Dose-Dependent Pharmacokinetics and First-Pass Metabolism of Acetaminophen in Rats

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(Received October 23, 1989)

In order to investigate the *in vivo* first-pass metabolism of acetaminophen (AAP) following the oral and intraduodenal administration in rats, a pharmacokinetic compartment model including absorption process was developed. Using the parameters for the disposition kinetics of AAP and its metabolites, sulfate and glucuronide, which were determined in the separate study, the extent of the first-pass metabolism and the contribution of sulfation and glucuronidation to the total first-pass metabolism *in vivo* were quantitatively estimated. As for the results, the first-pass metabolism of AAP following the oral and intraduodenal administration was mainly attributable to the sulfo-conjugation pathway in rats. The sulfation of AAP in the intestine and/or in the liver during the first-pass was proved to be a saturable process. Then, the sulfation in the first-pass metabolism showed the dose- and absorption rate-dependent kinetics.

Thus, the pharmacokinetic model including the absorption process proposed in the present study was proved to be valid and useful for the estimation of *in vivo* first-pass metabolism of AAP in rats.

Keywords — acetaminophen; rat; pharmacokinetics; oral administration; intraduodenal administration; first-pass metabolism; sulfation; glucuronidation

Introduction

Acetaminophen (AAP) has been reported to show a nonlinear disposition kinetics following intravenous administration in rats.^{1,2)} The nonlinearity in the pharmacokinetics of AAP in rats is known to be caused by the saturation in the sulfo-conjugation pathway, and the entire nonlinear pharmacokinetics of AAP including its metabolites is also reported by Watari *et al.*²⁾ On the other hand, AAP is also known to undergo extensive first-pass metabolism in the intestinal gut wall and/or in the liver,^{3,4)} which decreases the bioavailability of AAP following the oral administration.^{5–7)} In general, the extent of first-pass metabolism can be determined by comparing the area under the concentration-time curve (*AUC*) of AAP after enteral administration with that after intravenous administration. However, the use of *AUC* method for the estimation of the bioavailability or the extent of first-pass metabolism of the drug showing a nonlinear disposition kinetics such as AAP may occasionally lead to invalid results. Also the contribution of two pathways of conjugation, sulfation and glu-

curonidation, to the total first-pass metabolism can not be quantitatively estimated by the *AUC* method.

In the present study, in order to estimate the extent of the *in vivo* first-pass metabolism of AAP and the contribution of the two conjugation pathways to the total first-pass metabolism following the oral and intraduodenal administration of AAP, a dose-dependent pharmacokinetic model including an absorption process in rats was developed.

Materials and Methods

Materials — Acetaminophen of reagent grade and *p*-nitrophenyl- β -D-glucuronide were obtained from Nacalai Tesque Inc. (Kyoto, Japan). *p*-Nitrophenyl sulfate potassium salt was purchased from Sigma Chemical Company (St. Louis, U.S.A.). β -Glucuronidase (4400000 UF/ml) and β -glucuronidase-arylsulfatase (100000 UF/ml, 800000 UR/ml) were purchased from Boehringer Mannheim (Mannheim, F.R.G.). Acetaminophen glucuronide (AAP-G) and acetaminophen sulfate (AAP-S)

which are major metabolites of AAP were synthesized. Isolation and synthesis of AAP-G and AAP-S were reported by many investigators.⁸⁻¹¹⁾ In the present study, AAP-G was synthesized from *p*-nitrophenyl- β -D-glucuronide by the method as follows: *p*-Nitrophenyl- β -D-glucuronide was reduced to *p*-aminophenyl- β -D-glucuronide by hydrogenation in an aqueous solution using 5% palladium on charcoal at room temperature under atmospheric pressure, followed by drying with the freeze dryer, Eyla FD-5 (Tokyo Rikakikai Co. Ltd., Tokyo, Japan). Then, the resultant *p*-aminophenyl- β -D-glucuronide (*ca.* 1 g) was dissolved in the mixture of 0.45 ml acetic anhydride and 45 ml 0.05 M K₂CO₃ solution, and stirred for 5 h at room temperature for acetylation. The reaction mixture was lyophilized, and resultant AAP-G was redissolved in hot water and recrystallized by dropwise addition of ethanol, which was isolated as a potassium salt. AAP-S was synthesized from *p*-nitrophenylsulfate by the same reduction method used for AAP-G synthesis from *p*-nitrophenyl- β -D-glucuronide. The compounds obtained were assigned by ¹H- and ¹³C-nuclear magnetic resonance (NMR) spectrum, and were used after confirming purity with a single peak on a high performance liquid chromatography (HPLC) column. All other chemicals were of reagent grade, and were used without further purification.

