Pharmacokinetics of Propranolol and Its Metabolites in Horses after Intravenous or Oral Administration

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The pharmacokinetics characteristics of propranolol (PPL) in horses was studied by administering the drug intravenously or orally to the animals. The predominant primary pathway was ring oxidation, and 4-hydroxypropranolol glucuronide (4-OHPG) was the major metabolite in both plasma and urine. Side-chain glucuronidation and oxidation were not significant. A two-compartment model was employed for PPL followed by a one-compartment model for 4-OHPG. After oral administration, one-step absorption and two-step first pass metabolism were employed. The fraction absorbed of PPL was approximately 70% after oral administration, and the bioavailability varied among individual horses from 1 to 79% depending on the first pass metabolism. The biologic half-life ($T_{1/2}$) of PPL obeys the allometric equation in some animal species including rats and horses, except for human. $T_{1/2}$ of PPL in horses was approximately 2 h.

Key words pharmacokinetics; horse; propranolol; 4-hydroxypropranolol; first pass metabolism

Literature on pharmacokinetic studies in horses has recently increased. Few reports however, describe drug metabolites, although a large number of drugs are excreted in urine as their metabolites. Dope testing in horse races is carried out in Japan using urine samples, and blood samples are also used when urine samples cannot be collected. Drug metabolites are detectable in the plasma samples because they are tested a minimum of 3 h after the dosing. The metabolism of drugs and the types of metabolites are different among animal species, so it is important to study pharmacokinetics of doping drugs including their metabolites.

Although the factors that control pharmacokinetics tend to depend on an allometric equation, studies have been done on several species that are smaller than human. It is not clearly understood whether the allometric equations can be adapted to horses which are larger than human, therefore, studies with horses would supply useful information about the pharmacokinetics of a drug.

Propranolol (PPL) is a non-selective β -adrenergic blockade widely used in clinical practice for the treatment of cardiac disorders and hypertension. It is also misused as a doping agent in some sports because of its sedative effect^(1,2) and is prohibited for use by the International Olympic Committee. PPL affects the physical ability of racehorses by decreasing endurance when it is injected intravenously⁽³⁾; this is also true in human.^(4–7) The pharmacokinetics of PPL varies greatly in human following oral administration^(1,8) but there is no comparable report on horses. PPL metabolism follows three primary metabolic pathways,⁽⁶⁾ ring oxidation, side-chain oxidation and side-chain glucuronidation, and the metabolites excreted in the urine of rats, hamsters, dogs, and human have their own characteristics.^(9–11)

This paper reports the pharmacokinetics of PPL and its major metabolite, 4-hydroxypropranolol glucuronide (4-OHPG), in horses after intravenous or oral administration to learn its disposition in these animals, *i.e.* the elimination rate and the metabolites in plasma or urine under normal feeding.

MATERIALS AND METHODS

Materials Racemic-PPL hydrochloride, racemic-oxprenolol hydrochloride and racemic-labetalol hydrochloride were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.) and Inderal tablets (20 mg each) were from Zeneca (Osaka, Japan). β -glucuronidase (from *Pomacea canaliculata*, Type A-I, 22000 Fishman units/ml) was obtained from Nippon Bio-test Laboratories (Tokyo, Japan). Racemic-4-hydroxypropranolol (4-OHP) hydrochloride was synthesized in our laboratory. Bond Elut Certify LRC (130 mg) from Varian (Harbor, CA, U.S.A.) was used after conditioning with 3 ml of methanol, 2 ml of water and 2 ml of 0.1 M phosphate buffer (pH 6.0). All other chemicals were of HPLC grade or special grade.

Drug Administration Healthy geldings (castrated male horses) were used which were fed three times a day and had free access to water (Table 1). The horses were kept in stalls 12 h before and after drug administration, respectively. Eight mg/ml of PPL hydrochloride saline solution was intravenously injected *via* the jugular vein at the dose of 0.2 mg/kg, or Inderal tablets which were broken into powder were administered orally at the dose of 1.0 mg/kg as PPL hydrochloride with 2 l of water using a catheter. Blood was withdrawn through the jugular vein, and plasma was obtained from the blood immediately. Urine samples were collected at designated times. The samples were stored at -40°C . The administrations were done in the morning after an overnight fast. All animals were allowed to access feed and water freely after drug administration.

Table 1. Animal Parameters

Horse No.	Strain	Sex	Age (years)	Body weight (kg)
1	TB	G	9	520
2	TB	G	12	490
3	HB	G	13	600
4	TB	G	9	515
5	TB	G	7	542

TB, thoroughbred; HB, half-bred; G, gelding.

