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Analysis of hepatic metabolism affecting pharmacokinetics of propranolol in humans

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Abstract

We examined the metabolic kinetics of propranolol, constructed from saturable and non-saturable components, using liver microsomes. The metabolic activity in rat microsomes was much higher than that in human microsomes within the clinically observed plasma range. Using the physiologically based pharmacokinetic (PBPK) model incorporating the obtained metabolic parameters, the plasma kinetics of propranolol was well correlated with reported values, and then used to analyze the effect of hepatic first-pass metabolism on propranolol plasma pharmacokinetics in clinical doses.

The simulated plasma concentrations and AUC values of propranolol increased proportionally to its dose; these levels were almost equivalent to intrinsic clearance (CL_{int1}), presumed to be non-saturable. When Michaelis–Menten parameters were decreased to one twentieth, plasma concentrations slightly increased after 160 mg dosing. A similar result was obtained with steady-state plasma levels after repeated administration. On the other hand, the first-order absorption rate constant of propranolol did not affect AUC values. The dose-normalized AUC value started to increase about 10^3 mg dosing. When the dose exceed 10^6 mg dose, the CL_{int1} component hardly contributed to propranolol pharmacokinetics. Accordingly, under the conditions of the PBPK model, propranolol pharmacokinetics was considered to be dose-independent within the clinical dose range.

Keywords: Propranolol; Pharmacokinetics; Hepatic metabolism; Physiologically based pharmacokinetic model; Human

1. Introduction

Beta-blockers have long been used in the treatment of hypertension, angina pectoris and cardiac arrhythmias, as well as for a number of other indications, e.g., migraine, hyperthyroidism, tremor, etc. Propranolol was the first beta-blocker and is still used throughout the world. Propranolol is known to be a highly-extracted drug in the liver, and its pharmacokinetics has been studied quite extensively (Ludden, 1991); however, certain ambiguities remain, particularly in connection with the dose-dependent bioavailability (BA) after oral administration. The non-linear relationship between the dose and oral BA of propranolol has been described several times (Shand and Rangno, 1972; Routledge and Shand, 1979; Weidler et al., 1979; McAinsh et al., 1981). Other authors claim that there is no correlation between BA and propranolol dosage (Gomeni et al., 1977; Borgström et al., 1981). There are several discussions concerning the dose range, which causes the non-linear pharmacokinetics of orally administered propranolol. For instance, some authors have reported non-linear pharmacokinetics by comparing BA between sustained release dosage forms and conventional tablets (lower BA was obtained after sustained release dosing), because of a saturable first-pass effect (dose:

Abbreviations: BA, bioavailability; PBPK, physiologically based pharmacokinetics; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, NADP, reduced form; G6P, glucose-6-phosphate; G6PDH, G6P dehydrogenase; K_m, Michaelis–Menten constant; V_{max}, maximum velocity of metabolism; $K_{m,app}$, apparent K_m; CL_{int}, intrinsic clearance; $CL_{int,app}$, apparent CL_{int}; v, velocity of microsomal metabolic reaction; $C_{microsomes}$, initial drug concentration in microsomal suspension; f_m, unbound fraction in microsomes; Q, blood flow rate through the tissue; Q_{tot}, total blood flow though the body; V, tissue volume; K_p, tissue-to-blood distribution ratio; AUC, area under the plasma concentration–time curve; T_{max}, peak time; C_{max}, maximum plasma concentration; F_a, fraction absorbed from the intestinal tract; k_a, absorption rate constant; k_{el}, elimination rate constant; f_B, unbound fraction in blood; R_B, blood-to-plasma concentration ratio.

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40–160 mg) (McAinsh et al., 1981), whereas others reported linear propranolol pharmacokinetics between BAs and doses (dose: 40–120 mg) (Borgström et al., 1981).

In the present study, we examined metabolic kinetic parameters, such as the Michaelis constant (K_m) and maximum velocity of metabolism (V_{max}), in vitro using human liver microsomes, and the results were compared with rat data. The physiologically based pharmacokinetic (PBPK) model was constructed for propranolol in humans incorporating these metabolic parameters, reporting biochemical and physiological parameters, and it was confirmed whether the model was adequately correlated with reported data. Using this PBPK model, factors affecting propranolol pharmacokinetics, such as the first-pass effect based on in vitro metabolic parameters (K_m and V_{max}) and the absorption rate constant (k_a) were analyzed within clinically observed plasma levels.

