Acyl Glucuronidation and Glucosidation of Pranoprofen, a 2-Arylpropionic Acid Derivative, in Mouse Liver and Kidney Homogenates

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The formation of acyl glucuronide and glucoside of 2-(5*H*-[1]benzopyrano[2,3-*b*]pyridin-7-yl)propionic acid (pranoprofen), an anti-inflammatory drug, was studied in homogenates and microsomes of mouse tissues by using *S*(+)-, *R*(-)- and *RS*(±)-pranoprofen.

Acyl glucuronidation occurred mainly in the liver, whereas acyl glucosidation was predominant in the kidney. Furthermore, both conjugations occurred by enzymatic transfer of glucuronic acid of uridine diphosphate glucuronic acid (UDPGA) and glucose of uridine diphosphate glucose (UDPG) to pranoprofen, respectively. No conjugation reactions were observed in the lung, plasma or gut. The amount of conjugates in the liver and kidney increased by the prolongation of the incubation times and reached the maximum at 15—30 min for glucuronidation and 45 min for glucosidation. After that, both conjugates decreased with the lapse of time. Both acyl conjugates were the least stable in the liver, then in the kidney and the most stable in the plasma.

In the liver, acyl glucoside was converted to acyl glucuronide and pranoprofen in the presence of UDPGA, but only a small amount of acyl glucuronide was changed to acyl glucoside in the kidney in spite of the presence of UDPG.

In the kidney, acyl glucoside decreased relative to acyl glucuronide at increasing doses for both *S*(+)- and *R*(-)-pranoprofen, but the concentration of acyl glucoside was much higher for *S*(+)-pranoprofen than *R*(-)-enantiomer 1 h after the oral administration of *S*(+)- and *R*(-)-pranoprofen. No acyl glucoside was detected in the liver and plasma. Although only a small difference in acyl glucuronidation in the liver was observed between *S*(+)- and *R*(-)-enantiomers, acyl glucosidation in the kidney occurred more predominantly for *S*(+)-pranoprofen than for the *R*(-)-enantiomer.

Keywords — conjugation; glucuronidation; glucosidation; stereoselectivity; pranoprofen; *in vitro*; mouse tissue homogenate

Introduction

Although conjugation with glucuronic acid is a common metabolic pathway of many xenobiotics in mammals, more recently, glucoside formation of xenobiotics in mammals has been demonstrated for 3-(4-pyrimidinyl)-5-(4-pyridyl)-1,2,4-triazole,¹⁾ phenobarbital,²⁾ *p*-nitrophenol³⁾ and pantothenic acid.^{4,5)}

Previously, we demonstrated the first piece of evidence for the formation of acyl glucoside of pranoprofen in mice.^{6,7)} Furthermore, at low doses glucosidation was a major metabolic pathway, while at high doses glucuronidation was an important pathway for pranoprofen in mice, and glucosidation might occur stereoselectively, showing that it was more predominant for *S*(+)-isomer than for *R*(-)-antipode.^{8,9)}

Koster *et al.*¹⁰⁾ and Fry¹¹⁾ reported that a dose-dependent shift in sulfation and glucuroni-

dation of phenolic compounds was observed in mammals, and the limitation of sulfation of hamol and phenol relative to glucuronidation at increasing doses was caused by saturation of the overall sulfation process by the acceptor substrate. Furthermore, an alternative explanation for a dose-dependent shift in these conjugations is based on the observation that *in vitro* K_m values of many phenols are smaller for phenol-sulfotransferase than for uridine diphosphate (UDP)-glucuronyltransferase.

The present study describes in detail acyl glucuronidation and glucosidation of *S*(+), *R*(-) and *RS*(±)-pranoprofen in whole homogenates of mouse livers and kidneys and their microsomes to explain both the mechanism of a dose-dependent shift in acyl glucuronidation and glucosidation of pranoprofen, and stereoselective conjugations.

Materials and Methods

Materials — Racemic pranoprofen, *S*(+)-pranoprofen ($[\alpha]_D^{20} = +45.9^\circ$), *R*(-)-pranoprofen ($[\alpha]_D^{20} = -44.6^\circ$) were synthesized and supplied by the Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd. An internal standard, 2-[4-(2-pyridyl)phenyl] propionic acid, which was synthesized in the same laboratories mentioned above was used for the quantitative determination. Both acyl glucuronide and glucoside which we had isolated and purified were used.⁹⁾ All other chemicals used were the purest grade available.