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Instruments LC-10AD HPLC system with RF-535 fluorescence HPLC monitor (Shimadzu, Kyoto, Japan) was used. The column was an Asahipak ODP 50 packed column (4.6 mm i.d.×150 mm, Showa Denko, Tokyo) with an Asahipak ODP 50G guard column (4.6 mm i.d.×10 mm). The mobile phase consisted of water–acetonitrile–acetic acid (85:14:1) for plasma PPL, water–acetonitrile–tetrahydrofuran–acetic acid (88:6:5:1) for plasma 4-OHP and water–acetonitrile–tetrahydrofuran–acetic acid (83:14:2:1) for urinary 4-OHP. The flow rate was set at 1.0 ml/min at 40 °C. The fluorometer was set at an excitation wavelength of 280 nm for PPL and 300 nm for 4-OHP, and at an emission wavelength of 345 nm for PPL and 420 nm for 4-OHP.

The GC-MS system (Hewlett–Packard, Palo Alto, CA, U.S.A.) consisted of a gas chromatograph model 5890 and a mass-selective detector model 5989B. The column was a DB-5MS (0.25 mm i.d.×15 m, 0.25 μ m of film thickness, J and W Scientific, Folsom, CA, U.S.A.). The temperature was programmed as follows: injector 250 °C, and oven from 50 °C (2 min) to 170 °C at 30 °C/min and thereafter from 170 °C to 300 °C at 10 °C/min. The transfer line was kept at 280 °C. Detection was performed with selected ion monitoring at m/z 266.

Methods. Identification of Urinary Metabolites Identification of urinary metabolites was performed by the method described by Wynne *et al.*¹²⁾ as follows: 3 ml of urine was hydrolyzed with β -glucuronidase and was applied on a Bond Elut Certify, and then its basic extract and acidic extract were analyzed by GC-MS.

Sample Preparation for Assay. Plasma Levels of PPL or PPL Glucuronide The determination of PPL or PPL glucuronide (PPLG) in plasma was made by HPLC. The concentration was measured as follows: 1 ml of plasma was added with 5 μ g of oxprenolol (OXP) as an internal standard and 2 ml of methanol, after mixing by vortex for 20 s, the sample was centrifuged at 3000 rpm for 10 min. The supernatant was diluted with 20 ml of 0.1 M phosphate buffer (pH 6.0) and was loaded on a conditioned Bond Elut Certify column. The column was washed with 5 ml of 0.1 M phosphate buffer (pH 6.0), and the same amount 0.1 M acetic acid and

methanol; it was then dried *in vacuo* and eluted with 5 ml of ethylacetate containing 1% of ammonia solution. After drying in a stream of nitrogen at 40 °C, the residue was dissolved in 250 μ l of mobile phase of HPLC and 100 μ l of aliquot was injected. The concentration of PPLG was measured using 0.5 ml of plasma which had been hydrolyzed with β -glucuronidase by incubation at 37 °C for 17 h. The sample was added with 5 μ g of OXP and 2 ml of methanol, and then was treated using the same procedure as for PPL.

The calibration curves for plasma concentration of PPL and PPLG were linear in 1 (non-hydrolyzed) or 2 (hydrolyzed)–500 ng/ml of plasma ($r \leq 0.999$). The coefficients of variation (CV) in a day were less than 10%, and the recoveries were more than 73% (1 and 10 ng/ml plasma of PPL or 5 μ g/ml plasma of OXP).

Urine Levels of PPL or PPLG The determination of PPL or PPLG concentration in urine was made by GC-MS as follows: 0.5 ml of urine was added with 5 ng of OXP and adjusted to pH 12 with 2 M NaOH, then extracted with 2 ml of ethylacetate. After drying in a stream of nitrogen at 40 °C, the residue was derivatized with trifluoroacetic anhydride, dissolved in 100 μ l of ethylacetate and 1 μ l of aliquot was injected into GC-MS. The urinary concentration of PPLG was measured as follows: 0.2 ml of urine was added with 10 ng of OXP and hydrolyzed with 100 μ l of β -glucuronidase (2200 units) by incubating at 50 °C for 2 h. The hydrolyzed urine was treated by the same procedure as urinary PPL.

The calibration curves of urinary concentration of PPL and PPLG were linear in 1–1000 ng/ml ($r \leq 0.999$). The CV values in a day were less than 10%, and the recoveries were more than 71% (50 ng/ml urine of PPL or 10 ng/ml urine of OXP).

Plasma or Urine Levels of 4-OHPG Glucuronized 4-OHP in plasma or urine was extracted by the method of Kwong and Shen¹³⁾ and Pritchard *et al.*¹⁴⁾ with some modifications: 0.5 ml of plasma or urine was added with 200 ng/ml plasma or 500 ng/ml urine of labetalol (LAB) as an internal standard. Then, 1 ml of 1 M acetate buffer (pH 5.0), 100 μ l of 20% ascorbic acid solution and 100 μ l of β -glucuronidase were added. After incubating at 37 °C for 4 h, the sample was

Fig. 1. Model Describing Pharmacokinetics of PPL and Its Metabolites after Intravenous or Oral Administration of PPL-HCl

X 's with subscript are amount of drug in the respective compartment. k 's with subscript are first order rate constant of the respective process. V_{PPL} is the distribution volume of central compartment for PPL. V_{4-OHPG} is the distribution volume of 4-OHPG.

added with 100 μ l of 20% ascorbic acid solution and 1.2 ml of 1 M carbonate buffer (pH 10.0), then extracted with 4 ml of ethylacetate. The ethylacetate phase was back-extracted with 250 μ l (plasma) or 500 μ l (urine) of 0.25 M sulfuric acid containing 1% of ascorbic acid. The ethylacetate phase was discarded, the aqueous phase was washed with 3 ml of hexane, and 100 μ l (plasma) or 20 μ l (urine) of aliquot was injected into HPLC.