2. Materials and methods

2.1. Materials

Propranolol was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G6P) and G6P dehydrogenase (G6PDH) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Acetonitrile (HPLC grade) was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). All other reagents were of analytical grade and were commercially obtained.

2.2. In vitro metabolism in rat and human liver microsomes

2.2.1. Rat liver microsomal preparation

Male Wistar rats (SLC, Shizuoka, Japan), weighing 300–350 g, were used. The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Doshisha Women's College of Liberal Arts. The rats were housed in pairs under controlled environmental conditions and fed commercial feed pellets. All rats had free access to food and water.

Rat liver was freshly obtained after the removal of blood from the liver, and homogenized in ice-cold 1.15% KCl solution. The homogenate was centrifuged at $9000 \times g$ for 15 min, and the supernatant was centrifuged at $105{,}000 \times g$ for 60 min to obtain a microsomal pellet, which was resuspended in 0.1 M phosphate buffer (pH 7.4) to a concentration of 2.0 mg protein/mL. Protein concentration was determined by the method of Lowry et al. (1951).

The obtained microsomal samples were immediately frozen after collection and stored in a freezer at −80 °C until analysis.

2.2.2. Metabolic assay method

Pooled human liver microsomes (20 mg protein/mL in 250 mM sucrose) were purchased from Daiichi Pure Chemicals Co., Ltd. (Osaka, Japan). The incubation mixture contained an NADPH generating system (0.5 mM NADP, 5 mM G6P, 2 unit/mL G6PDH and 5 mM $MgCl_2$), 0.1–20.0 μM of propranolol and 0.2 mg protein/mL of rat or 0.5 mg protein/mL of human liver microsomal suspensions in 1.0 mL (rat) or 0.5 mL (human) of 0.1 M phosphate buffer (pH 7.4). The time courses of the unchanged propranolol in both microsomes were obtained in advance.

After 5 min preincubation at 37 °C in a water bath, the enzyme reactions were initiated by the addition of 100 μL (rat) or 50 μL (human) of 2.0 mg (rat) or 5.0 mg (human) protein/mL microsomal suspension. The reaction mixture was incubated for 3 min (rat) or 5 min (human), and then the reaction was terminated by the addition of 3.0 mL of ethyl acetate. Moreover, a control incubation containing no NADPH was also run at the initial concentration and ethyl acetate was added immediately. Each concentration group was run in duplicate to quadruplicate.

2.2.3. Analytical procedures of propranolol in microsomal suspensions

After termination of the reaction by adding ethyl acetate, 1.0 mL (rat) or 0.5 mL (human) of 0.25 M Na_2CO_3 was added to a reaction sample. After extraction by shaking the sample tube and centrifugation at 3000 rpm for 15 min, the aqueous phase was frozen at −40 °C, and then the organic liquid was transferred to a new tube and evaporated. The residue was reconstituted in the mobile phase (see below), of which an aliquot was injected into the following HPLC system.

The HPLC system was equipped with two detectors, SPD-20A UV and RF-10AXL fluorometric detectors (both Shimadzu Co., Kyoto, Japan). This HPLC system consisted of a Shimadzu LC-20AD pump and a Shimadzu SIL-20A automatic sample injector. The flow rate of the pump was 0.8 mL/min. The analytical column was a Shim-pack VP-ODS (150 mm × 4.6 mm i.d., Shimadzu Co.) and was maintained at 40 °C by column oven (Shimadzu CTO-20A). The composition of the mobile phase was 0.1 M KH_2PO_4 buffer (pH 3.0):CH_3OH (50:50). Propranolol was detected by UV (228 nm) and fluorescence (emission = 300 nm, excitation = 370 nm) detectors for high and low concentration ranges, respectively, and the data were loaded onto Shimadzu LC solution analytical software by connecting to a Shimadzu CBM-20A communication bus module.