Animals — Male ddY mice weighing 20–30 g supplied by the Kyushu Laboratory Animal Center (Fukuoka, Japan) were used. The animals had free access to food and water.

Pranoprofen was suspended in 0.5% methylcellulose, and orally administered directly by intubation at a single dose of 25, 100 or 200 mg/kg.

Preparation of Whole Homogenates and Microsomes — The animals were sacrificed by exsanguination and 25% (w/v) tissue homogenates were prepared in ice-cold 0.25 M sucrose in 100 mM Tris-HCl buffer pH 7.2, with a Teflon-glass homogenizer.

The liver and kidney microsomal fractions were collected by differential centrifugation ($9000 \times g$ for 10 min, $105000 \times g$ for 60 min) in a Hitachi (85P-72 type) ultracentrifuge. The microsomal pellets obtained were resuspended in 0.25 M sucrose in 100 mM Tris-HCl buffer pH 7.2. All procedures for the whole homogenate preparation and the isolation of microsomal fractions were done at 0–4 °C. Microsomal protein was determined according to the method of Lowry *et al.*¹²⁾ Since enzyme activities for conjugations were unstable, whole homogenates of tissues were used immediately after preparation except for the liver and kidney microsomes. The latter were used for the determination of apparent kinetic constant for conjugations.

High Performance Liquid Chromatographic (HPLC) Analysis — A Shimadzu LC-3A HPLC equipped with a fluorescence detector (Shimadzu RF-530, excitation wave length; 300 nm, emission wave length; 370 nm) was used to measure pranoprofen, and its acyl glucuronide and

glucoside.

Chromatography was carried out on a TSK-GEL ODS 80TM column (Tosoh, Tokyo, Japan, 4.6×150 mm) connected to a TSK-GEL ODS 120T column (Tosoh, Tokyo, Japan, 4.6×150 mm). The mobile phase contained 100 mM ammonium acetate pH 5.0–acetonitrile (3:1, v/v). The flow rate was 1 ml/min.

The calibration curves for pranoprofen and its acyl glucuronide and glucoside were obtained from the peak area by the injection of pranoprofen (125 μ g/ml in ethanol), its acyl glucuronide (64 μ g/ml in 100 mM acetate buffer pH 5.0), and glucoside (44.6 μ g/ml in 100 mM acetate buffer pH 5.0) using the internal standard.

For the recovery test, several fixed amounts of pranoprofen, its acyl glucuronide, and glucoside were added to each 0.5 ml of tissue whole homogenates. Then 0.5 ml of 0.4 N perchloric acid with 10 μ l of internal standard (1 mg/ml) was added and each mixed with a vortex mixer. After the centrifugation of the homogenates, 4 μ l of the supernatant was injected into HPLC. Pranoprofen, its acyl glucuronide and glucoside were determined by a calibration curve from the peak area ratio (sample/internal standard), and each compound was quantitatively recovered from the tissue homogenates.

Enzyme Assay — The standard incubation medium contained 150 μ l of tissue homogenates, 100 mM Tris-HCl buffer pH 7.2, 5 mM $MgCl_2$, 2 mM UDPGA, 2 mM UDPG and 0.2 mM pranoprofen in a total volume of 500 μ l. The standard incubation was carried out for 30 min at 37 °C and terminated by adding 0.5 ml of 0.4 N perchloric acid. After the centrifugation of the incubation mixtures, 2–10 μ l of the supernatant was injected directly into HPLC.

Conjugations in Tissue Homogenates — A capacity for acyl glucuronide and glucoside formation was assayed in the liver, kidney, lung and gut homogenates, and in the 4-times-diluted plasma in the UDPGA and UDPG generating system by the use of *RS*($\pm$)-pranoprofen as substrate.

Effect of Co-factors on Conjugation Reactions — To elucidate the effect of the Co-factors on conjugation reactions, acyl glucuroni-

dation in the liver and acyl glucosidation in the kidney were investigated in the presence or absence of UDPGA, UDPG and MgCl_2 and also studied using inactivated enzymes obtained from adding 0.4 N perchloric acid to the incubation mixture prior to the incubation.

Effect of Incubation Time on Conjugation Reactions — To elucidate the effect of incubation time on conjugation reactions of pranoprofen in the liver and kidney homogenates, incubations were carried out for 5, 15, 30, 45 min, 1, 1.5 and 2 h at 37 °C, respectively.