Animal Study — Male Wistar rats (220 — 280 g body weight) were fasted overnight but water was allowed *ad libitum*. The rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and were fixed supine on a surface kept at 37 °C to maintain normal body temperature during experiments. The left femoral vein was cannulated with polyethylene tubing (PE-50) for the collection of blood. Prior to the drug administration, the rat penis was ligated to prevent a possible leakage of the urine. In oral administration, AAP was dissolved in a 40 w/v% polyethylene glycol 300-saline solution to make a 100 mM. The solution was administered orally *via* stomach tubing at a volume of 0.5 ml/kg. Intraduodenal administration was performed *via* polyethylene tubing (PE-10), which was cannulated through the bile duct into the

duodenum. Before the intraduodenal administration, the intestine was tied at the pylorus and ileo-caecal valve. AAP solution (100 mM) was administered at a volume of 0.5 or 2 ml/kg. The intestinal loop was removed 3 h after the administration to measure the amount of AAP remaining unabsorbed in the intestine. For the intravenous administration, AAP was dissolved in the above solvent at a concentration of 66.2, 198.5, or 330.8 mM and each solution was administered intravenously at a dosing volume of 1 ml/kg *via* the polyethylene tubing (PE-50) which was cannulated into the right femoral artery. AAP-S or AAP-G was dissolved in the saline at a concentration of 66.2 mM and the solution was intravenously administered at a volume of 1 ml/kg. About 200 μ l of blood were collected in a heparinized-capillary at appropriated time intervals. After each collection of blood, an equivalent volume of saline was infused to supplement the loss of blood. Plasma was separated by centrifugation at 10000 rpm for 5 min with a micro-centrifuge. At 3 h after the drug administration, the urinary bladder was removed to collect the urine and AAP and its excreted metabolites were measured.

Analytical Method — The concentration of AAP in plasma was determined by HPLC. One ml of pH 5.0 acetate buffer solution was added to a 20 μ l of plasma. Then, AAP in the solution was extracted with 6 ml of ethylacetate by vigorously shaking for 15 min. After centrifugation, 5 ml of the organic phase was removed and the solvent was evaporated under reduced pressure. The residue was dissolved in 100 μ l of 50% methanol aqueous solution. Twenty μ l of the solution was injected on a HPLC column equipped with an injection valve unit (Toso Manufacturing Co., Ltd., Tokyo, Japan). A column of TSKgel ODS-80Tm (150 $\times$ 4.6 mm i.d.) (Toso Manufacturing Co., Ltd., Tokyo, Japan) was used. A mixture of methanol and 0.15% formic acid aqueous solution (pH 2.6) (3:7, v/v) was used as a mobile phase at a flow rate of 0.9 ml/min. AAP was detected at 254 nm with UV-8000 ultraviolet detector (Toso Manufacturing Co., Ltd., Tokyo, Japan).

The concentration of AAP-G was determined after hydrolyzing with β -glucuronidase. β -Glu-

curonidase was diluted 100 fold with pH 5.0 acetate buffer solution. One ml of the diluted enzyme solution was added to 20 μ l of plasma sample, and incubated at 39°C for 2 h to liberate AAP from a glucuronide form. Total AAP was extracted and determined in the same manner as described above. The concentration of AAP-G was calculated by subtracting the concentration of AAP before hydrolysis from that after hydrolysis with β -glucuronidase.