The calibration curves were linear ($r \leq 0.999$) in 10–200 ng/ml (plasma) or 0.1–50 μ g/ml (urine). The CV values in a day were less than 8%, respectively. The recoveries of 4-

OHP were more than 78% (50 or 100 ng/ml plasma) or more than 63% (0.5 or 50 μ g/ml in urine), and those of LAB were 52% (400 ng/ml plasma) or 59% (10 μ g/ml urine).

Pharmacokinetic Analysis Model adaptation was carried out using Scientist (MicroMath Scientific Software, Salt Lake City, U.S.A.), after being pre-analyzed by Win Nonlin (Scientific Consulting, U.S.A.). The model used for pharmacokinetic analysis is illustrated in Fig. 1. A two-compartment model was assumed for PPL followed by a one-compartment model for 4-OHPG in plasma. After oral administration, one-step absorption and two-step first pass metabolism were em-

Fig. 2. Plasma Concentration of PPL and Its Metabolites after Intravenous Administration at 0.2 mg/kg of PPL-HCl or Oral Administration at 1.0 mg/kg of PPL-HCl

●, PPL; ○, PPLG; □, 4-OHPG. Symbols are observed data. Solid curves and broken curves are model estimations for PPL and 4-OHPG, respectively.

ployed.

Mathematical expression of the model is as follows:

intravenous administration

$$C_{PPL} = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

$$C_{4\text{-OHPPG}} = K_0 \left\{ \frac{A}{k_{el} - \alpha} (e^{-\alpha t} - e^{-k_{el} t}) + \frac{B}{k_{el} - \beta} (e^{-\beta t} - e^{-k_{el} t}) \right\} \quad (2)$$

oral administration

$$C_{PPL} = Ae^{-\alpha t} + Be^{-\beta t} - (A+B)e^{-K_A t} \quad (3)$$

$$C_{4\text{-OHPPG}} = K_0 \left[\frac{A}{k_{el} - \alpha} (e^{-\alpha t} - e^{-k_{el} t}) + \frac{B}{k_{el} - \beta} (e^{-\beta t} - e^{-k_{el} t}) - \frac{A+B}{k_{el} - K_A} (e^{-K_A t} - e^{-k_{el} t}) + K_{11} \left\{ \frac{e^{-K_A t}}{(K_F - K_A)(K_{el} - K_A)} + \frac{e^{-K_{el} t}}{(K_F - K_A)(K_{el} - K_{el})} \right\} \right] \quad (4)$$

where the definitions of parameters are given in the appendix. We assumed that the ratio of individual metabolite formed by pre-systemic metabolism was equal to that formed by systemic metabolism: $k_g/(k_g + k_{\text{other}3}) = k_{4\text{-OHPPG}}/(k_{4\text{-OHPPG}} + k_{\text{other}2})$. PPL and 4-OHPPG were fitted simultaneously in each administration route, and K_0 value and volume of distribution for PPL obtained from the intravenous administration data were used in the oral administration data analysis.

RESULTS

Plasma Levels and Urinary Excretion of PPL and Its Metabolites In plasma, PPL and 4-OHPPG were found after both intravenous and oral administration (Fig. 2), while unconjugated 4-OHP was not found after either. After oral ad-

ministration, PPL concentration in plasma reached a peak at 45 min—1 h, a very short period, especially in horse No. 1 it quickly reached a peak within 15 min. Plasma levels of 4-OHPPG reached their peaks at 1—3 h after intravenous injection, and 45 min—2 h after oral administration. Plasma half-life (β phase) of PPL was 1.9—2.9 h, and that of 4-OHPPG was 1.8—3.2 h. The bioavailability varied strikingly from 1 to 79 % among individual horses.

In urine, the major basic metabolite was 4-OHPPG, and there were trace amounts of other metabolites; acidic metabolite was not found. The cumulative amount of metabolites excreted in urine until 12 h after dosing are shown in Table 2 as % of the dose. The extent of 4-OHPPG excreted was at 15—36% and 12—26% of the dose in the urine of intravenously or orally administered horses, respectively. PPL was excreted at a maximum 0.1% of the dose following both types of administration.

Pharmacokinetics of PPL and Its Metabolites Since the major metabolite was 4-OHPPG in both plasma and urine, the pharmacokinetics of PPL and 4-OHPPG were analyzed. Preliminary calculation using the Win Nonlin program showed that the area under the concentration—time curve (AUC) ratio of 4-OHPPG/PPL was 4.5 after intravenous injection.