Stability of Acyl Glucuronide and Glucoside in the Tissues — In order to clarify the stability of conjugates in the tissues, a known quantity of acyl glucuronide and glucoside was added to 0.5 ml of the liver and kidney homogenates and to the diluted plasma (diluted 4 times with 100 mM Tris-HCl buffer pH 7.2), and incubated at 37 °C for the indicated intervals.

Re-conjugations of Acyl Glucuronide and Glucoside in the Kidney and Liver Homogenates — 0.1 mM of acyl glucuronide and glucoside, which were isolated from 24 h urine samples of mice after the administration of pranoprofen by the method reported previously,⁹⁾ were incubated in the kidney and liver homogenates containing UDPGA and UDPG.

Concentration of Pranoprofen, and Its Acyl Glucuronide and Glucoside in the Tissues —

One hour after the oral administration of the test drug at doses of 25, 100 and 200 mg/kg, the blood was collected from the tail vein, the mice were killed by exsanguination, and the liver and kidney were excised. Three volumes of 100 mM

Tris-HCl buffer solution (pH 7.2) were added to the tissues and the tissue homogenates were prepared in ice-cold vessels as described above. Then 0.5 ml of 0.4 N perchloric acid and 10 μl of the internal standard (0.1 mg/ml) were added to 0.5 ml of the homogenates, and then pranoprofen, its acyl glucuronide and glucoside were determined by HPLC.

Stereoselective Glucuronidation and Glucosidation — Acyl glucuronidation and glucosidation in the liver and kidney homogenates were determined by the use of 0.2 mM *S*(+)-, *R*(-)- and *RS*($\pm$)-pranoprofen as substrates.

Determination of Apparent Kinetic Constants for Glucuronidation and Glucosidation — The substrate concentrations used were 0.05, 0.07, 0.1, 0.2, 0.5, 1 and 2 mM of *S*(+)- and *R*(-)-pranoprofen. After the incubation of the substrate at 37 °C for 30 min in the liver and kidney microsomes (0.5–1 mg of protein), the generated acyl glucuronide and glucoside were determined by HPLC analysis. The K_m and $V_{\max}$ values for the formation of acyl glucuronide and glucoside by the liver and kidney microsomes were determined by extrapolation of the linear portion of the Lineweaver-Burk plots.

Results

Conjugations in the Tissue Homogenates

In the liver, the activity of glucuronidation was 80 times that of glucosidation, but the former was only one sixth that of the latter in the kidney. Neither of the conjugation reactions

TABLE I. Acyl Glucuronidation and Glucosidation of *RS*($\pm$)-Pranoprofen in Mouse Liver, Kidney, Lung, Gut Homogenates, and Plasma

	Rate of formation (nmol/min/g of tissue)	
	Acyl glucuronide formed	Acyl glucoside formed
Liver	28.8 $\pm$ 0.08	0.36 $\pm$ 0.25
Kidney	2.1 $\pm$ 0.24	12.10 $\pm$ 0.24
Lung	N.D.	N.D.
Gut	N.D.	N.D.
Plasma	N.D.	N.D.

N.D., not detected. Incubation mixture contains 150 μl of tissue whole homogenates, 100 mM Tris-HCl buffer pH 7.2, 5 mM MgCl_2 , 2 mM UDPGA, 2 mM UDPG, and 0.2 mM *RS*($\pm$)-pranoprofen in a total volume of 500 μl . The incubation was carried out for 30 min at 37 °C and terminated by adding 0.5 ml of 0.4 N perchloric acid.

TABLE II. Acyl Glucuronidation and Glucosidation of *RS*(±)-Pranoprofen in Mouse Liver and Kidney Homogenates

	Rate of formation (nmol/min/g tissue)			
	Liver		Kidney	
	Acyl glucuronide formed	Acyl glucoside formed	Acyl glucuronide formed	Acyl glucoside formed
Complete	15.7 ± 0.8	N.D.	0.3 ± 0.1	8.5 ± 0.0
— MgCl ₂	11.9 ± 0.8	N.D.	0.4 ± 0.1	7.1 ± 0.1
— UDPG, UDPGA	N.D.	N.D.	N.D.	N.D.
Inactivated enzyme	N.D.	N.D.	N.D.	N.D.
— MgCl ₂ , UDPG, UDPGA	N.D.	N.D.	N.D.	N.D.

