Effect of Chronic Administration of Phenobarbital on the Hepatobiliary Transport of Phenol Red: Assessment by Statistical Moment Analysis

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The effect of enzyme induction on the hepatobiliary transport of phenol red (PR) in rats was investigated by application of a new analytical system to determine local drug disposition based on statistical moment theory (T. Kakutani *et al., J. Pharmacokin. Biopharm.* 13:609–631, 1985). Employing the moment parameters obtained from the time courses of plasma and biliary concentrations of PR and its metabolite after intravenous injection, the hepatobiliary transport of PR was theoretically assessed by separating it into component subprocesses such as hepatic uptake, hepatobiliary transfer, and intrahepatic metabolism. The results demonstrated that the acceleration of plasma disappearance of PR caused by pretreatment with phenobarbital (PB), known to induce hepatic enzyme systems, could be attributed to elevation of both hepatic and extrahepatic clearances. While PB did cause bile flow elevation (choleresis) and increased metabolism, these effects were shown to make little contribution to accelerated plasma disappearance of PR, since it was shown that the hepatobiliary excretion of PR was rate-limited by the intrahepatic transfer process, which was unaffected by PB treatment. From the results of this study, this experimental/analysis methodology seems to be useful in obtaining detailed information about hepatobiliary transport of the drug from *in vivo* data.

KEY WORDS: hepatobiliary transport; phenol red; enzyme induction; statistical moment analysis.

INTRODUCTION

Chemicals known to induce microsomal or cytosol enzymes in animal species often affect the pharmacokinetic fate of other xenobiotics (2). For example, chronic administration of phenobarbital (PB)[4] increases hepatic cytochrome P-450 content (3) and increases the plasma clearance on antipyrine (4). PB also increases the activity of UDP-glucuronosyl transferase (5–7) and enhances the plasma disappearance of hexachlorophene (8) and valproic acid (6). The predominant mechanism underlying this effect is thought to be increased enzyme activity, but other factors concerning hepatobiliary transport are also affected by PB pretreatment (2,9,10). Liver weight (4), hepatic blood flow (9), hepatic uptake (11), biliary excretion (10), bile flow (3,6), and cytosolic ligandin content (12,13) have been reported to be elevated by PB treatment, while plasma protein binding has been shown to decrease (14). Since each of these factors may play an important role in the pharmacokinetics of drugs, it is difficult to identify their individual contributions to drug elimination (2,9,10), and an appropriate analytical/experimental system is necessary to estimate the details of local drug disposition from *in vivo* experiments.

In order to assess local drug disposition, an analytical system has been proposed in which moment analysis is applied to venous concentration–time curves after single-pass muscle perfusion following bolus arterial injection (1). Organic anions such as phenol red (PR) are known to be actively excreted into bile and urine (2,15). However, few details of the disposition of PR in liver and kidney have been reported. In a previous report (16), a new analytical system (1) was applied to a liver perfusion system to assess hepatic uptake of PR. The present study applies this method to biliary concentration–time curves after intravenous injection of PR to assess hepatobiliary transport processes. Moreover, the effect of microsomal enzyme induction on the pharmacokinetics of PR was also investigated.

MATERIALS AND METHODS

Chemicals

Phenol Red (PR) and phenobarbital (PB) sodium were purchased from Nacalai Tesque and Wako Chemicals, Japan, respectively. All other chemicals were of analytical grade and were obtained commercially.

Animals

Male Wistar rats weighing 215.4 ± 17.4 g (Shimizu Experimental Animals Co., Japan) were used. Animals were fed a standard laboratory diet and tap water *ad libitum*. For the enzyme induction experiment (5,6,8,11), rats pretreated with PB (75 mg/kg/day, i.p.) once daily for 4 days were sacrificed 24 hr after the last PB treatment. PB was administered in saline in a volume of 5.0 ml/kg.

In Vivo Experiment

Animal experiments were performed in two ways: (i) simultaneous collection of plasma and bile (System A) and (ii) simultaneous collection of bile and urine (System B). In both experimental systems, a median lapatomy was performed under pentobarbital anesthesia (30 mg/kg body

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[4] Abbreviations used: AUBC, area under biliary concentration–time curve; AUC, area under plasma concentration–time curve; C_B, blood concentration; C_{max}, peak concentration; CL_{EH}, extrahepatic clearance; CL_H, hepatic clearance; FH, unmetabolized ratio (ratio of free compounds recovered from bile to those transferred into liver); F_{PR} and $F_{PR\text{-}G}$, ratios of excreted amounts of PR and PR-G in bile to injected dose; f_u, plasma unbound fraction; k_H, first-order hepatic uptake rate constant; MBT, mean biliary excretion time; MHT, mean hepatobiliary transfer time; MHT_{cor}, corrected hepatobiliary transfer time; MHT_{el}, mean intrahepatic residence time; MRT, mean residence time in plasma; MUT, mean urinary excretion time; PB, phenobarbital; PR, phenol red; PR-G, glucuronic acid conjugate of phenol red; R_B, blood-to-plasma concentration ratio; t_{max}, peak time; VBT, variance of biliary excretion time; VHT, variance of hepatobiliary transfer time; VRT, variance of residence time in plasma; V_{ss}, steady-state distribution volume; VUT, variance of urinary excretion time.