Table 2. Cumulative Amounts of Urinary Excretion of PPL and Its Metabolites until 12 Hours after Administration (% of Dose)

Horse No.	Intravenous administration			Oral administration		
	PPL	PPLG	4-OHPPG	PPL	PPLG	4-OHPPG
1	0.02	0.18	22.61	0.07	0.05	12.27
2	0.03	0.16	33.67	0.02	0.24	20.27
3	0.05	0.32	36.46	0.01	0.23	26.16
4	0.02	0.10	15.44	0.01	0.12	12.88
5	0.03	0.19	30.53	0.01	0.10	22.35
Mean	0.03	0.19	27.74	0.02	0.15	18.79
± S.D.	0.011	0.083	8.610	0.025	0.082	6.053

Table 3. Estimated and Derived Parameters after Intravenous Administration at 0.2 mg/kg of PPL-HCl in Horses^{a)}

		Horse No.					Mean ± S.D.
		1	2	3	4	5	
Dose (mg PPL)		91.2	86.0	105.3	90.4	95.1	93.6 ± 7.3
Estimated parameters							
A	(ng/ml)	78.77	56.74	78.12	89.71	61.71	73.01 ± 13.51
α	(h ⁻¹)	2.009	0.8818	1.892	2.268	0.7192	1.554 ± 0.7035
B	(ng·ml)	20.88	19.96	26.39	40.03	14.75	24.40 ± 9.663
β	(h ⁻¹)	0.3346	0.2992	0.3647	0.3231	0.2314	0.3106 ± 0.05015
K_0	(h ⁻¹)	1.077	1.473	2.074	0.6268	1.196	1.289 ± 0.5344
k_{el}	(h ⁻¹)	0.2237	0.3177	0.3761	0.2172	0.2566	0.2783 ± 0.06764
Derived parameters							
AUC_{PPL}	(ng·h/ml)	101.6	131.1	113.7	163.4	149.5	131.9 ± 25.29
CL_{tot}	(l/h/kg)	1.726	1.339	1.544	1.074	1.173	1.371 ± 0.2666
V_{PPL}	(l/kg)	1.760	2.288	1.679	1.353	2.295	1.875 ± 0.4096
$V_{ss,PPL}$	(l/kg)	3.500	3.024	2.992	2.634	3.097	3.049 ± 0.3087
$V_{\beta,PPL}$	(l/kg)	5.158	4.476	4.234	3.324	5.070	4.453 ± 0.7420
$AUC_{4\text{-OHPPG}}$	(ng·h·ml)	489.2	607.6	626.7	471.7	697.0	578.5 ± 95.66
$CL_{R,4\text{-OHPPG}}$	(l/h/kg)	0.08103	0.09716	0.1020	0.05739	0.07680	0.0829 ± 0.01774
$V_{4\text{-OHPPG}}$	(l/kg)	0.3622	0.3058	0.2712	0.2642	0.2993	0.3006 ± 0.03878
K_E	(h ⁻¹)	0.9807	0.5852	0.9196	0.7938	0.5113	0.7581 ± 0.2048
$k_{4\text{-OHPPG}}$	(h ⁻¹)	0.2217	0.1969	0.3349	0.1224	0.1560	0.2064 ± 0.08130

a) The parameters were estimated by converting 4-OHPPG into PPL equivalent.

Table 4. Estimated and Derived Parameters after Oral Administration at 1.0 mg/kg of PPL-HCl in Horses^{a)}

	Dose (mg PPL)	Horse No.					Mean ^{b)} ±S.D.
		1	2	3	4	5	
		456.0	430.0	526.0	452.0	475.0	470.8±41.2
Estimated parameters ^{c)}							
K_A	(h ⁻¹)	4.699	2.449	1.269	2.237	6.165	3.030±2.152
A	(ng/ml)	550.1	58.38	91.18	10.36	2.873	40.70±41.68
α	(h ⁻¹)	1.982	1.143	1.157	— ^{d)}	— ^{d)}	1.150±0.009899
B	(ng/ml)	82.56	5.066	3.019	— ^{d)}	— ^{d)}	4.043±1.447
β	(h ⁻¹)	0.3036	0.3175	0.2363	0.5100	0.3457	0.3524±0.1149
K_F	(h ⁻¹)	15.50	10.25	1.276	2.404	2.826	4.189±4.0933
K_H	(ng/ml/h)	4742	9399	597.1	2374	11720	6023±5373
k_{el}	(h ⁻¹)	0.2395	0.2259	0.2569	0.2053	0.2600	0.2370±0.02616
Derived parameters							
AUC_{PPL}	(ng·h/ml)	414.8	41.13	17.35	15.68	7.845	20.50±14.36
AUC_{4-OHPG}	(ng·h/ml)	2158	2710	3117	2872	3131	2957±203.4
$CL_{R,4-OHPG}$	(l/h/kg)	0.04983	0.06558	0.07357	0.03932	0.06259	0.06026±0.01471
V_{4-OHPG}	(l/kg)	0.2080	0.2903	0.2864	0.1915	0.2407	0.2522±0.04629
k_{ab}	(h ⁻¹)	3.729	0.2272	0.02553	0.05375	0.05906	0.09138±0.09172
k_{fp}	(h ⁻¹)	0.5852	2.068	0.5426	1.513	5.065	2.297±1.950
k_B	(h ⁻¹)	2.071	2.216	0.7455	0.4422	0.7599	1.041±0.7971
F	(%)	79.36	9.277	2.012	2.403	0.9581	3.662±3.792
Fraction absorbed (%)		91.81	93.74	44.77	70.04	83.12	72.92±21.12
K_E	(h ⁻¹)	0.9529	0.8642	0.6053	0.5100	0.3457	0.5813±0.2170
k_{4-OHPG}	(h ⁻¹)	0.1273	0.1869	0.3537	0.09380	0.09296	0.1818±0.1228