The complete incubation mixture contains 150 μ l of tissue whole homogenates, 100 mM Tris-HCl buffer pH 7.2, 5 mM MgCl₂, 2 mM UDPGA, 2 mM UDPG, and 0.2 mM *RS*(±)-pranoprofen in a total volume of 500 μ l. The incubation was carried out for 30 min at 37 °C and terminated by adding 0.5 ml of 0.4 N perchloric acid.

occurred in the lung, plasma and gut (Table I). Effect of Co-factors on Conjugation Reactions in the Liver and Kidney Homogenates

The complete assay system containing MgCl₂, UDPGA and UDPG showed the highest conjugation activity toward *RS*(±)-pranoprofen, and the activity was reduced to 76% for glucuronidation, and to 84% for glucosidation in the absence of MgCl₂. Neither pranoprofen acyl glucuronide or glucoside were

formed without UDPGA and UDPG or by the inactivation of enzymes (Table II).

Effect of Incubation Time on the Conjugation Reactions

The amount of acyl glucuronide in the liver and of acyl glucoside in the kidney increased by the prolongation of the incubation, and reached the maximum at 15–30 min for glucuronide and 45 min for glucoside. After that, glucuronide and glucoside decreased with the lapse of time. Disappearance rates of conjugates were faster in the liver than in the kidney (Fig. 1).

Fig. 1. Effect of Incubation Time on Pranoprofen Acyl Glucuronide and Glucoside Formation in Mouse Liver and Kidney Homogenates

●, glucuronidation in the liver; ▲, glucosidation in the kidney. Incubation mixture contains 150 μ l of tissue whole homogenates, 100 mM Tris-HCl buffer pH 7.2, 5 mM MgCl₂, 2 mM UDPGA, 2 mM UDPG, and 0.2 mM *RS*(±)-pranoprofen in a total volume of 500 μ l. The incubation was carried out at 37 °C and terminated by adding 0.5 ml of 0.4 N perchloric acid.

Fig. 2. Stability of Pranoprofen Acyl Glucuronide and Glucoside in Mouse Liver, Kidney Homogenates and Plasma

▲, glucuronide in liver; △, glucoside in liver; ●, glucuronide in kidney; □, glucoside in kidney; ●, glucuronide in plasma; ○, glucoside in plasma.

TABLE III. Formation of Acyl Glucuronide and Glucoside, and Pranoprofen from *S*(+)- and *R*(-)-Acyl Glucuronide and Glucoside in Mouse Liver and Kidney Homogenates

Tissue	Substrate	Co-factor (UDPG, UDPGA)	Composition (%)		
			Acyl glucuronide	Acyl glucoside	Pranoprofen
Kidney	<i>S</i> (+)-Acyl glucuronide	+	90.7	2.8	6.5
		-	87.0	0	13.0
	<i>R</i> (-)-Acyl glucuronide	+	79.0	0.7	20.3
		-	73.0	0	27.0
Liver	<i>S</i> (+)-Acyl glucoside	+	38.7	0.5	60.8
		-	5.7	0	94.3
	<i>R</i> (-)-Acyl glucoside	+	37.9	3.7	58.4
		-	5.0	1.7	93.3

Incubation mixture contains 150 μ l of tissue whole homogenates, 100 mM Tris-HCl buffer pH 7.2, 5 mM MgCl₂, 2 mM UDPGA, 2 mM UDPG, and 0.2 mM *S*(+) and *R*(-) acyl glucuronide and glucoside in a total volume of 500 μ l. The incubation was carried out for 30 min at 37 °C and terminated by adding 0.5 ml of 0.4 N perchloric acid.

Stability of Acyl Glucuronide and Glucoside in the Tissues

Both acyl conjugates were unstable in the liver, kidney and plasma. The disappearance rate was the greatest in the liver, slower in the kidney, and the slowest in the plasma. In all tissues, the disappearance of acyl glucoside was a little faster than that of acyl glucuronide (Fig. 2).

Re-conjugations of Acyl Glucuronide and Glucoside in the Kidney and Liver Homogenates

Re-conjugation reactions of *S*(+)-, *R*(-)-glucuronide and glucoside which were obtained from mouse urine after the oral administration

of *S*(+)- and *R*(-)-pranoprofen were investigated and the result was shown in Table III.