2.2.4. Metabolic kinetic analysis

In vitro metabolic activities of propranolol were estimated from the decrease of propranolol by using rat and human liver microsomes under linear conditions. The apparent K_m ($K_{m,app}$), V_{max} and apparent hepatic intrinsic clearance (CL_{int}), $CL_{int,app}$, of propranolol were estimated from the total propranolol concentration by fitting the obtained data to the following equation. This equation contains both Michaelis–Menten type saturable and non-saturable components using non-linear least-squares regression analysis, MULTI (Yamaoka et al., 1981), with a weighting factor of zero.

$$v = \frac{V_{max\,1} \times C_{microsomes}}{K_{m1,app} + C_{microsomes}} + CL_{int2,app} \times C_{microsomes}$$

where v and $C_{microsomes}$ indicate the reaction velocity of the microsomal metabolic reaction and initial propranolol concentration in microsomal suspension, respectively.

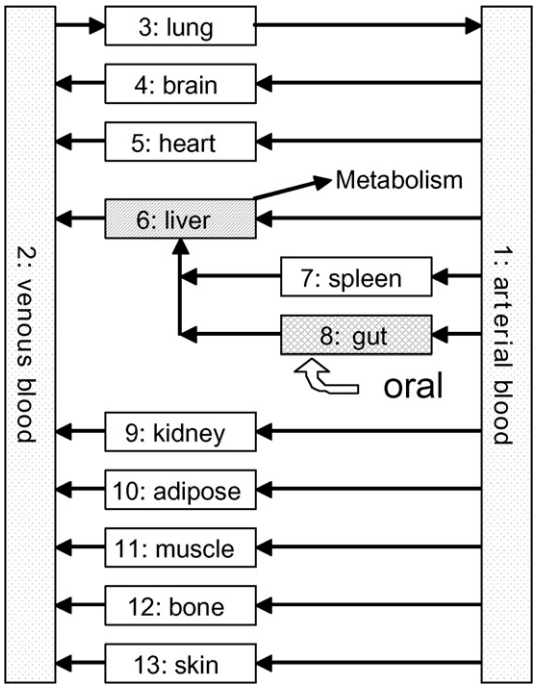

Fig. 1. Schematic diagram of PBPK model developed for propranolol kinetics in humans.

To obtain the K_{m1} and CL_{int2} of propranolol in microsomal suspensions, $K_{m1,app}$, and $CL_{int2,app}$ were corrected to its unbound concentrations using the reported unbound fraction in microsomes (f_m) (Obach, 1997). Results are expressed as the mean or mean ± S.E.

2.3. Model development

A PBPK model was developed to describe the pharmacokinetics of propranolol in humans. As shown in Fig. 1, it depicted the body as being composed of 11 tissue/organ compartments and 2 blood compartments with the lungs closing the loop. The assumptions of the model are: (1) elimination takes place only in the liver, (2) drug transport occurs solely via blood flow and each tissue acts as a well-stirred compartment (Pang and Rowland, 1977), (3) the k_a of propranolol is a constant and is not affected by the dose or its plasma concentration, (4) hepatic metabolism of propranolol is considered to occur according to the mixture profiles of saturable and non-saturable components from the results of in vitro experiments and (5) plasma and microsomal bindings of propranolol were constant within the experimental propranolol concentration range.

The physiological parameters of each tissue in humans, such as tissue volume (V) and blood flow rate through the tissue (Q), and tissue-to-blood partition coefficient (K_p) values are quoted from previous reports (Poulin and Theil, 2002) and listed in Table 1. The unbound fraction in blood (f_B) (Obach, 1997), f_m (Obach, 1997) and the blood-to-plasma concentration ratio (R_B) (Goodman and Gilman, 1996) are quoted from the literature. The V_{max1} from in vitro experiments expressed as nmol/mg protein/min was multiplied by the term, 259.34 (MW), 45 mg protein/g liver (Houston, 1994) and liver tissue volume as a scaling factor to yield estimates for the whole liver. The obtained V_{max1}, K_{m1} and CL_{int2} values were incorporated into the metabolic term of the PBPK model described in the differential equation. The k_a was calculated from the reported peak time (T_{max}) and the eliminating rate constant (k_{el}) values (Yasuhara et al., 1990) using the following equation: $T_{max} = 2.303/(k_a - k_{el}) \times \log(k_a/k_{el})$.