a) The parameters were estimated by converting 4-OHPG into PPL equivalent. b) Calculated with four horses (horse No. 2—horse No. 5), because A , α , B , AUC_{PPL} , k_{ab} and F for horse No. 1 were statistically different from those for the other four. c) K_0 value was fixed to that obtained with intravenous administration data. However, for horse No. 4 the average value of the other four horses was used, because the small K_0 value in horse No. 4 (due to the small AUC_{4-OHPG} after intravenous administration) led to $K_A < k_{ab} + k_{fp}$, which was unrealistic. d) One-compartment model assumed for PPL, since the two-compartment model was not applicable.

Fig. 3. Scheme Describing PPL Disposition after Oral Administration in Horses (Average Value of Four Horses)

tion and 5—400, which was extensively large, after oral administration, suggesting pre-systemic formation of 4-OHPG.

Based on these results, the model illustrated in Fig. 1 was constructed. As shown in Fig. 2, the observed values agreed well with predicted curves that were drawn using the non-linear least squares method. Accordingly, the pharmacokinetic parameters obtained are shown in Tables 3 and 4. Definitions of estimated and derived parameters are given in the appendix. Figure 3 illustrates the disposition of PPL in horses using the average parameters estimated by the model. The diverse profile of horse No. 1 is attributed to the large bioavailability and small first pass absorption. Since several parameters for this animal were statistically different from those for the other four horses (Smirnov-Grubbs' test, $p < 0.05$), we calculated the average and standard deviation of parameters in Table 4 and illustrated Fig. 3 using the parameters for the other four horses.

DISCUSSION

There are many reports on the determination of PPL in plasma,^{15–19} however, the detection limits are 1 ng/ml or higher. The plasma levels of PPL could not be determined by those methods when PPL was orally administered to horses at the usual dose used for human, because the plasma level was very low. The method using a solid phase extraction with Bond Elut Certify followed by analysis of HPLC with a fluorometer improved the detection limit, which was 0.1 ng/ml.

The major metabolite of PPL in horse urine was 4-OHPG, which accounted for approximately 23% of the dose after intravenous or oral administration (Table 2). The cumulative amount of urinary excretion of 4-OHPG in horses was twice as large as that in human,⁹ and three times as large as that in dogs.^{11,20} On the contrary, PPLG, which was excreted at 17% and 7—16% of the dose in man and dogs,^{9,20,21} was found to be less than 0.3% of the dose. Naphtoxylactic acid, reported to be the major metabolite in both human and dogs^{10,11,20} was not found.

The AUC ratio of PPLG/PPL was 0.0002—0.013 after oral administration, and PPLG was not found in the plasma after intravenous administration in horses; in contrast, the ratio in human was reported to be 1 or 7 after intravenous or oral administration, respectively.²¹

These results demonstrated that the primary metabolism pathway of PPL in horses is predominantly ring oxidation as true in rats,¹⁰ and the side-chain glucuronidation and oxidation are not significant. The facts that the pathway from PPL to 4-OHPG could be described as a single step, although it is a two step pathway, and that unconjugated 4-OHP was not found in plasma suggest that the rate of metabolism from 4-

Table 5. Systemic and Pre-systemic Formed Fractions of 4-Hydroxypropranolol Glucuronide (4-OHPG) Calculated by *AUC* Values of Propranolol and 4-OHPG

	Horse No.				
	1	2	3	4	5
<i>AUC</i> _{i.v.} ratio ^{a)}	4.81	4.64	5.51	2.89	4.66
Systemic <i>AUC</i> _{4-OHPG, p.o.} (ng · h/ml) ^{b)}	2000	191	95.7	45.3	36.6
Pre-systemic <i>AUC</i> _{4-OHPG, p.o.} (ng · h/ml) ^{c)}	158	2519	3021	2827	3084
Pre-systemic <i>AUC</i> _{4-OHPG, p.o.} fraction ^{d)}	0.0733	0.930	0.969	0.980	0.988
Systemic <i>AUC</i> _{4-OHPG, p.o.} fraction ^{e)}	0.927	0.0704	0.0307	0.0158	0.0117

a) $AUC_{4-OHPG, i.v.}/AUC_{PPL, i.v.}$, b) $a \times AUC_{PPL, p.o.}$, c) $AUC_{4-OHPG, p.o.} - b$, d) $c/AUC_{4-OHPG, p.o.}$, e) $b/AUC_{4-OHPG, p.o.}$.