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weight, i.p.), the right renal artery and vein were ligated, and a 5% (w/v) glucose solution was constantly infused into the right femoral vein at a rate of 0.0580 ± 0.0098 ml/min to prevent dehydration and to maintain a constant urine flow rate. The standard-dose experiment (PR dose, 8 mg/kg, System A-I), high-dose experiment (80 mg/kg, System A-II), whole-kidney (right plus left) ligation experiment (8 mg/kg, System A-III), and PB-treated experiment (8 mg/kg, System A-IV) were carried out using System A. In addition, whole-kidney ligation was also performed on the PB-treated rats (8 mg/kg, System A-V). System B experiments utilized the standard dose of PR (8 mg/kg) to compare the results with those of System A-I. Since the urinary recovery of PR was highly variable when plasma samples were collected simultaneously, only urine and bile were collected.

The bile duct and the left renal ureter were cannulated with polyethylene tubing (PE-10). The bile duct cannula was anchored close to the liver to avoid contamination with pancreatic secretions. Bile and urine samples were collected in weighed test tubes at appropriate time intervals (first 1 min, then 2–10 min) following intravenous injection of PR dissolved in pH 7.4 phosphate-buffered saline into the femoral vein. This solution was injected for 40 sec; the midinjection time point was defined to be time 0. Blood samples were obtained from the jugular vein using a heparinized syringe and were centrifuged at 2000g for 2 min. The total blood sample volume was 1.5–2.0 ml for each experiment. The liver was removed and weighed at the end of the experiment. The plasma, bile, and urine samples were weighed and the volumes were calculated. In these cases, a specific gravity of 1.0154 ± 0.0023 (n = 5) was used for plasma; the specific gravities of bile and urine were assumed to be 1.0.

Assays

Free PR in plasma, bile, and urine was measured spectrophotometrically at 560 nm after dilution with saline and 1.0 N NaOH solution. To avoid fading, the measurement was done immediately after the addition of NaOH. Blank plasma, bile, and urine were collected before administration of PR in each run for subtraction of blank absorbance values. It was confirmed from preliminary experiments that this method had a high specificity and sensitivity for PR (absorbance of samples was more than 20, 12, and 20 times of blank values, respectively), had a high reproducibility (CV value was usually less than 0.5% in triplicates), and was not affected by PB pretreatment (with the exception of plasma PR determination at the 8 mg/kg dose; the absorbance was 3.3–12 times of blank plasma absorbance).

The determination of the metabolite, PR–glucuronic acid conjugate (PR-G), was carried out according to the method of Hart and Schanker (17). A 0.5-ml aliquot of 4.0 N HCl was added to 1.0 ml of saline diluted sample solution. After incubation at 90°C for 30 min, 1.0 ml of 5.0 N NaOH was added, and the total concentration of PR and PR-G was determined. The difference between this concentration and the concentration of PR was assumed to represent the PR-G concentration. PR was confirmed to be stable throughout these procedures.

Theory

Statistical Moment Analysis

For plasma PR concentration–time curves, the plasma moments are defined as follows:

$$\mathrm{AUC} = \int_0^\infty C dt \quad (1)$$

$$\mathrm{MRT} = \int_0^\infty t\, C dt/\mathrm{AUC} \quad (2)$$

$$\mathrm{VRT} = \int_0^\infty (t - \mathrm{MRT})^2\, C dt/\mathrm{AUC} \quad (3)$$

where t is the sampling time and C is the concentration of drug. AUC, MRT, and VRT are the area under the plasma concentration–time curve, the mean residence time in plasma, and the variance of residence time in plasma, respectively. The plasma moments were calculated by fitting the data to a biexponential equation using the nonlinear least-squares method using the personal computer program MULTI (18). The equations used for biliary and renal excretion of PR and PR-G were analogous; e.g., AUBC, MBT, and VBT are the area under the biliary concentration–time curve, the mean biliary excretion time, and the variance of biliary excretion time, respectively. The product of AUBC and bile flow rate is the ratio of mass recovered in bile to inject dose (presented as F). The moment calculation was made using the linear trapezoidal method and extrapolation from the last sampling time to infinite time using the terminal log-linear slope. The average number of time points used for the extrapolation was 14.6.