The differential equations listed in Appendix were integrated numerically by the Runge–Kutta–Gill method, which was programmed with Visual Basic 6.0 in Microsoft Excel 2003 and then the AUC values were calculated as a value extrapolated to infinity from simulated plasma concentrations by noncompartmental analysis using Winnonlin® professional version 5.0.1. (Pharsight® Corporation, Mountain View, CA, USA). In the case of repeated administration, AUC values were calculated as the time range from immediately after administration to just before the next administration in the steady-state.

Table 1
Physiological parameters and K_p values in various human tissues

	Compartment	Q (L/min)[a]	V (L)[a]	K_p
1	Arterial blood	5.27[b]	1.72	
2	Venous blood	5.27[b]	3.44	
3	Lung	5.27[b]	0.32	6.46
4	Brain	0.11	0.36	13.54
5	Heart	0.26	0.21	4.38
6	Liver	0.93[c]	2.31	5.67
7	Spleen	0.11	0.13	2.98
8	Gut	0.69	1.70	8.22
9	Kidney	0.07	0.46	5.18
10	Adipose	0.37	4.80	0.18
11	Muscle	1.46	25.53	3.20
12	Bone	0.64	2.62	6.90
13	Skin	0.31	12.00	7.22

[a] Values for Q and V fractions, and K_p values for rats were quoted from a previous report (Poulin and Theil, 2002) and scaled up to humans.
[b] Total cardiac output was calculated with an allometric equation (Brown et al., 1997) (=0.235 × (body weight = 63.2 kg)$^{0.75}$).
[c] Sum of hepatic artery plus portal vein flow.

3. Results

Fig. 2 shows the rate of propranolol metabolism normalized to protein content as a function of propranolol concentration in rat and human liver microsomes after 3 or 5 mins' incubation, respectively. The obtained $K_{m1,app}$ and V_{max1} calculated from the decrease of propranolol concentration were 0.424 ± 0.032 μM and 0.899 ± 0.027 nmol/mg protein/min in rat, and 9.05 ± 4.34 μM and 0.170 ± 0.097 nmol/mg protein/min in human liver microsomes. To calculate the K_{m1} values, $K_{m1,app}$ values were corrected with f_m, namely 0.44 in rat and 0.38 in human microsomes. The K_{m1} values were 0.187 ± 0.014 in rat and 3.44 ± 1.65 μM (0.892 μg/mL) in human microsomes. Moreover, the CL_{int2} values were 0.210 ± 0.005 in rats and 0.0886 ± 0.0071 mL/min/mg protein in humans. Obtained parameters are listed in Table 2. Propranolol was more extensively and sensitively metabolized in rat microsomes than human microsomes. To incorporate

Table 2
Summary of enzyme kinetic parameters for propranolol in rat and human liver microsomes

	K_{m1}[a] (μM)	V_{max1} (nmol/mg protein/min)	CL_{int1}[a,b] (mL/mg protein/min)	CL_{int2}[a] (mL/mg protein/min)
Rat	0.187 ± 0.014	0.899 ± 0.027	4.82	0.210 ± 0.005
Human	3.44 ± 1.65	0.170 ± 0.097	0.0426	0.0886 ± 0.0071

[a] K_{m1}, CL_{int1} and CL_{int2} were values based on unbound propranolol corrected by f_m (Obach, 1997).
[b] CL_{int1} was calculated as V_{max1}/K_{m1}.