Fig. 4. Relationship between the Fraction of 4-OHPG Formed Systemically after Oral Administration of PPL and Bioavailability of PPL

The bioavailability of PPL was calculated by $AUC_{PPL, p.o.} \times Dose_{i.v.}/AUC_{PPL, i.v.} \times Dose_{p.o.}$.

OHP to 4-OHPG is instantaneous.

It is generally known that the bioavailability of PPL is low because of its great first pass metabolism, and the bioavailability reported for rats,²²⁾ rabbits,²³⁾ dogs^{20,24,25)} and human^{26–28)} is approximately 10–30%. In horses, the bioavailability of 4% in Table 4 was smaller than other animal species. The variation of bioavailability, however, was also pronounced among horses; these variations might be due to changes in hepatic blood flow. The horses were allowed to feed after dosing in this study. It has been reported that hepatic blood flow is significantly increased by the intake of food.²⁹⁾ The cause for this should be explored further.

The fraction absorbed of PPL after oral administration in the individual horse was estimated. First, the fraction of 4-OHPG formed systemically to the total appearance of 4-OHPG after oral administration, which was calculated by the ratios of *AUC* values of PPL and 4-OHPG after intravenous and oral administration, is shown in Table 5. The fractions of 4-OHPG formed systemically were 93% for horse No. 1 and 1–7% for horse Nos. 2–5. The relationship between these fractions and the bioavailability of PPL is represented in Fig. 4; their relationships can be expressed as a straight line went through origin. This suggested that the fraction absorbed of PPL being expressed as the slope of the plot remained constant among horses, with a value of 88%. Consequently, the

Fig. 5. Relationships between Pharmacokinetic Parameters and Body Weights of Mammalian Species

A, $T_{1/2}$ and body weight; B, CL_{tot} and body weight; C, V_{β} and body weight. Lines are arbitrary.

variation of bioavailability of PPL in horses could be explained by pre-systemic metabolism, not incompleteness of absorption.

To examine the relationship between the pharmacokinetic

parameters, *i.e.* CL_{tot} , V_β and $T_{1/2}$, of PPL, and body weights in various animal species,^{1,20–22,24–28,30–37} the parameters are represented in Fig. 5 as functions of body weights using the logarithmic scale. The curve for medians of $T_{1/2}$ of each animal except human was linear, $T_{1/2}=AW^B$ ($A=0.88787$, $B=0.15753$, $r=0.996$, the unit of $T_{1/2}$ is h and that of W is kg). The correlation of $T_{1/2}$ and body weight was also seen when caffeine was dosed.³⁸ However, the $T_{1/2}$ of human deviated from this correlation, and the $T_{1/2}$ of horses was shorter than that of human. CL_{tot} tend to be smaller as the body weight becomes heavier, but there was no correlation. There was no clear relationship between V_β and body weight.

This study was carried out with racemic PPL. An Individual optical enantiomer of PPL has its own specific activities,³⁹ and studies have shown that $S(-)$ -PPL is 100 times more potent as a β -receptor antagonist than the $R(+)$ -PPL.⁴⁰ The stereoselective disposition of PPL is varied in species, *e.g.* human,⁴¹ dogs,⁴² rabbits⁴² and rats,⁴³ and doses.^{37,44} There has been no report on stereoselective pharmacokinetics in horses, and a stereoselective study is necessary.

CONCLUSION

The following pharmacokinetic characteristics in horses were recognized. The ring oxidation was the predominant primary pathway and 4-OHPG was the major metabolite in both plasma and urine. Side-chain glucuronidation and oxidation were not significant. The fraction absorbed of PPL was approximately 70% after oral administration. The bioavailability was low in comparison with other animal species, and the large clearance of PPL might cause $T_{1/2}$ of PPL in these animals to be shorter than that of human.

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Appendix 1. Definition of Model Parameters

(1) Intravenous administration

$$K_E = k_{other2} + k_{4-OHPG}$$

$$\alpha + \beta = k_{12} + k_{21} + K_E$$

$$\alpha\beta = k_{21} \times K_E$$

$$A = \frac{\text{Dose}_{i.v.}(\alpha - k_{21})}{(\alpha - \beta)V_{PPL}}$$

$$B = \frac{\text{Dose}_{i.v.}(k_{21} - \beta)}{(\alpha - \beta)V_{PPL}}$$

$$K_0 = k_{4-OHPG} \times \frac{V_{PPL}}{V_{4-OHPG}}$$

(2) Oral administration

$$K_A = k_{ab} + k_{fp} + k_{other1}$$

$$K_F = k_g + k_{other3}$$

$$A = \frac{\text{Dose}_{p.o.}(k_{21} - \alpha) \times k_{ab}}{(\alpha - \beta)(\alpha - K_A)V_{PPL}}$$

$$B = \frac{\text{Dose}_{p.o.}(k_{21} - \beta) \times k_{ab}}{(\alpha - \beta)(K_A - \beta)V_{PPL}}$$