Deconvolution Using Plasma and Biliary Moments

The biliary concentration–time profile of PR is a hybrid of hepatobiliary transport and the plasma PR concentration–time profile. Therefore, deconvolution was performed using two sets of the plasma and biliary moments, and hepatobiliary transfer moments were calculated according to

$$\mathrm{FH} = \mathrm{AUBC_{PR}}/(\mathrm{AUBC_{PR}} + \mathrm{AUBC_{PR\text{-}G}}) = F_{PR}/(F_{PR} + F_{PR\text{-}G}) \quad (4)$$

$$\mathrm{MHT} = \mathrm{MBT_{PR}} - \mathrm{MRT} \quad (5)$$

$$\mathrm{VHT} = \mathrm{VBT_{PR}} - \mathrm{VRT} \quad (6)$$

where FH, MHT, and VHT are the unmetabolized ratio, the mean hepatobiliary transfer time, and the variance of hepatobiliary transfer time, respectively. In general, FH is the ratio of free compounds recovered from bile to those transferred into liver, and cannot be obtained from the biliary concentration profile alone. In this experiment, it was confirmed that PR transferred into the liver was recovered as PR and PR-G from bile, and Eq. (4) could be assumed to hold true.

Assessment of the Hepatobiliary Transfer Process for PR

The theoretical background for these derivations is shown in the Appendix and in previous reports (1,19).

Hepatic uptake can be assessed by the hepatic clearance, $\mathrm{CL_H}$ [$=(F_{PR} + F_{PR\text{-}G})$ CL], and the first-order hepatic

uptake rate constant, k_H [$=(F_{PR} + F_{PR\text{-}G})$ MRT], where CL is total-body clearance (= dose/AUC). CL_H and k_H supply information on hepatic uptake based on unidirectional transport (19). In order to obtain accurate CL_H and k_H values, accurate AUBCs were obtained by terminal extrapolation. The extrahepatic clearance, CL_{EH} was determined as $CL_{EH} = CL - CL_H$.

Hepatobiliary transfer can be assessed by the corrected mean hepatobiliary transfer time, MHT_{cor} (= MHT/FH). MHT_{cor} is the true mean time value necessary for intrahepatic transfer (including transfer through the bile duct and cannulae).

Intrahepatic metabolism can be assessed with respect to rate and extent. The rate of intrahepatic metabolism (conjugation to PR-G) can be assessed by the mean intrahepatic residence time, MHT_{el} [= MHT/(1 − FH)]. MHT_{el} is the mean residence time in the intrahepatic space. MHT_{el} is equal to the reciprocal of the first-order metabolism rate constant based on the well-stirred assumption (1,19). The extent of intrahepatic metabolism can be assessed by the unmetabolized ratio, FH.

All results are expressed as mean ± one standard deviation.

RESULTS

Effects of PB Treatment on the Physiological Condition and Basic Pharmacokinetic Parameters of PR

The effect of PB treatment on the physiological parameters of the rats is summarized in Table I. PB treatment resulted in a significant elevation in liver weight. However, the blood density, plasma density, hematocrit, blood-to-plasma concentration ratio of PR (R_B), and unbound fraction of PR (f_u) values were not changed. The sums of R_B and the hematocrits were near-unity, suggesting that PR is located in plasma regardless of PB treatment at a low blood PR concentration (40 μg/ml). At the high blood PR concentration (400 μg/ml), plasma protein binding was saturated and PR also distributed into the red blood cells. Bile flow rates during the experiments are given in Table II and were shown to be elevated about 1.5-fold by chronic PB treatment.

Table I. Effect of Phenobarbital Treatment on the Physiological Condition of Rats and the Binding of PR in Blood

	Control	PB treatment
Body weight (BW) (g)	213.3 ± 16.1 (39)[a]	233.4 ± 23.3 (17)[b]
Liver weight (LW) (g)	8.15 ± 1.53 (30)	10.49 ± 1.75 (17)*
BW/LW (%)	3.79 ± 0.58 (30)	4.48 ± 0.42 (17)*
Blood density (g/ml)	1.0416 ± 0.0069 (5)	1.0423 ± 0.0046 (4)
Plasma density (g/ml)	1.0154 ± 0.0023 (5)	1.0157 ± 0.0020 (5)
Hematocrit	0.420 ± 0.021 (12)	0.441 ± 0.008 (4)
R_B (C_B = 40 μg/ml)	0.586 ± 0.024 (13)	0.578 ± 0.010 (4)
R_B (C_B = 400 μg/ml)	0.711 ± 0.026 (4)**	—
f_u (C_B = 40 μg/ml)	0.147 ± 0.004 (4)	0.138 ± 0.009 (4)
f_u (C_B = 400 μg/ml)	0.647 ± 0.020 (4)**	—

[a] Numbers of experiments in parentheses.
[b] Not significantly different from the control-group body weight or body weight prior to PB treatment (220.2 ± 19.8 g).
* Compared with the no-PB treatment group by Student's t test, $P < 0.01$.
** Compared with the low-C_B group by Student's t test, $P < 0.01$.