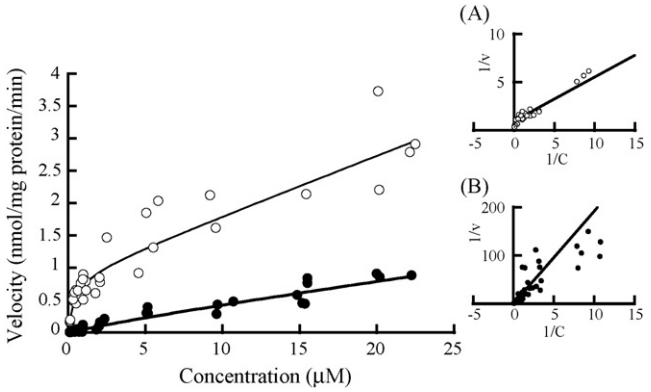

Fig. 2. Rate of propranolol metabolism as a function of propranolol concentration in rat and human liver microsomes.
Inset figures are the Lineweaver–Burk plots in rat (A) and human (B) liver microsomes; (◯) rat and (●) human microsomes.

these parameters into the PBPK model, the V_{max1} and CL_{int2} values in human microsomes were converted into those per human. These values were obtained as 4572 μg/min/human and 9208 mL/min/human using scaling factors, respectively.

Fig. 3 shows the plasma concentration–time curves predicted according to the PBPK model (lines) and observed values reported by Yasuhara et al. (1990) (symbols). At first, the T_{max} values were around 120 min, and the k_a values were presumed to be 0.0125 and 0.0142 min^{-1} at 10 and 20 mg oral doses, respectively. However, the predicted curves were more rapidly absorbed and decreased than the observed values (dotted lines). Then k_a was estimated to be 0.00837 or 0.00964 min^{-1} of each dose when the T_{max} was presumed to be 150 min. The predicted elimination phases were similar to the observed elimination phase in the slope at both doses (broken lines). To fit the predicted curves close to the observed points, F_a was assumed to be 0.65 (solid lines).

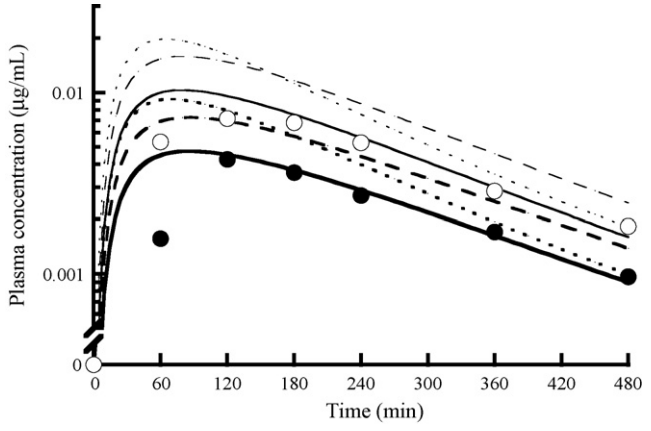

Fig. 3. Simulated and reported plasma concentrations of propranolol after oral administration in humans to estimate k_a and F_a values.
Simulated concentrations were obtained according to the PBPK model.
Lines indicate simulated curves: (‥‥) $k_a = 0.0125\ min^{-1}$ and $F_a = 1$ (10 mg dose), (‥‥) $k_a = 0.0142\ min^{-1}$ and $F_a = 1$ (20 mg dose), (– –) $k_a = 0.00837\ min^{-1}$ and $F_a = 1$ (10 mg dose), (– –) $k_a = 0.00964\ min^{-1}$ and $F_a = 1$ (20 mg dose), (——) $k_a = 0.00837\ min^{-1}$ and $F_a = 0.65$ (10 mg dose), (——) $k_a = 0.00964\ min^{-1}$ and $F_a = 0.65$ (20 mg dose). (●) 10 mg dose, (◯) 20 mg dose, and both observed points were quoted from the literature (Yasuhara et al., 1990).

Fig. 4 shows the simulated plasma concentration–time curves (lines) and observed data reported by Kopitar et al. (1986) (symbols). The simulation employed $k_a = 0.009\ min^{-1}$, which was the average of the above k_a values (0.00837 and 0.00964 min^{-1}). The simulated curves at three doses (40, 80 and 160 mg) indicated a relatively similar slope to the observed points in the terminal elimination phase; however, the observed points at 10 mg dose were rapidly decreased as compared to other observed data and simulated curves.

The following simulations were performed using $k_a = 0.009\ min^{-1}$ and $F_a = 0.65$, in addition to the obtained metabolic parameters described above.