$$K_H = \frac{\text{Dose}_{p.o.} \times k_{fp} \times K_F}{V_{PPL} \times K_E}$$

Appendix 2. Derived Parameters of Table 3

$$C_0 = A + B$$

$$k_{21} = \frac{A\beta + B\alpha}{C_0}$$

$$K_E = \frac{\alpha\beta}{k_{21}}$$

$$k_{12} = \alpha + \beta - (k_{21} + K_E)$$

$$V_{PPL} = \frac{\text{Dose}_{i.v.}}{C_0}$$

$$V_{ss, PPL} = V_{PPL} \left(1 + \frac{k_{12}}{k_{21}} \right)$$

$$V_{\beta, PPL} = V_{PPL} \left(\frac{\alpha}{k_{21}} \right)$$

$$V_{4-OHPG} = \frac{V_{PPL} \times K_E \times X_{u, 4-OHPG}^\infty}{\text{Dose}_{i.v.} \times K_0}$$

$$CL_{tot, PPL} = V_{PPL} \times K_E$$

$$CL_{R, 4-OHPG} = V_{4-OHPG} \times k_{el}$$

$$k_{4-OHPG} = \frac{K_0 \times V_{4-OHPG}}{V_{PPL}}$$

$$AUC_{PPL} = \frac{\text{Dose}_{i.v.}}{V_{PPL} \times K_E}$$

$$AUC_{4-OHPG} = AUC_{PPL} \times \frac{K_0}{k_{el}}$$

Appendix 3. Derived Parameters of Table 4

$$k_{21} = \frac{\beta A(K_A - \alpha) + \alpha B(K_A - \beta)}{A(K_A - \alpha) + B(K_A - \beta)}$$

$$K_E = \frac{\alpha\beta}{k_{21}}$$

$$k_{12} = (\alpha + \beta) - (k_{21} + K_E)$$

$$AUC_{PPL, p.o.} = \frac{A}{\alpha} + \frac{B}{\beta} - \frac{A+B}{K_A}$$

$$AUC_{4-OHPG, p.o.} = \frac{AUC_{PPL, p.o.} \times K_0}{k_{el}} + \frac{K_0 \times K_H}{K_A \times K_F \times k_{el}}$$

$$V_{4-OHPG} = \frac{X_{u, 4-OHPG}^\infty}{AUC_{4-OHPG, p.o.} \times k_{el}}$$

$$k_{ab} = \frac{K_A \times AUC_{PPL, p.o.} \times K_E \times V_{PPL}}{\text{Dose}_{p.o.}}$$

$$k_{fp} = \frac{K_H \times K_E \times V_{PPL}}{\text{Dose}_{p.o.} \times K_F}$$

$$\text{bioavailability (F)} = \frac{k_{ab}}{K_A}$$

$$\text{fraction absorbed} = \frac{k_{ab} + k_{fp}}{K_A}$$

REFERENCES

- Gomeni R., Bianchetti G., Sega R., Morselli P.L., *J. Pharmacokinet. Biopharm.*, **5**, 183–192 (1977).
- Park J., Park S., Lho D., Dhoo H.P., Chung B., Yoon C., Min H., Choi M. J., *J. Anal. Toxicol.*, **14**, 66–72 (1990).
- Snow D. H., Summers R. J., Guy P. S., *Res. Vet. Sci.*, **27**, 22–29