Plasma and Biliary PR Concentration–Time Profiles

Typical concentration–time curves of PR in bile and plasma and PR-G in bile after intravenous bolus injection of PR at a dose of 8 mg/kg (System A-I) are shown in Fig. 1. The concentrations were normalized to dose (% dose/ml). Plasma PR concentration–time curves were biexponential and PR-G was undetectable in plasma.

The moments obtained from the plasma concentrations of PR and the pharmacokinetic parameters obtained from these moments are summarized in Table III.

Compared with the standard dose (System A-I, 8 mg/kg), the high-dose group (System A-II, 80 mg/kg) seemed to show a larger V_{ss} and a lower k_H, and no alteration of CL_H. The renal ligation (System A-III) caused an elevation of AUC, but CL_H, k_H, and V_{ss} were not changed.

PB treatment showed similar effect with or without renal ligation. The AUC and MRT values were decreased; i.e., enhancement of plasma PR disappearance was caused by PB treatment, as in previous reports (2,4,6,8). This was explained by elevation of both of CL_H, CL_{EH}, and k_H.

In all experimental systems, the $(VRT)^{0.5}$/MRT value [the dispersion ratio (20)] was near-unity, indicating that the distribution of PR between blood and interstitial fluids was rapid and that dose elevation and PB treatment had no significant effect on this distribution.

Pharmacokinetic Parameters Determined from Biliary Concentration–Time Curves

C_{max}, t_{max}, and the biliary moments of PR and PR-G for all experimental systems are summarized in Table IV.

Table II. Bile Flow Rates During Experiments A–I to A–V[a]

Bile flow rate	μl/min	μl/min/kg	μl/min/g liver
A–I (5)[b]	14.7 ± 2.3	63.9 ± 10.3	1.53 ± 0.27
A–II (4)	13.9 ± 4.5	66.5 ± 15.6	1.74 ± 0.39
A–III (4)	11.5 ± 1.5	54.7 ± 9.1	1.35 ± 0.20
A–IV (4)	23.0 ± 5.5*,[c]	96.4 ± 21.2*	2.10 ± 0.30*
A–V (4)	19.3 ± 2.1**	83.6 ± 12.6**	1.91 ± 0.10**

[a] A–I: PR dose, 8 mg/kg. A–II: PR dose, 80 mg/kg. A–III: PR dose, 8 mg/kg, and renal ligation. A–IV: PR dose, 8 mg/kg, and PB pretreatment. A–V: PR dose, 8 mg/kg, renal ligation, and PB pretreatment.
[b] Numbers of experiments in parentheses.
[c] Comparisons between System A–I and System A–IV, or System A–III and System A–V by Student's t test.
* $P < 0.05$.
** $P < 0.01$.

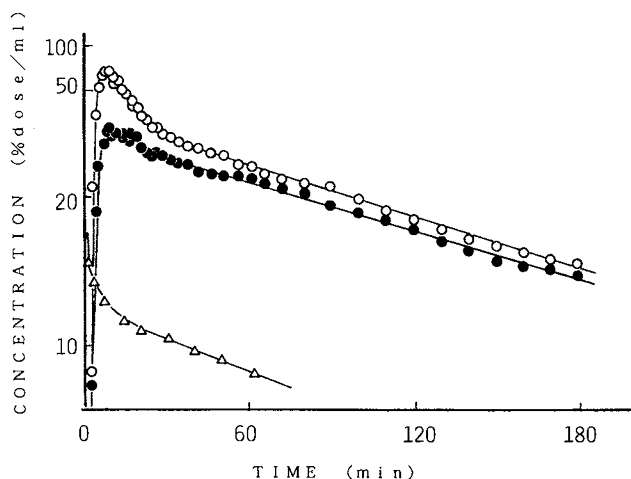

Fig. 1. Typical concentration–time curves of PR in bile (○) and plasma (△), and PR-G in bile (●) after intravenous bolus injection of PR at a dose of 8 mg/kg (System A-I).