Fig. 5 shows the predicted plasma concentration–time curves of propranolol after single (A) and repeated (B) doses, respectively. The daily doses were all 160 mg after repeated doses, namely 4 times of 40 mg, 2 times of 80 mg or one dose of 160 mg per day. These curves are shown at steady-state concentrations.

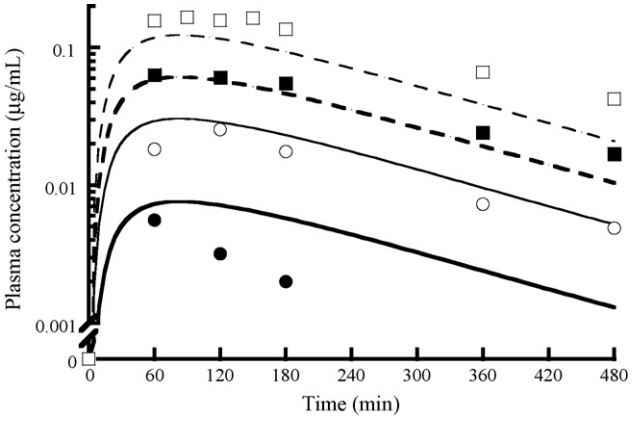

Fig. 4. Simulated and reported plasma concentrations of propranolol after oral administration in humans.
Simulated concentrations were obtained according to the PBPK model.
Lines indicate predicted curves: (——) 10 mg dose, (——) 40 mg dose, (– –) 80 mg dose, (– –) 160 mg dose. The k_a is obtained as the appropriate mean value in Fig. 3, namely 0.009 min^{-1}. $F_a = 1$; (●) 10 mg dose, (◯) 40 mg dose, (■) 80 mg dose, (□) 160 mg dose, and these symbols represent the reported data (Kopitar et al., 1986).

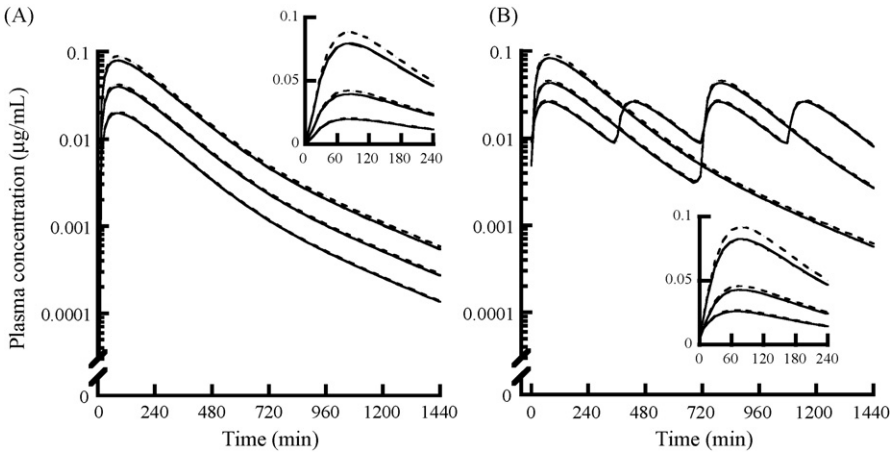

Fig. 5. Simulated plasma propranolol concentrations according to the PBPK model after single (A) and repeated (B) oral administration in humans as the CL_{int1} value fluctuated and its normal value was around C_{max} value (each inset figure).
(▬) $CL_{int1} = V_{max1} \times C_{liver}/(K_{m1} + C_{liver})$, (▬ ▬) $CL_{int1} = V_{max1} \times C_{liver}/K_{m1}$ (linear condition), (▪▪▪▪▪) $CL_{int1} = V_{max1}/20 \times C_{liver}/(K_{m1}/20 + C_{liver})$ (both metabolic parameters decreased to one twentieth).
Metabolic parameter values listed in Table 2 are scaled up to the whole body as described in Section 2.
(A) Oral doses were 40 mg: lower concentration band, 80 mg: intermediate concentration band, 160 mg: higher concentration band. (B) Oral daily doses were all 160 mg. Namely 40 mg 4 times, 80 mg twice or 160 mg once, and these curves indicated simulated concentrations at the steady-state.