In the liver, both *S*(+)- and *R*(-)-glucoside were converted to acyl glucuronide and pranoprofen in the presence of UDPGA and UDPG; however they were partly hydrolyzed to form pranoprofen in the absence of UDPGA and UDPG. On the other hand, most of the *S*(+)- and *R*(-)-glucuronide did not change to acyl glucoside but a little changed to pranoprofen in the kidney both in the presence and absence of UDPGA and UDPG.

Concentration of Pranoprofen, Its Acyl

TABLE IV. Tissue Concentration of Pranoprofen (PNPF), and Its Acyl Glucuronide (GN) and Glucoside (GS) in Mice 1 h after Oral Administration of *S*(+)- and *R*(-)-Pranoprofen

Dose (mg/kg)	Tissue	Tissue concentration (μ g/g or ml)					
		<i>S</i> (+)-PNPF			<i>R</i> (-)-PNPF		
		GN	GS	PNPF	GN	GS	PNPF
25	Kidney	N.D.	2.7	16.3	N.D.	N.D.	18.5
	Liver	0.9	N.D.	19.7	0.5	N.D.	16.4
	Plasma	2.7	N.D.	50.0	3.3	N.D.	52.6
100	Kidney	15.9	21.5	35.0	13.8	2.5	35.3
	Liver	41.3	N.D.	46.1	88.6	N.D.	31.3
	Plasma	26.5	N.D.	180.6	12.2	N.D.	117.4
200	Kidney	60.8	30.9	83.8	60.6	6.4	50.2
	Liver	127.8	N.D.	124.6	206.0	N.D.	47.0
	Plasma	88.9	N.D.	303.0	39.2	N.D.	186.0

n = 3, pooled sample.

TABLE V. Stereoselective Acyl Glucuronidation and Glucosidation of *R*(-)-, *S*(+)- and *RS*(±)-Pranoprofen in Mouse Liver and Kidney Homogenates

Substrate	Rate of formation (nmol/min/g tissue)			
	Liver		Kidney	
	Glucuronide	Glucoside	Glucuronide	Glucoside
<i>S</i> (+)	30.5 ± 2.2	0.8 ± 0.1	1.7 ± 0.1	19.2 ± 0.8
<i>R</i> (-)	31.4 ± 2.2	N.D.	2.7 ± 0.03	3.0 ± 0.1
<i>RS</i> (±)	28.8 ± 1.1	0.4 ± 0.3	2.1 ± 0.2	12.1 ± 0.2

Incubation mixture contains 150 μ l of tissue whole homogenates, 100 mM Tris-HCl buffer pH 7.2, 5 mM MgCl₂, 2 mM UDPGA, 2 mM UDPG, and 0.2 mM *S*(+), *R*(-) and *RS*(±)-pranoprofen in a total volume of 500 μ l. The incubation was carried out for 30 min at 37 °C and terminated by adding 0.5 ml of 0.4 N perchloric acid.

Glucuronide and Glucoside in the Tissues

Concentrations of pranoprofen, its acyl glucuronide and glucoside in the liver, kidney and plasma were determined 1 h after the oral administration of *S*(+)- and *R*(-)-pranoprofen at three doses (25, 100, 200 mg/kg), and the results are shown in Table IV.

At the lowest dose, the concentration of acyl glucuronide was a low level of less than 1 μ g/g in the liver and kidney. In the plasma, the concentration of acyl glucuronide was 2.7 μ g/ml for *S*(+)-pranoprofen and 3.3 μ g/ml for *R*(-)-pranoprofen, but they were also at low levels in comparison to pranoprofen levels of 50.0 and 52.6 μ g/g, respectively. Acyl glucoside with a concentration of 2.7 μ g/g, was detected only for *S*(+)-pranoprofen in the kidney. In the liver, kidney and plasma, the concentration of acyl glucuronide increased relative to pranoprofen at increasing doses. Acyl glucuronide in the liver was higher for *R*(-)-pranoprofen than for *S*(+)-enantiomer, but in the plasma an opposite result was obtained. In the kidney, acyl glucoside decreased relative to acyl glucuronide at increasing doses for both *S*(+)- and *R*(-)-pranoprofen,

but the concentration of acyl glucoside was much higher for *S*(+)-pranoprofen than *R*(-)-enantiomer.