Compared to the standard dose (System A-I, 8 mg/kg), the high dose (System A-II, 80 mg/kg) resulted in a low $C_{max_{PR}}$, $C_{max_{PR\text{-}G}}$, and $AUBC_{PR\text{-}G}$. The $t_{max_{PR}}$ was not altered, but the MBT_{PR} seemed to be increased. Renal obstruction (System A-III, 8 mg/kg) showed an elevation of ratio transported into liver to dose and resulted in larger C_{max} and AUBC values.

PB treatment showed similar effects on the biliary concentration–time profiles of PR and PR-G with and without renal ligation. PB treatment caused a decrease in t_{max} and MBT values of PR and PR-G. Although $C_{max_{PR}}$ and $AUBC_{PR}$ were also decreased, $C_{max_{PR\text{-}G}}$ and $AUBC_{PR\text{-}G}$ were not remarkably altered by PB treatment.

Table III. Pharmacokinetic Parameters for PR Calculated from the Plasma–Concentration Time Curves

	AUC (% dose min/ml)	MRT (min)	VRT (min^2)
A-I (5)[a]	94.6 ± 15.7	38.9 ± 10.2	1928 ± 947
A-II (4)	91.4 ± 16.9	42.6 ± 1.8	2063 ± 168
A-III (4)	148.2 ± 4.1**,[b]	55.1 ± 5.2*	3428 ± 632*
A-IV (4)	62.4 ± 7.5++,[c]	30.7 ± 2.0	1107 ± 163
A-V (4)	95.4 ± 14.5++	35.8 ± 5.5++	1450 ± 432++

	CL_H (ml/min/kg)	CL_{EH} (ml/min/kg)	V_{ss} (ml/kg)	k_H ($\times 10^3$; min^{-1})
A-I	3.27 ± 0.35	1.45 ± 0.51	177 ± 16	18.8 ± 3.5
A-II	3.15 ± 0.50	2.27 ± 0.94	230 ± 28**	13.9 ± 3.0
A-III	3.22 ± 0.37	−0.02 ± 0.11**	175 ± 7	18.4 ± 1.9
A-IV	4.31 ± 0.83+	2.60 ± 1.20	212 ± 30	20.5 ± 4.1
A-V	4.22 ± 0.83	0.38 ± 0.24+	162 ± 15	26.2 ± 6.2

[a] Numbers of experiments in parentheses.
[b] Compared with results of System A–I by Student's t test: (*) $P < 0.05$; (**) $P < 0.01$.
[c] Compared between System A–I and System A–IV, or System A–III and System A–V, by Student's t test: (+) $P < 0.05$; (++) $P < 0.01$.

Assessment of Pharmacokinetic Fate of PR Based on the Present Analysis

Although the plasma and biliary moment values are themselves valuable in analyzing hepatobiliary transport of PR, the biliary moments are hybrid functions of the plasma concentration profile and many related processes in the liver. Therefore, the pharmacokinetic parameters corresponding to several of these processes are calculated in Table V.

Compared to the standard dose (System A-I, 8 mg/kg), the high dose (System A-II, 80 mg/kg) resulted in decreased or unaltered hepatic uptake extent ($F_{PR} + F_{PR\text{-}G}$) and saturated metabolic processes [FH (extent) and MHT_{el} (rate)]. These effects resulted in a decrease in $F_{PR\text{-}G}$. However, the time necessary to transport through the liver (MHT_{cor}) was not affected. Renal ligation (System A-III, 8 mg/kg) caused the elevation of the ratio transported into liver to dose and resulted in larger F_{PR} and $F_{PR\text{-}G}$; however, the metabolic process was not saturated (FH and MHT_{el}), and MHT_{cor} was also unaffected.

PB treatment resulted in elevation of metabolism (FH); however, PB treatment did not accelerate the transfer of PR through the liver (MHT_{cor}), with and without renal ligation.

For experimental Systems A-I to A-V, $(VHT)^{0.5}/MHT$ were 2.37 ± 0.35, 1.87 ± 0.19, 2.49 ± 0.28, 2.75 ± 0.49, and 2.39 ± 0.41, respectively, indicating the existence of an intrahepatic nonequilibrium compartment (19). This nonequilibrium effect (20) was decreased only by dose elevation.

Comparison of Biliary and Urinary Moments

The results of System B experiments are shown in Table VI. In this case, the urine flow rate was 147.7 ± 56.2 µl/min/kg ($N = 4$). Since the variance in urine flow rate was large, F_{PR} and $F_{PR\text{-}G}$ values are presented in Table VI as zeroth moments.

