line represents the simulation curve calculated according to the model shown in Chart 1 by using the recalculated parameters of the mean plasma concentration of APAP.

Table 3
Pharmacokinetic parameters of APAP after intravenous administration of APAP with and without continuous infusion of APAPS in rats

Parameters	Control ($n=3$)	APAPS ($n=4$)
k_{A12} (1/h)	9.71 ± 0.45	11.05 ± 0.29
k_{A21} (1/h)	5.70 ± 0.30	5.89 ± 0.42
k_{A10} (1/h)	0.33 ± 0.08	0.49 ± 0.13
K_m (μM)	70.1 ± 10.2	118.4 ± 18.2
V_{max} (μmol/min/mg)	4.8 ± 0.3	6.8 ± 0.3
V_{max}/K_m (ml/min/kg)	67.8 ± 7.7	57.0 ± 6.2
Vd_{A1} (ml/kg)	372.5 ± 6.4	457.1 ± 5.7*
Vd_{A2} (mi/kg)	643.5 ± 9.9	858.5 ± 32.0*
Vd_{ss} (ml/kg)	1016.0 ± 11.8	1315.6 ± 32.5*
$AUC_{0-\infty}$ (μg·h/ml)	20.5 ± 1.4	13.8 ± 0.8
CL_{total} (ml/min/kg)	24.4 ± 1.6	36.1 ± 2.0*

The dose of APAP was 30 mg/kg. Each value represents the mean ± S.E. of 3–4 rats.
Infusion rate of APAPS was 118 mg APAP eq/h/kg. Plasma concentration of APAPS was kept to about 200 μg APAP eq/ml. Saline was infused in control study.
*$P<0.05$, significantly different from saline infusion.

($\beta = 1.63-1.86$ h^{-1}) was not so altered by the continuous infusion of APAPS (Fig. 3). The CL_{total} and Vd_{ss} were significantly increased under the continuous infusion of APAPS. Accordingly, it could be considered that the increase of CL_{total} may contribute to the distribution volume of APAP.

These results indicate that the effect of APAPS on the plasma elimination of APAP is different between the coadministration by bolus and infusion. The reasons for these differences will be discussed later.

3.4. Effect of APAPS on the tissue distribution of APAP

Tissue-to-plasma concentration ratios (k_p) were estimated under the steady-state of APAPS according to the following equations;

$$k_p = C_t/C_p = (C_t \cdot V_t - C_p \cdot V_e)/V_i/C_p \quad (5)$$

$$V_e = V_t \cdot IS \quad (6)$$

$$V_t = V_i + V_e \quad (7)$$

where C_t and C_p are the concentrations of APAP in the tissue and in the plasma, respectively; V_t is the tissue volume; V_i and V_e are the intracellular volume and the extracellular volume, respectively. The specific gravities of all tissues were assumed as equal to 1. The k_p values of APAP corrected by inulin space (IS) are shown in Table 4, but the value for kidney was quoted from the reported value (Tsuji et al., 1983). Plasma concentration of APAP at 5 min after i.v. bolus coadministration with APAPS at 30 mg APAP eq/kg was decreased from 32.7 to 28.5 μg/ml, although the concentrations of APAP in various tissues were increased. The k_p values of APAP were increased in various tissues, i.e. liver, kidney and brain, while IS values in these tissues were not changed by the coadministration of APAPS. The increase in k_p value shows the increase in distribution volume of APAP. It was clarified that APAPS increased the distribution of APAP.

3.5. Serum protein binding of APAP in the presence of APAPS

As one of the factors controlling the distribution, serum protein binding of APAP was estimated under the continuous infusion of APAPS. The in vivo serum protein binding (%) of APAP at 5 min after i.v. administration of APAP was significantly decreased by the presence of APAPS from 51.2 ± 3.0 to 21.6 ± 2.2% ($p<0.01$), although that of APAPS was not significantly changed from 59.1 to 55.1% without and with APAPS infusion, respectively. Fig. 4 shows the effect of APAPS on the in vitro serum protein binding of APAP. Sandberg-Rosenthal plot was used to simply characterize serum protein binding of APAP. The dissociation constant (K_d) of APAP (233.2 μM) in the presence of APAPS was larger than that in the absence of APAPS (108.8 μM), while the apparent number of the binding site (n) was not changed; 0.830 and 0.832 in the absence and presence of APAPS, respectively. These results suggest that the displacement of APAP by its main metabolite, APAPS, is competitive and the concomitant displacement of APAPS is slight.

Protein binding of APAP was competitively displaced by APAPS in vivo and in vitro. The increase in unbound concentration will depend on

Table 4
Tissue-to-plasma concentration ratio (k_p) of APAP with and without continuous infusion of APAPS in rats

Tissue	Control		APAPS		Ratio of k_p APAPS/Control
	Concentration (μg/ml or g)	k_p	Concentration (μg/ml or g)	k_p	
Plasma	32.7 ± 0.5	—	28.5 ± 1.8	—	—
Lung	21.5 ± 2.0	0.55 ± 0.10	26.6 ± 0.4	0.92 ± 0.07**	1.67
Liver	13.6 ± 1.7	0.37 ± 0.11	19.5 ± 2.9	0.62 ± 0.08*	1.68
Kidney	32.2 ± 3.5	1.02 ± 0.14	42.5 ± 4.1	1.62 ± 0.15*	1.59
Heart	22.9 ± 0.6	0.67 ± 0.04	23.8 ± 2.1	0.85 ± 0.03	1.27
Spleen	17.7 ± 0.3	0.46 ± 0.02	21.3 ± 5.0	0.68 ± 0.15	1.48
Brain	9.8 ± 0.6	0.29 ± 0.02	12.6 ± 0.9	0.43 ± 0.01*	1.48

Each concentration represents the mean ± S.E. of three experiments at 5 min after intravenous administration of APAP (30 mg/kg) under steady-state concentration of APAPS (200 μg APAP eq/ml).
*$P<0.05$, significantly different from control (saline infusion).
**$P<0.01$, significantly different from control (saline infusion).

many factors including the rate of displacer input and the pharmacokinetic characteristics of the displaced drug. Rapid intravenous injection of the displacing drug will result in a higher maximum unbound concentration of displaced drug than a slow input of displacing drug (Oie and Levy, 1979).