Concentration of AAP-S was determined by hydrolyzing with β -glucuronidase-arylsulfatase. β -Glucuronidase-arylsulfatase was diluted by 100 fold with pH 5.0 acetate buffer solution. One ml of the diluted enzyme solution was added to a 20 μ l of plasma sample to hydrolyze the sulfate to AAP. Total AAP was extracted and determined in the same manner as described above. The concentration of AAP-S was calculated as the difference between AAP concen-

tration obtained after hydrolysis by β -glucuronidase-arylsulfatase and that by β -glucuronidase. The concentration of AAP and its metabolites in the urine were determined by the same procedures as described above after an appropriate dilution with distilled water.

Data Analysis — A pharmacokinetic analysis was made by the use of MULTI or MULTIRUNGE, a nonlinear least squares analysis program for microcomputer developed by Yamaoka *et al.*¹²⁾ with minor modifications. The Student's *t*-test was utilized to estimate a significant difference with $p = 0.05$ as the minimal level of significance.

Results and Discussion

AAP was administered orally at a dose of 50 μ mol/kg, and into the duodenum at doses of 50 and 200 μ mol/kg to rats. After the administra-

Fig. 1. Plasma Concentration of AAP, AAP-S and AAP-G Following the Oral and Intraduodenal Administration of AAP (A) oral administration (50 μ mol/kg), (B) intraduodenal administration (50 μ mol/kg), (C) intraduodenal administration (200 μ mol/kg). Each point represents the mean $\pm$ S.E. ($n = 3$). Key: AAP ($\circ$), AAP-S ($\square$), AAP-G ($\triangle$).

TABLE I. The AUC Value and the Urinary Excretion of Acetaminophen Following the Intravenous, Oral, and Intraduodenal Administration

Dose ($\mu\text{mol/kg}$)	50			200	
Administration	i.v.	<i>p.o.</i>	i.d.	i.v.	i.d.
<i>AUC</i> ($\mu\text{mol}\cdot\text{min/ml}$)	1.375 $\pm$ 0.097	0.535 $\pm$ 0.116	1.048 $\pm$ 0.080	6.951 $\pm$ 0.851	6.794 $\pm$ 0.728
Urinary recovery (%) ^{a)}	83.5 $\pm$ 3.70	85.5 $\pm$ 5.6	85.6 $\pm$ 2.6	84.9 $\pm$ 2.1	76.8 $\pm$ 5.9
Percent in urine (%) ^{b)}					
AAP	0.51 $\pm$ 0.51	0.85 $\pm$ 0.04	0.69 $\pm$ 0.39	1.24 $\pm$ 0.77	0.75 $\pm$ 0.38
AAP-S	95.1 $\pm$ 4.8	93.8 $\pm$ 6.9	92.3 $\pm$ 4.8	92.8 $\pm$ 1.5	87.9 $\pm$ 2.46
AAP-G	4.47 $\pm$ 1.16	5.44 $\pm$ 0.98	7.05 $\pm$ 0.98	5.89 $\pm$ 0.54	11.5 $\pm$ 1.12

a) Ratio of total amount excreted including metabolites for 3 h after the administration to the dose. b) Ratio to the total amount excreted. Each value represents the mean $\pm$ S.E. of 3 rats.