- (1979).
- 4) Epstein S. E., Robinson B. F., Kahler R. L., Braunwald E., *J. Clin. Invest.*, **44**, 1745—1753 (1965).
 - 5) Kaiser P., *Acta Physiol. Scand. Suppl.*, **536**, 1—53 (1984).
 - 6) Laustiola K., Uusitalo A., Koivula T., Sovijärvi A., Seppälä E., Nikkari T., Vapaatalo H., *Eur. J. Clin. Pharmacol.*, **25**, 293—297 (1983).
 - 7) Lundborg P., Åström H., Bengtsson C., Fellenius E., Schenck H. V., Svensson L., Smith U., *Clin. Sci.*, **61**, 299—305 (1981).
 - 8) Lho D.-S., Shin H.-S., Kang B.-K., Park J., *J. Anal. Toxicol.*, **14**, 73—76 (1990).
 - 9) Walle T., Walle U. K., Olanoff L. S., *Drug Metab. Dispos.*, **13**, 204—209 (1985).
 - 10) Bargar E. M., Walle U. K., Bai S. A., Walle T., *Drug Metab. Dispos.*, **11**, 266—272 (1983).
 - 11) Walle T., Wilson M. J., Walle U. K., Bai S. A., *Drug Metab. Dispos.*, **11**, 544—549 (1983).
 - 12) Wynne P. M., Dyke T. M., Vine J. H., Lind K. L., Jenkins M. A., Wickramasinghe I., Proceedings of the 11th International Conference of Racing Analysis and Veterinarians, Queensland, Australia, 1996, pp. 470—477.
 - 13) Kwong E. C., Shen D. D., *J. Chromatogr.*, **414**, 365—379 (1987).
 - 14) Pritchard J. F., Schneek D. W., Hayes A. H., Jr., *J. Chromatogr.*, **162**, 47—58 (1979).
 - 15) Caccia S., Guiso G., Ballabio M., Ponte P. De., *J. Chromatogr.*, **172**, 457—462 (1979).
 - 16) Lo M., Riegelman S., *J. Chromatogr.*, **183**, 213—220 (1980).
 - 17) Albani F., Riva R., Baruzzi A., *J. Chromatogr.*, **228**, 362—365 (1982).
 - 18) Yamamura Y., Uchino K., Kotaki H., Isozaki S., Saitoh Y., *J. Chromatogr.*, **374**, 311—319 (1986).
 - 19) Hedeén K. M., Tyczkowska K., Aucoin D. P., Norton R. M., *J. Chromatogr.*, **572**, 239—245 (1991).
 - 20) Vu V. T., Bai S. A., Abramson F. P., *J. Pharm. Exp. Ther.*, **224**, 55—61 (1983).
 - 21) Walle T., Fagan T. C., Conradi E. C., Walle U. K., Gaffney T. E., *Clin. Pharmacol. Ther.*, **26**, 167—172 (1979).
 - 22) Shibasaki S., Asahina M., Kawamata Y., Kojo M., Nishigaki R., Umemura K., *J. Pharmacobio-Dyn.*, **12**, 549—557 (1989).
 - 23) Corbo M., Liu J.-C., Chien Y. W., *J. Pharm. Sci.*, **79**, 584—587 (1990).
 - 24) Bai S. A., Wilson M. J., Walle U. K., Walle T., *J. Pharm. Exp. Ther.*, **227**, 360—364 (1983).
 - 25) Bai S. A., Easterling D. E., Wilson M. J., Walle U. K., Haney C. A., Peet N. P., Pruett J. K., Walle T., *Drug Metab. Dispos.*, **17**, 495—505 (1989).
 - 26) Kornhauser D. M., Wood A. J. J., Vestal R. E., Wilkinson G. R., Branch R. A., Shand D. G., *Clin. Pharmacol. Ther.*, **23**, 165—174 (1978).
 - 27) Borgström L., Johansson C.-G., Larsson H., Lenander R., *J. Pharmacokinet. Biopharm.*, **9**, 419—429 (1981).
 - 28) Sowinski K. M., Lima J. J., Burlew B. S., Massie J. D., Johnson J. A., *Br. J. Clin. Pharmacol.*, **42**, 339—346 (1996).
 - 29) Olanoff L. S., Walle T., Cowart T. D., Walle U. K., Oexmann M. J., Conradi E. C., *Clin. Pharmacol. Ther.*, **40**, 408—414 (1986).
 - 30) Shand D. G., Nuckolls E. M., Oates J. A., *Clin. Pharmacol. Ther.*, **11**, 112—120 (1970).
 - 31) Walle T., Conradi E. C., Walle U. K., Fagan T. C., Gaffney T. E., *Clin. Pharmacol. Ther.*, **27**, 22—31 (1980).
 - 32) Kawashima K., Ishikawa H., *J. Pharm. Exp. Ther.*, **213**, 628—631 (1980).
 - 33) Silber B. E., Holford N. H. G., Riegelman S., *J. Pharm. Sci.*, **72**, 725—732 (1983).
 - 34) Yoshimura M., Kojima J., Ito T., Fujii M., Suzuki J., *J. Pharmacobio-Dyn.*, **8**, 503—512 (1985).
 - 35) Belpaire F. M., Smet F. De., Vynckier L. J., Vermeulen A. M., Rosseel M. T., Bogaert M. G., Chauvelot-Moachon L., *J. Pharm. Exp. Ther.*, **254**, 116—122 (1990).
 - 36) Souich P. D., Maurice H., Héroux L., *Drug Metab. Dispos.*, **23**, 279—284 (1995).
 - 37) Marier J.-F., Pichette V., Souich P. D., *Drug Metab. Dispos.*, **26**, 164—169 (1998).
 - 38) Aramaki S., Suzuki E., Ishidaka O., Momose A., Umemura K., *Chem. Pharm. Bull.*, **39**, 2999—3002 (1991).
 - 39) Stoschitzky K., Lindner W., Egginger G., Brunner F., Obermayer-Pietsch B., Passath A., Klein W., *Clin. Pharmacol. Ther.*, **51**, 445—453 (1992).
 - 40) Barrwtt A. M., Cullum V. A., *Br. J. Pharmacol.*, **34**, 43—55 (1968).
 - 41) Silber B., Holford N. H. G., Riegelman S., *J. Pharm. Sci.*, **6**, 699—704 (1982).
 - 42) Laethem M. E., Belpaire F. M., Wijnant P., Bogaert M. G., *Chirality*, **7**, 616—622 (1995).
 - 43) Piquette-Miller M., Jamali F., *Pharm. Res.*, **10**, 294—299 (1993).
 - 44) Paradiso-Hardy F. L., Walker S. E., Bowles S.K., *Int. J. Clin. Pharmacol. Ther.*, **36**, 370—375 (1998).