Furthermore, the k_p values in various tissues including liver, target organ of metabolism, were significantly increased in the presence of high level of APAPS (Table 4). The hepatic extraction ratio (E_h) was reported to be in the wide range from 0.14 to 0.41 at lower doses, and the extrahepatic metabolism of APAP in systemic circulation may be minor and/or negligible in rats (Pang and Gillette, 1980; Belanger et al., 1987; Hirate et al., 1990). The E_h of APAP was calculated to be about 0.31 when APAP is metabolized in liver alone and the hepatic blood flow rate is 13.8 ml/min/0.25 kg rat (Davies and Morris, 1993). In general, the plasma profiles of these low hepatic extraction drugs may depend on both the unbound fraction in blood and the hepatic intrinsic clearance (Rowland and Tozer, 1995). The increase of unbound fraction of such drugs would cause the increase in CL_{total}, resulting in the lower plasma concentration. As shown in Table 3, CL_{total} of APAP was increased under steady state concentration of APAPS. However, plasma concentration of APAP was increased at bolus injection of APAPS (Fig. 1a). Such different plasma profiles of APAP between the coadmimstration by bolus injection and constant infusion of APAPS may be partly explained as follows. Unbound fraction of APAP just after bolus injection of APAPS is higher than APAPS infusion. However, plasma concentration of APAPS was time-dependently decreased after bolus injection, but was constant in the case of infusion. The distribution of APAP changes according to the APAPS concentration-dependent serum protein binding of APAP. An increase in the unbound fraction of

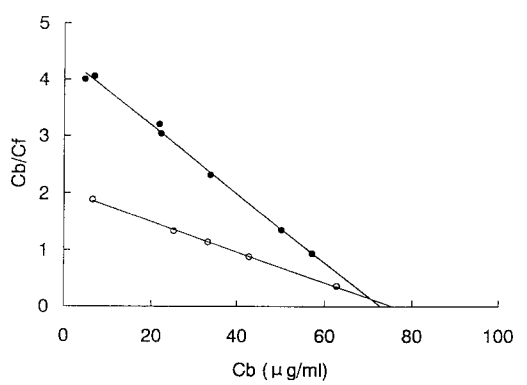

Fig. 4. Effect of APAPS on serum protein binding of APAP. APAPS was added to 10 μg APAP eq/ml in serum. Control (○); With APAPS (●). Cb is the concentration of bound drug obtained from substracting unbound (Cf) from total drug concentrations.

APAP will occur immediately after injection of APAPS, but it will gradually return to its control level. On the other hand, rapid increase in unbound fraction of APAP at bolus injection of APAPS may cause the saturation of conjugative metabolism. Furthermore, APAPS inhibits the metabolism of APAP, that is product inhibition (results shown below). The reduced metabolic clearance would be the reason for high plasma concentration of APAP after simultaneous bolus injection of APAPS.

Moreover, it should also be noted that displacement of protein binding will have no effect on the elimination half-lives of displaced drugs with moderate to large volumes of distribution (0.4 l/kg), but will shorten the half-life of drugs with small volumes of distribution which is nearly equal to the extracellular fluid volume (74.2 ml/0.25 kg rat) (MacKichan, 1989; Herve et al., 1994). Accordingly, the terminal elimination phase of APAP which had a Vd_{ss} of 819.8 ml/kg (Table 1), may not be directly affected by the changes in unbound APAP (Figs. 1 and 3). On the other hand, any increased unbound concentration in the plasma will also lead to increased drug elimination by glomerular filtration and metabolism (McElnay and D'Arcy, 1983; Grainger-Rousseau et al., 1989), however, renal clearance of APAP was low and was not apparently altered.

3.6. Effect of APAPS on conjugative metabolism of APAP

The effect of APAPS on APAP sulfation and glucuronidation was investigated using the cytosol and microsomal fractions of rat liver. In the presence of APAPS, APAP sulfation and glucuronidation were inhibited. The rate of APAP sulfation was decreased from 0.469 to 0.405 nmol/mg protein/15 min ($n = 2$) and also that of APAP glucuronidation was decreased from 0.333 to 0.263 nmol/mg protein/15 min ($n = 2$). The possibility of product inhibition on the conjugative metabolism of APAP by APAPS was speculated.

4. Conclusion

In the present study, it was clarified that the pharmacokinetics of APAP was affected by its major metabolite, APAPS. The available data suggested that the pharmacokinetic interactions of APAP and APAPS were mainly due to the displacement of serum protein binding of APAP by APAPS.

It was indicated that not only drug-drug interactions but drug/conjugated metabolite interactions are necessary to ensure the effectiveness and safety of drugs in multiple-drug therapy, while the protein binding of the parent drugs is not always displaced by their conjugated metabolites.

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