tions, the plasma concentrations of AAP and its major metabolites, AAP-S and AAP-G, were determined periodically and are presented in Fig. 1 (A — C). The area under the plasma concentration–time curve (*AUC*), total urinary excretion including its metabolites and the ratios of AAP and its metabolites excreted in the urine to the total excretion were listed in Table I. The *AUC* values of AAP following the oral and intraduodenal administration at a dose of 50 $\mu\text{mol/kg}$ were much lower than that after intravenous administration at the same dose of AAP. The *AUC* value following the intraduodenal administration at the lower dose (50 $\mu\text{mol/kg}$) was greater than that after oral administration, whereas the *AUC* value after intraduodenal administration at a higher dose (200 $\mu\text{mol/kg}$) was almost the same as that after in-

travenous administration at the same dose. On the other hand, AAP remained in the gastrointestinal tract was not detected 3 h after the oral and intraduodenal administration, and the ratios of total urinary excretion of AAP including its metabolites to the dose were the same extent to that after intravenous administration. Then, the absorption of AAP from the gastrointestinal tract was considered to be almost complete. Therefore, the smaller *AUC* values found after oral or intraduodenal administration at the lower dose indicate that AAP must be metabolized during the first-pass through the liver and intestinal gut wall before reaching the systemic circulation.

In order to evaluate the first-pass metabolism of AAP after the oral and intraduodenal administration, the nonlinear pharmacokinetic model including an absorption process as shown in Fig. 2 was proposed.

In the model, AAP was assumed to be absorbed from the intestine (compartment 7) with a first-order absorption rate constant, k_a . Thereafter, the absorbed AAP was conjugated in the intestinal gut wall and/or in the liver before reaching the systemic circulation at the ratios of M_g and M_s , for AAP-G and AAP-S, respectively. Then, AAP which escaped from first-pass metabolism after absorption reached the systemic circulation at a ratio of F . Since the absorption of AAP from the intestine in the present study is considered to be complete as mentioned above, the sum of F , M_g and M_s corresponds to unity, then, F represents the extent of bioavail-

Fig. 2. Pharmacokinetic Model of AAP and Its Metabolites Including Absorption Process in Rats

ability. The model for the disposition of AAP and its metabolites after reaching the systemic circulation are almost the same as that reported by Watari *et al.*,²⁾ in which the compartment 1 to 3 are the central compartments for AAP, AAP-S, and AAP-G, respectively, and compartment 4 to 6 are the peripheral compartments for AAP, AAP-S, and AAP-G, respectively. The rate constant of the conjugation to AAP-G, k_{13} , was assumed to be first-order. Since the process for the conjugation of AAP to AAP-S was considered to be capacity-limited, Michaelis-Menten constants, K_m and V_{max} were applied to this pathway. The elimination of AAP *via* urinary excretion was excluded from the model, because the excretion of unmetabolized AAP into the urine was negligible in the present study. The differential equations employed for the simulation were as follows:

$$\frac{dX_7}{dt} = -k_a \cdot X_7$$

$$\begin{aligned} \frac{dX_1}{dt} = & k_a \cdot F \cdot X_7 + k_{41} \cdot X_4 \\ & - \left(\frac{V_{max}}{K_m + X_1} + k_{13} + k_{14} \right) \cdot X_1 \end{aligned}$$

$$\begin{aligned} \frac{dX_2}{dt} = & k_a \cdot M_s \cdot X_7 + \frac{V_{max}}{K_m + X_1} X_1 + k_{52} \cdot X_5 \\ & - (k_{20} + k_{25}) \cdot X_2 \end{aligned}$$

$$\begin{aligned} \frac{dX_3}{dt} = & k_a \cdot M_g \cdot X_7 + k_{13} \cdot X_1 + k_{63} \cdot X_6 \\ & - (k_{30} + k_{36}) \cdot X_3 \end{aligned}$$

$$C_1 = X_1/V_1, \quad C_2 = X_2/V_2, \quad C_3 = X_3/V_3$$

$$F + M_s + M_g = 1$$

Where, X , C and V represent the amount, concentration of drug and volume of distribution in each compartment described by the number of subscript, respectively. In order to confirm the validity of the model and to determine the

parameters for the disposition of AAP and its metabolites after reaching the systemic circulation, AAP and its metabolites were administered intravenously.