The metabolic parameters were ranged in three stages to estimate its effect on plasma levels. Although the plasma concentration was slightly higher when both V_{max1} and K_{m1} were decreased to one twentieth as shown in their inset figures, simulated curves almost overlapped in all simulations.

The relationship between the propranolol dose and its AUC/dose value at several CL_{int1} model assumptions is shown in Fig. 6. AUCs were calculated from the data in Fig. 5. When decreasing V_{max1} and K_{m1} values to one twentieth, AUC/dose values tended to increase with the dose after both single and repeated administrations.

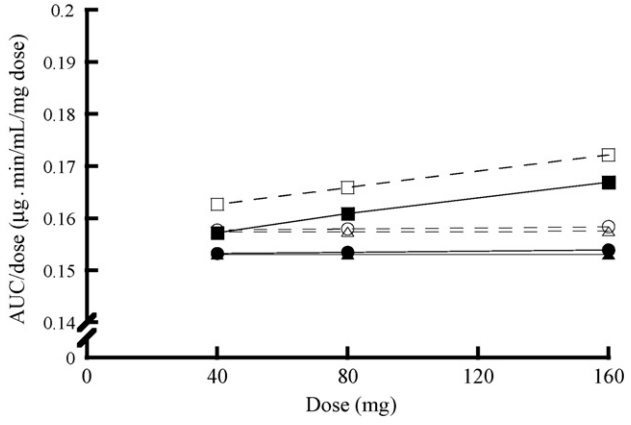

Fig. 6. Dose-normalized propranolol AUC value vs. dose plot after single and repeated oral simulations as the CL_{int1} value fluctuated.
Closed and open symbols indicate data obtained after single and repeated administration, respectively; (●) and (○) $CL_{int1} = V_{max1} \times C_{liver}/(K_{m1} + C_{liver})$, (▲) and (△) $CL_{int1} = V_{max1} \times C_{liver}/K_{m1}$ (linear condition), (■) and (□) $CL_{int1} = V_{max1}/20 \times C_{liver}/(K_{m1}/20 + C_{liver})$ (metabolic parameters decreased to one twentieth).
Metabolic parameter values listed in Table 2 are scaled up to the whole body as described in Section 2.

The effect of the k_a value on propranolol pharmacokinetics was considered. Fig. 7(A) shows the plasma concentration–time curves of propranolol after 40 mg dose, which varied only the k_a value. When the k_a value decreased, T_{max} delayed and C_{max} and k_{el} decreased. Fig. 7(B) shows the correlation between AUC values after oral administration and the dose of propranolol. From this figure, it is considered that the k_a value did not affect the AUC value of propranolol. Moreover, although k_a, V_{max1} and K_{m1} values all decreased to one tenth, the AUC value within a similar dose range increased dose-dependently in a linear fashion as did the others (data not shown).

Fig. 8 shows the alteration of the calculated dose-normalized AUC value as a function of the propranolol dose after oral administration. The AUC/dose was almost constant under 1000 (10^3) mg dose, and then increased. Further, when the dose was increased to 10^6 mg, the AUC/dose approached but never