Since all of the biliary moments were the same as those obtained in System A-I (Table III), it was assumed that the plasma moment values of System A-I in Table III could be used for analyzing the urinary moments obtained in System B. Using MRT values of 38.9 min, the mean times for PR transfer across the liver and kidney were calculated to be 20.2 and 15.7 min, respectively. Although the variability was rather large, the mean urinary excretion time of PR-G was about nine times longer than the mean residence time of PR in plasma (MRT_{PR}). This may be explained by either a slow conjugation rate in the kidney or retarded arrival of PR-G from the liver (production site) to the kidney through the systemic circulation.

DISCUSSION

PR is known to be eliminated from plasma by active secretion into urine and bile and by hepatic metabolism in a variety of species (2,15). The pharmacokinetic characteristics of PR (21,22) and the nature of its metabolite (17,23) have been previously reported. Intravenously administered PR recovered from bile and urine is primarily as PR and PR-G, indicating that PR is a good model compound to verify the proposed analytical system and to investigate the effect of enzyme induction on its pharmacokinetics. Statistical mo-

Table IV. T_{max}, C_{max}, and Moments Determined from the Biliary Concentration–Time Curves

	t_{max} (min)	C_{max} (% dose/ml)	AUBC (% dose min/ml)	MBT (min)	VBT (min^2)
			(a) PR		
A–I	8.9 ± 0.5	81.2 ± 13.7	3,133 ± 503	61.9 ± 9.5	4,848 ± 1,535
A–II	9.3 ± 1.9	59.7 ± 11.0*,[a]	3,735 ± 1,082	77.0 ± 10.7	6,238 ± 1,790
A–III	10.5 ± 2.2	123.3 ± 38.6	6,136 ± 879**	79.1 ± 13.5	7,044 ± 2,444
A–IV	6.0 ± 1.0++,[b]	53.6 ± 14.6+	1,635 ± 518++	47.9 ± 10.4	3,304 ± 1,835
A–V	6.0 ± 0.6++	72.3 ± 11.0+	2,888 ± 391++	60.5 ± 17.2	5,010 ± 3,472
			(b) PR-G		
A–I	12.9 ± 2.1	26.6 ± 7.0	1,744 ± 456	84.3 ± 12.5	7,259 ± 2,491
A–II	14.6 ± 3.3	4.9 ± 2.4**	782 ± 470*	166.0 ± 55.1*	18,491 ± 12,498
A–III	12.8 ± 2.5	26.6 ± 4.6	2,696 ± 771	131.3 ± 21.3**	17,021 ± 5,481**
A–IV	8.8 ± 2.2+	27.4 ± 9.3	1,255 ± 541	77.7 ± 36.4	7,835 ± 8,230
A–V	11.3 ± 3.3	30.4 ± 6.7	1,884 ± 160	88.6 ± 29.1	8,965 ± 6,480

[a] Compared with results of System A–I by Student's *t* test: (*) $P < 0.05$; (**) $P < 0.01$.
[b] Compared between System A–I and System A–IV, or System A–III and System A–V, by Student's *t* test: (+) $P < 0.05$; (++) $P < 0.01$.

ment analysis has many significant applications in the determination of whole body drug disposition. It has previously been applied to a single-pass organ perfusion system (1,16,24), and an application to hepatobiliary transport analysis using *in vivo* data is proposed in this paper. The existence of a lag time in biliary excretion of PR and PR-G after intravenous injection of PR has been reported (16,17,25–28). The present analysis provides theoretical support for lag times.

A schematic summary of the hepatobiliary disposition of PR and the effects of dose elevation and PB pretreatment are indicated plainly in Fig. 2.

The effect of PR dose elevation on its disposition may be summarized as follows: due to the saturation of plasma protein binding (f_u), the extravascular distribution volume is enlarged (V_{ss}). In addition, hepatic uptake [k_H (rate) and $F_{PR} + F_{PR\text{-}G}$ (extent)] is saturated. Cancellation between these effects results in no apparent alteration of AUC and hepatic clearance (CL_H). The mean time necessary for hepatobiliary transfer is also unchanged (MHT_{cor}). The saturation of metabolic processes is observed (MHT_{el}, FH), and saturation of both hepatic uptake and metabolism processes results in lower biliary recovery as PR-G. Although hepatic uptake of PR is decreased, recovered PR in bile is apparently increased (F_{PR}), and this is explained by saturation of metabolism. High dose also induces temporal choleresis, which is indicated by low $C_{max_{PR}}$ and $C_{max_{PR\text{-}G}}$ values.