To simulate the plasma concentration of the metabolites after dosing of AAP, the parameters for the disposition kinetics of the metabolites (k_{20} , k_{25} , k_{52} , V_2 , k_{30} , k_{36} , k_{63} , V_3) should be determined separately by administering the individual metabolite. Each metabolite of AAP has been reported to demonstrate linear disposition kinetics in rat,²⁾ then, pharmacokinetic parameters of each metabolite were estimated following the intravenous administration at one dose of 66.2 $\mu\text{mol/kg}$. As shown in Fig. 3, disposition of each metabolite in rats could be described by a two-compartment model and the estimated parameters are summarized in Table II.

Since AAP was reported to show the dose-dependent disposition kinetics, AAP was administered intravenously at three doses of 66.2, 198.5, and 330.8 $\mu\text{mol/kg}$ in rats for determination of the parameters such as K_m , V_{max} , k_{13} , k_{14} , k_{41} , and V_1 . The plasma concentration of AAP, AAP-S and AAP-G following the intravenous administration of AAP are shown in Fig. 4. The pharmacokinetic parameters of AAP at each dose were calculated on a basis of a two-compartment model and summarized in Table

Fig. 3. Plasma Concentration of AAP-G ($\blacktriangle$) and AAP-S ($\blacksquare$) Following Intravenous Administration of AAP-G and AAP-S

Dose: 66.2 $\mu\text{mol/kg}$, respectively. Each point represents the mean $\pm$ S.E. ($n = 3$).

TABLE II. Pharmacokinetic Parameters of Metabolites of Acetaminophen Following Intravenous Administration of Metabolite

Conjugate	AAP-S		AAP-G	
k_{25}	0.020 ± 0.007	k_{36}	0.107 ± 0.022	(min ⁻¹)
k_{52}	0.070 ± 0.015	k_{63}	0.135 ± 0.025	(min ⁻¹)
k_{20}	0.053 ± 0.004	k_{30}	0.053 ± 0.005	(min ⁻¹)
V_2	205.7 ± 12.0	V_3	176.6 ± 16.0	(ml/kg)
AUC	6.127 ± 0.210	AUC	7.405 ± 0.625	(μmol · min/ml)
$Cl^a)$	10.8 ± 0.4	$Cl^a)$	8.3 ± 1.1	(ml/min/kg)

a) $Cl = \text{dose}/AUC$, dose: 66.2 μmol/kg. Each value represents the mean ± S.E. of 4 rats.

TABLE III. Pharmacokinetic Parameters of Acetaminophen Following Intravenous Administration

Dose (μmol/kg)	66.2	198.5	330.8
k_{12} (min ⁻¹)	0.069 ± 0.022	0.072 ± 0.017	0.041 ± 0.001
k_{21} (min ⁻¹)	0.085 ± 0.009	0.149 ± 0.038	0.122 ± 0.024
k_{10} (min ⁻¹)	0.083 ± 0.013	0.063 ± 0.005	0.044 ± 0.001 ^{b,c)}
V_1 (ml/kg)	476.4 ± 78.5	461.9 ± 34.7	492.0 ± 13.2
AUC (μmol · min/ml)	1.774 ± 0.094	6.951 ± 0.851	15.427 ± 0.671
$Cl^a)$ (ml/min/kg)	37.5 ± 2.0	29.5 ± 4.1	21.5 ± 0.9 ^{b)}

a) $Cl = \text{dose}/AUC$. b) $p < 0.05$ (vs. 66.2 μmol/kg). c) $p < 0.05$ (vs. 198.5 μmol/kg). Each value represents the mean ± S.E. of 3 rats.