Stereoselective Acyl Glucuronidation and Glucosidation

Acyl glucuronide and glucoside formation were determined in the liver and kidney homogenates by the use of *S*(+)-, *R*(-)- and *RS*(±)-pranoprofen as substrates (Table V). The rate of acyl glucuronide in the liver was 30.5 nmol/min/g of tissue for *S*(+)-pranoprofen, 31.4 nmol/min/g of tissue for *R*(-)-enantiomer, resulting in no difference in acyl glucuronidation between the enantiomers. On the other hand, in the kidney the rate of glucoside formation was 19.2 nmol/min/g of tissue for *S*(+)-pranoprofen and 3.0 nmol/min/g of tissue for *R*(-)-enantiomer, respectively. That is, acyl glucosidation in the kidney for *S*(+)-pranoprofen was 6 times greater than that for the *R*(-)-enantiomer.

Determination of Apparent Kinetic Constants for Glucuronidation and Glucosidation of *S*(+)- and *R*(-)-Pranoprofen

Apparent kinetic constants for conjugations

TABLE VI. Apparent Kinetic Constants for Acyl Glucuronidation and Glucosidation of *R*(-)- and *S*(+)-Pranoprofen in Mouse Liver and Kidney Microsomes

Substrate	Glucuronidation in liver			Glucosidation in kidney		
	$V_{\max}$	K_m	$V_{\max}/K_m$	$V_{\max}$	K_m	$V_{\max}/K_m$
<i>S</i> (+)	0.33	1.11	0.30	0.46	0.13	3.54
<i>R</i> (-)	0.53	0.77	0.69	0.18	0.26	0.69

$V_{\max}$ (nmol/min/mg of protein), K_m (mM).

obtained from Lineweaver–Burk plots were listed in Table VI.

For glucuronidation in the liver microsomes, the apparent K_m values for $S(+)$ - and $R(-)$ -pranoprofen were 1.11 and 0.77 mM, and the V_{max} value were 0.33 and 0.53 nmol/min/mg of protein, respectively. On the other hand, for glucosidation in the kidney microsomes, the apparent K_m values for $S(+)$ - and $R(-)$ -pranoprofen were 0.13 and 0.26 mM, and V_{max} values were 0.46 and 0.18 nmol/min/mg of protein, respectively. The V_{max}/K_m values for glucuronidation of $S(+)$ - and $R(-)$ -pranoprofen were 0.30 and 0.69, and these values for glucosidation of $S(+)$ - and $R(-)$ -pranoprofen were 3.54 and 0.69, respectively.

Discussion

In previous studies,^{8,9)} we showed a dose-dependent shift in the acyl glucuronidation and glucosidation of pranoprofen in mice, and glucosidation decreased relative to glucuronidation at increasing doses. Furthermore, it was estimated that the stereoselective conjugation might markedly occur for glucosidation, but not for glucuronidation *in vivo*.

The present investigation showed that the rate of glucuronidation of pranoprofen was higher in the liver than in the kidney, whereas that of glucosidation was more predominant in the kidney than in the liver. No conjugation activities were observed in the lung, plasma and gut (Table I). These findings indicate that conjugation reactions of pranoprofen are not significant in the process of the absorption and of the blood circulation. Glucuronidation and glucosidation of pranoprofen might occur by the enzymatic transfer of glucuronic acid of UDPGA and glucose of UDPG to aglycones since no conjugates were formed because of the inactivation of enzymes or the removal of UDPGA and UDPG. $MgCl_2$ enhanced the enzyme activities for both conjugation reactions, but it is not an essential factor (Table II). These results revealed that pranoprofen was nearly all glucuronidated in the liver and glucosidated in the kidney by enzymes following the administration to mice.

Although the amount of acyl glucuronide and

glucoside formed in the liver and kidney gradually increased with the prolongation of the incubation time, glucuronide in the liver and glucoside in the kidney reached the maximum at 15–30 min and 45 min, respectively (Fig. 1). After that, both generated conjugates decreased with the lapse of time and the elimination rate was greater in the liver than in the kidney. On the other hand, acyl glucuronide and glucoside which were isolated from mouse urine were unstable in the kidney and plasma, and especially in the liver. Both conjugates reduced to less than 50% of the initial amount in the liver, but 70–90% of conjugates were still intact in the plasma 1 h after the incubation (Fig. 2). These findings indicate that the diminution of formed conjugates according to the lapse of time might be caused by the instability of both conjugates in the liver and kidney.