The effect of PB treatment on PR disposition may be summarized as follows: PB treatment results in an increase in liver weight. Accelerated plasma disappearance of PR can be attributed to the increase in both hepatic and extrahepatic clearance (CL_H and CL_{EH}). Plasma disappearance acceleration is explained not only by an increase in liver weight but also by an elevation in hepatic uptake rate (k_H) and/or hepatic blood flow. PB treatment also results in choleresis. However, since the hepatobiliary excretion of PR is rate-limited by the intrahepatic transfer and since this is not altered (MHT_{cor}), choleresis is concluded to lead only to di-

Table V. Hepatobiliary Disposition Parameters for PR Calculated from the Moments

	F_{PR} (% dose)	$F_{PR\text{-}G}$ (% dose)	$F_{PR} + F_{PR\text{-}G}$ (% dose)	FH (%)
A–I	45.1 ± 3.5	25.0 ± 5.2	70.1 ± 6.9	64.5 ± 4.6
A–II	49.3 ± 10.5	9.8 ± 5.3**,[a]	59.1 ± 13.3	83.8 ± 6.7**
A–III	69.9 ± 4.9**	30.8 ± 7.6	100.7 ± 3.7**	69.6 ± 6.6
A–IV	36.5 ± 10.6	26.8 ± 6.9	63.3 ± 15.8	57.4 ± 6.4
A–V	55.3 ± 4.1++,[b]	36.2 ± 2.0	91.4 ± 5.6+	60.4 ± 1.2+
	MHT (min)	**VHT (min^2)**	**MHT_{cor} (min)**	**MHT_{el} (min)**
A–I	23.0 ± 9.6	2921 ± 1639	35.5 ± 14.6	66.7 ± 30.5
A–II	34.4 ± 9.1	4175 ± 1695	41.5 ± 11.3	246.1 ± 115.7*
A–III	24.0 ± 10.2	3617 ± 2109	35.3 ± 16.1	78.2 ± 27.1
A–IV	17.1 ± 9.0	2197 ± 1707	31.5 ± 20.7	38.8 ± 14.5
A–V	24.7 ± 13.6	3560 ± 3197	40.8 ± 22.5	62.7 ± 34.6

[a] Compared with results of System A–I by Student's *t* test: (*) $P < 0.05$; (**) $P < 0.01$.
[b] Compared between System A–I and System A–IV, or System A–III and System A–V, by Student's *t* test: (+) $P < 0.05$; (++) $P < 0.01$.

Table VI. Biliary and Urinary Moments Calculated After Bolus Injection of PR (8 mg/kg) in System B ($N = 4$)

	Bile	Urine
	(a) PR	
F_{PR} (%)	39.6 ± 9.3	25.5 ± 8.8
MBT_{PR} (MUT_{PR}) (min)	59.1 ± 14.2	54.6 ± 10.8
VBT_{PR} (VUT_{PR}) (min^2)	3799 ± 1533	4130 ± 975
	(b) PR-G	
$F_{PR\text{-}G}$ (%)	26.4 ± 5.8	6.0 ± 4.3
$MBT_{PR\text{-}G}$ ($MUT_{PR\text{-}G}$) (min)	82.4 ± 28.1	304.0 ± 236.0
$VBT_{PR\text{-}G}$ ($VUT_{PR\text{-}G}$) (min^2)	5980 ± 3719	82600 ± 109700

lution of PR and PR-G in bile, not to acceleration of its plasma disappearance. Moreover, since the mean residence time in the intrahepatic space (MHT_{el}) is larger than the mean residence time in plasma (MRT), the enhancement of metabolism is considered to have little relation to the accelerated plasma disappearance of PR.

In conclusion, the proposed analytical method can identify individual contributions of many factors in hepatobiliary transport system to drug elimination, and for the effect of PB treatment on PR disposition, it was elucidated that increased plasma disappearance of PR caused by PB treatment was due to increase in liver weight and hepatic uptake rate (and probably blood flow rate) but was not due to metabolism enhancement and choleresis.

APPENDIX

In the present analysis, the hepatobiliary transport process was considered as a "black-box," and the drug disposition function of the liver is thought to be reflected in the biliary concentration–time profile following the input function of the plasma concentration–time profile. Then, the deconvolution analysis was performed using two sets of moments, and the hepatobiliary transfer moments were calculated. The obtained moments were the same as those calculated from the biliary concentration–time profile after pulse input from the sinusoidal membrane of the hepatocyte (16). In general linear kinetics, the Laplace transform of the output function obtained by an unit pulse input is defined by the transfer function and the moments are also defined by the transfer function as

$$FH = \lim_{s \to 0} f(s) \quad (A1)$$

$$MHT = -\lim_{s \to 0} d/ds\,[\ln f(s)] \quad (A2)$$

$$VHT = \lim_{s \to 0} d^2/ds^2\,[\ln f(s)] \quad (A3)$$

where $f(s)$ is the transfer function and s is the Laplace operator.