III. The total clearance of AAP declined gradually as the dose of AAP increased. AAP has been reported to be eliminated from plasma mainly

by biotransformation in the liver and/or in the other tissues.^{3,4,13,14)} The present results also represent the decrease in the elimination rate constant, k_{10} , of AAP from plasma at a higher dose (330.8 μmol/kg) compared to that at a lower dose (66.2 μmol/kg), while there was no change in the volume of the central compartment, V_1 . To describe the entire dose-dependent disposition of AAP and its metabolites following the intravenous administration of AAP at three different doses, a simultaneous fitting analysis was performed on the time profiles of the plasma concentration of AAP, AAP-S, and

Fig. 4. Plasma Concentration of AAP and Its Metabolites Following the Intravenous Administration of AAP

Dose of AAP: 66.2 μmol/kg (○, AAP; □, AAP-S; △, AAP-G), 198.5 μmol/kg (●, AAP; ■, AAP-S; ▲, AAP-G), 330.8 μmol/kg (●, AAP; ■, AAP-S; ▲, AAP-G). Each point represents the mean ± S.E. ($n = 3$).

TABLE IV. Pharmacokinetic Parameters of Acetaminophen Estimated by Simultaneous Simulation Following Intravenous Administration at Various Doses of Acetaminophen

$V_{\max}$	6.189	(μmol · min/kg)
k_m	56.673	(μmol · /kg)
k_{13}	0.004	(min ⁻¹)
k_{14}	0.075	(min ⁻¹)
k_{41}	0.160	(min ⁻¹)
V_1	505.8	(ml/kg)

AAP-G.

The parameters for the distribution and excretion of both metabolites, AAP-S and AAP-G, were held constant as listed in Table II. The parameters for AAP such as K_m , V_{max} , k_{13} , k_{14} , k_{41} , and V_1 were estimated by a nonlinear least squares analysis with Runge-Kutta-Gill method by using a microcomputer program MULTI (RUNGE)¹²⁾ according to the model in Fig. 2 excluding absorption process. The estimated parameters are listed in Table IV. The best-fitted simulation curves are shown as solid lines in Fig. 4, which indicate that the pharmacokinetic model describes well the entire dose-dependent disposition of AAP and its metabolites in rats in the present study, as well as reported previously by Watari *et al.*²⁾ The values of the Michaelis-Menten constants, K_m and V_{max} , for AAP-S formation obtained in this study by the simultaneous fitting are in close agreement with those obtained by Watari *et al.*²⁾ and, Lin and Levy.¹⁴⁾ Thus, AAP-S formation in the present study is considered to be under a saturated stage.

Using the parameters for the disposition of AAP and its metabolites, the extent of first-pass metabolism following the oral or intraduodenal administration of AAP and the contribution of sulfation and glucuronidation to the first-pass metabolism were estimated according to the proposed pharmacokinetic model shown in Fig. 2. Since the absorption of AAP from the gastrointestinal tract are considered to be complete in the present study as mentioned already, the sum of F , M_g and M_s corresponds to unity, and the ratio

escaped from first-pass metabolism after absorption, F , represents the extent of bioavailability. The parameters for the disposition of AAP and its metabolites were held constant at the values listed in Table II and IV, then the parameters during the absorption process such as k_a , M_g , M_s , and F were estimated by nonlinear regression analysis and summarized in Table V. The solid lines in Fig. 1 represent the estimated regression lines, indicating good fittings to the observed values. In Table V, the bioavailability that calculated by the *AUC* method, BA (%), comparing the *AUC* to that after the intravenous administration at the same dose were also listed. The estimated bioavailability, F , after the intraduodenal administration at a higher dose (200 $\mu\text{mol/kg}$) was slightly different from the BA (%) value. Since AAP shows a nonlinear disposition kinetics at a higher dose, then, the bioavailability calculated by the *AUC* method might be unreliable. On the other hand, the F values following the oral and intraduodenal administration of AAP at a lower dose (50 $\mu\text{mol/kg}$) were quite close to the bioavailability, BA (%), calculated by the *AUC* method, since the disposition kinetic at the dose is considered to be linear. Consequently, the pharmacokinetic model including an absorption process proposed in Fig. 2 is considered to be useful to estimate the first-pass metabolism of AAP.