Furthermore, acyl glucuronide of $S(+)$ - and $R(-)$ -pranoprofen were not converted to acyl glucoside in the kidney in spite of the presence of UDPG (Table III). This result indicates that the hydrolysis of acyl glucuronide in the body to pranoprofen is essential for the formation of acyl glucoside in the kidney. In contrast to the kidney, glucuronidation of acyl glucoside occurred in the liver in the presence of UDPGA (Table III) because of a rapid hydrolysis of the conjugate in the liver (Fig. 2).

When $S(+)$ - and $R(-)$ -pranoprofen were administered to mice at a dose of 25 mg/kg, the concentration of pranoprofen was the highest in the liver, kidney and plasma, and its glucuronide and glucoside were at very low levels. However, the glucuronide increased at high doses in all tissues tested. The absence of the glucoside in the liver and plasma at all tested doses strongly suggests that acyl glucosidation of pranoprofen might actually occur mainly in the kidney. On the other hand, acyl glucoside decreased relative to glucuronide in the kidney at increasing doses (Table IV). These results corresponded to a dose-dependent shift in acyl glucuronidation and glucosidation of pranoprofen in the urine.⁸⁾

Stereoselective glucosidation in mice has been estimated from urinary excretion of acyl glucoside in which the aglycone was mainly $S(+)$ -pranoprofen after the administration of

Fig. 3. Postulated Mechanism of a Dose-Dependent Shift in Acyl Glucuronidation and Glucosidation of Pranoprofen in Mice after Oral Administration of Pranoprofen

RS-PNPF, *RS*(±)-pranoprofen; S-GN, *S*(+)-pranoprofen acyl glucuronide; R-GN, *R*(-)-pranoprofen acyl glucuronide; S-GS, *S*(+)-pranoprofen acyl glucoside; R-GS, *R*(-)-pranoprofen acyl glucoside.---, low dose; —, high dose.

pranoprofen.⁹⁾ The present studies reveal that the extent of glucuronidation in the liver is similar between *S*(+)- and *R*(-)-isomers, whereas glucosidation in the kidney was greater for *S*(+)-isomer than for *R*(-)-antipode (Tables V, VI).

Furthermore, the $V_{\max}/K_m$ value for acyl glucuronidation of *R*(-) enantiomer was only 2 times higher than that of *S*(+)-antipode, but the $V_{\max}/K_m$ value for acyl glucosidation of *S*(+)-enantiomer was 5 times higher than that of *R*(-)-antipode, showing that the stereoselective glucosidation in the kidney was much greater for the *S*(+)-enantiomer (Table VI). These results corresponded to the evidence that *S*(+)-enriching glucuronide was largely excreted in urine after the administration of pranoprofen and that the concentration of acyl glucoside in the kidney was higher for *S*(+)-pranoprofen than for the *R*(-)-enantiomer.⁹⁾

In the previous study,⁹⁾ we demonstrated that the urinary recovery of conjugates was less for *R*(-)-pranoprofen. The present study indicates that stereoselective biliary excretion of pranoprofen, as well as other profens,¹³⁾ might occur with extensive biliary excretion for *R*(-)-pranoprofen. This is because the concentration of acyl glucuronide in the liver was higher for *R*(-)-enantiomer but that in plasma was higher for *S*(+)-antipode. Nevertheless, further investigation must be carried out to elucidate this stereoselective biliary excretion.

From the present studies, the mechanism of a dose-dependent shift in acyl glucuronidation and glucosidation was proposed as follows: the pranoprofen absorbed was glucuronidated non-stereoselectively in the liver for *S*(+)- and *R*(-)-pranoprofen, but at a low dose glucuronide was easily hydrolyzed to form an unchanged pranoprofen in the body. Then pranoprofen, formed by hydrolysis in the body, is glucosidated in the kidney stereoselectively (with *S*(+)-isomer being superior) and is excreted in the urine as *S*(+)-enriching acyl glucoside. At high doses acyl glucuronide, which escapes from the hydrolysis in the body, increases in the systemic circulation and its glucuronide is excreted in urine without receiving glucosidation in the kidney, showing a dose-dependent shift in acyl glucuronidation and glucosidation of pranoprofen (Fig. 3).

The present investigation provides the first piece of evidence for the stereoselective glucosidation in the kidney and the mechanism of a dose-dependent shift in the acyl glucuronidation and glucosidation of pranoprofen in mice.

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