This theoretical background was essentially discussed by Weiss *et al.* (29,30), using the well-stirred and finite mass transfer models. Other types of finite mass transfer are also dealt with (19). In the present case, the hepatobiliary transport seems to be described by finite mass transfer with central elimination (19,30). Denoting the input (sinusoidal) and output (biliary) intrahepatic concentrations of PR by C_p and C_b, the mass balance equation is defined as

$$V_1\, dCb/dt = CL_{trans}(C_p - C_b) - (k_{10} + k_{12})\, V_1\, C_b + k_{21}\, V_2\, C_h \quad (A4)$$

$$V_2\, dCh/dt = k_{12}\, V_1\, C_b - k_{21}\, V_2\, C_h \quad (A5)$$

where V_1 and V_2 are the distribution volumes of the intrahepatic central and peripheral compartments, C_h is the intrahepatic peripheral concentration of PR, CL_{trans} is the hepatobiliary transfer clearance, and k_{10}, k_{12}, and k_{21} are the metabolic and intrahepatic transfer rate constants between the two compartments, respectively. If rapid equilibrium is attained between plasma and tissue, V_1 in Eq. (A4) should include the distribution volumes for all tissues connected with plasma. Consequently, this analysis can be applied only to drugs which show unidirectional transport from plasma to bile or urine. Laplace transformation of Eqs. (A4) and (A5) gives the transfer function, $f(s)$, as

$$f(s) = \frac{CL_{trans}\,(s + k_{21})}{[V_1\, s + CL_{trans} + V_1(k_{10} + k_{12})](s + k_{21}) - V_1\, k_{12}\, k_{21}} \quad (A6)$$

and substitution into Eqs. (A1) – (A3) yields

$$FH = CL_{trans}/(CL_{trans} + V_1\, k_{10}) \quad (A7)$$

$$MHT = V_1(k_{12} + k_{21})/[k_{21}(CL_{trans} + V_1\, k_{10})] \quad (A8)$$

$$VHT = MHT^2 + 2FH\, V_1\, k_{12}/(CL_{trans}\, k_{21}^2) \quad (A9)$$

The extent of elimination can be assessed by moments such as $AUBC_{PR}$ (F_{PR}), $AUBC_{PR\text{-}G}$ ($F_{PR\text{-}G}$), and FH. The parameter of intrahepatic elimination rate, mean intrahepatic residence time (MHT_{el}), is model-independently derived as *in vivo* or single-pass organ perfusion systems (29,30).

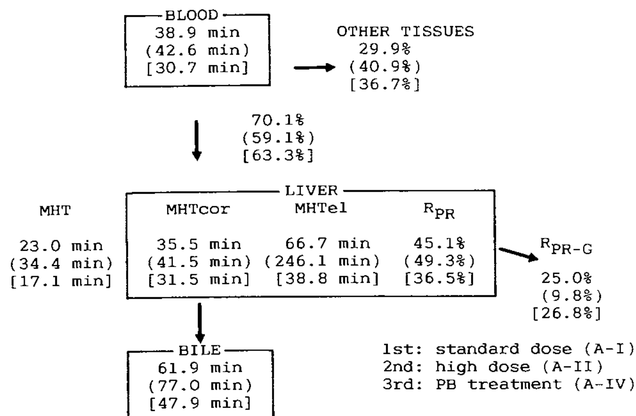

Fig. 2. A scheme explaining hepatobiliary transport processes of PR and the effect of dose elevation (System A-II, parentheses) and PB treatment (System A-IV, brackets). It is noted MBT = MHT + MRT, $MHT^{-1} = MHT_{cor}^{-1} + MHT_{el}^{-1}$.

Therefore, MHT_{el} is defined by using Eqs. (A7) and (A8) as follows:

$$MHT_{el} = MHT/(1 - FH) = (k_{12} + k_{21})/(k_{10}\, k_{21}) \quad (A10)$$

MHT_{el} has the same meaning as MRT in the *in vivo* system (19), and MRT is well-known to be a useful index for drug elimination.

The first moment MHT is the apparent time value necessary for intrahepatic transfer. That is, as the elimination rate increases, MHT decreases. MHT can be divided into MHT_{el} and the true index of intrahepatic transfer (MHT_{cor}), and MHT_{cor} is expressed as $MHT_{cor} = MHT/FH$ (30). In this definition,

$$MHT_{cor} = MHT/FH = V_1\,(1 + k_{12}/k_{21})/CL_{trans} \quad (A11)$$

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