Following the oral and intraduodenal administration at a lower dose (50 $\mu\text{mol/kg}$), the fraction of first-pass metabolism by sulfation, M_s , was greater than that by glucuronidation, M_g (Table V). These findings indicate that the sulfo-

TABLE V. Pharmacokinetic Parameters of First-Pass Metabolism of Acetaminophen Following Oral and Intraduodenal Administration

Dose ($\mu\text{mol/kg}$) Administration	50		200
	<i>p.o.</i>	<i>i.d.</i>	<i>i.d.</i>
k_a (min^{-1})	0.038 ± 0.005	$0.365 \pm 0.055^a)$	$0.172 \pm 0.031^a)$
M_s (min^{-1})	0.528 ± 0.096	$0.191 \pm 0.043^a)$	$0.058 \pm 0.050^a)$
M_g (min^{-1})	$0.064 \pm 0.029^b)$	$0.040 \pm 0.018^b)$	0.046 ± 0.007
F	0.408 ± 0.104	$0.769 \pm 0.031^a)$	$0.896 \pm 0.040^a)$
BA (%) ^{c)}	38.9 ± 8.4	76.2 ± 5.8	96.7 ± 10.3

a) $p < 0.05$ (vs. *p.o.*). b) $p < 0.05$ (vs. M_s). c) Bioavailability calculated by the *AUC* method. Each value represents the mean $\pm$ S.E. of 3 rats.

conjugation was mostly responsible for the first-pass metabolism of AAP during the absorption from the intestinal tract in rats as well as the metabolism after the intravenous injection. Furthermore, the extent of total metabolism and the metabolism by sulfation during first-pass were quite different between the oral and intraduodenal administration, with little changes in the M_g value. AAP has exhibited a dose-dependent disposition after the intravenous administration because of a saturation in the pathway of sulfo-conjugation. The sulfation of AAP is known to be a capacity-limited process with a depletion of endogenous sulfate (3'-phosphoadenosine 5'-phosphosulfate, PAPS).^{15,16} Following the intraduodenal administration, the extent of sulfation, M_s , was decreased at a higher dose (200 $\mu\text{mol/kg}$), compared to a lower dose (50 $\mu\text{mol/kg}$), while no change in the extent of glucuronidation, M_g , between the two doses was observed. These results indicate that sulfation of AAP in the intestine and/or in the liver during first-pass was also saturable process as well as after intravenous administration.^{1,2)}

On the other hand, the degree of the first-pass metabolism of a drug with high hepatic clearance has been reported to be reduced by rapid absorption, because a rapid absorption resulted in a high concentration temporarily in the portal vein.^{17,18)} As shown in Table I, the absorption rate constant, k_a , after the intraduodenal administration was greater than that after oral administration. Heading *et al.*¹⁹⁾ demonstrated the importance of the rate of gastric emptying in the rate of AAP absorption. As intraduodenal administration is independent from the gastric emptying rate unlike that in the oral administration, then, AAP is considered to be absorbed rapidly following the intraduodenal administration. The fraction of first-pass sulfation following the intraduodenal administration was significantly decreased, compared to that after oral administration at the same dose (50 $\mu\text{mol/kg}$). These results suggest that the rapid absorption might cause a transient saturable kinetic of AAP-S formation through that first-pass. Thus, it is considered that the bioavailability of AAP following the oral administration is affected by

gastric emptying rate and the dose of administration.

As a conclusion, the first-pass metabolism following the oral and intraduodenal administration of AAP was analyzed pharmacokinetically by using the proposed pharmacokinetic model. The contribution of sulfation and glucuronidation to the total first-pass metabolism *in vivo* were quantitatively determined. From the results, the first-pass metabolism of AAP following the oral and intraduodenal administration was mainly attributable to the sulfo-conjugation pathway in rats. The sulfation of AAP in the intestine and/or in the liver during the first-pass was proved to be saturable process. Then, the sulfation in the first-pass metabolism showed the dose- and absorption rate-dependent kinetics.

Thus, the pharmacokinetic model including the absorption process proposed in the present study was proved to be valid and useful for the estimation of *in vivo* first-pass metabolism of AAP in rats.

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