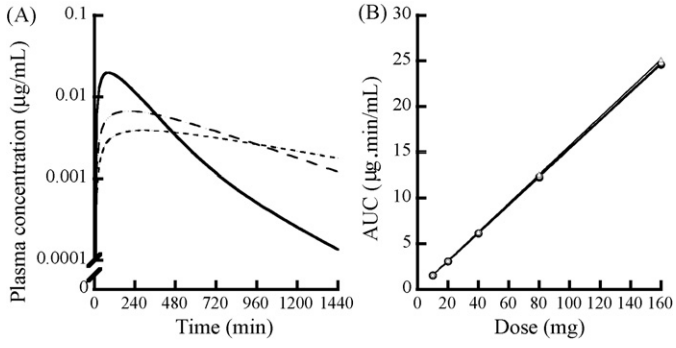

Fig. 7. Simulated plasma concentration–time curves after 40 mg dose (A), and correlation between AUC and dose (B) after oral administration of propranolol in humans as the k_a values fluctuated.
(A) (▬) $k_a = 0.009\ min^{-1}$, (▬ ▬) $k_a = 0.0018\ min^{-1}$ (k_a decreased to one fifth), (▪▪▪▪▪) $k_a = 0.0009\ min^{-1}$ (k_a decreased to one tenth). (B) (●) $k_a = 0.009\ min^{-1}$, (○) $k_a = 0.0018\ min^{-1}$ (k_a decreased to one fifth), (△) $k_a = 0.0009\ min^{-1}$ (k_a decreased to one tenth).

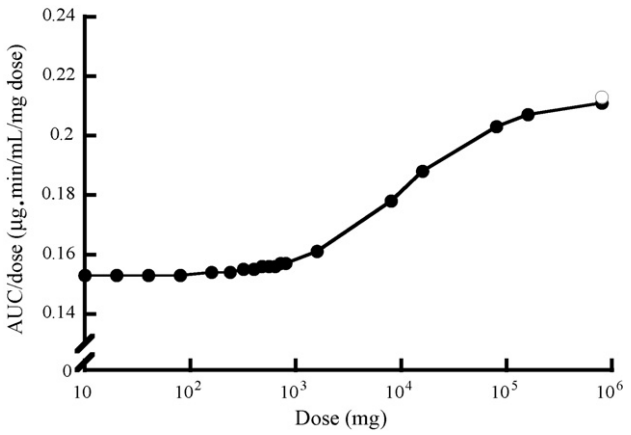

Fig. 8. Dose-normalized propranolol AUC value vs. dose curve after oral administration in humans.
Open circles indicate when CL_{int1} was negligible and propranolol was metabolized by only the CL_{int2} component.

exceeded in theory the value obtained when CL_{int1} was negligible and propranolol was metabolized by only the CL_{int2} component (open circle).

4. Discussion

The metabolic activity of propranolol in rat liver microsomes was much higher than that obtained from human liver microsomes within the clinically observed plasma levels (at most 0.5 μg/mL). It is reported that propranolol binding to liver microsomes increased with the increase of the microsomal protein concentration, but showed almost no variation from the propranolol concentration (Obach, 1997). The bindings in rat and human liver microsomes were around 60% and almost equal. It is considered that in vitro metabolic parameters, such as K_m and V_{max}, should be corrected to unbound substrate concentrations; therefore, $K_{m,app}$ and $CL_{int,app}$ values were corrected by these free fractions to obtain K_m and CL_{int} values. Propranolol CL_{int} was previously reported to be 2.4 mL/mg protein/min in rat liver microsomes (Masubuchi et al., 1993), though it was calculated from total propranolol concentrations as the sum CL_{int} of four metabolic pathways, namely 4-, 5-, 7-hydroxylation and N-dealkylation. This value was corrected by reported f_m, and then CL_{int} in rat liver microsomes was obtained as about 6 mL/mg protein/min. This value is similar to our sum CL_{int} value (CL_{int1} plus CL_{int2}: 5.03 mL/mg protein/min) as shown in Table 2. Komura et al. (2005) reported the CL_{int} value estimated from propranolol depression assay in female Wistar rat liver microsomes (5.33 mL/mg protein/min based on total propranolol). This value was calculated by dividing the first elimination phase rate constant by the microsomal protein concentration. This value is about 2.4-fold larger than our results. Additionally, the $CL_{int,app}$ value was previously reported to be 0.053 mL/mg protein/min in pooled human liver microsomes (Obach, 1997); and the CL_{int} value, 0.139 mL/mg protein/min, was obtained by correcting f_m and this value was similar to our data. From the above evidence, our results of metabolic kinetics in in vitro experiments were considered to be reasonable.

In general, CL_{int} is estimated from in vitro K_m and V_{max} values based on measuring metabolite formation. On the other hand, the CL_{int} in this study is calculated from substrate decrease. Comparing the methods, the advantages of this method are as follows: (1) simple to conduct, (2) metabolites do not need to be known and (3) can yield enzyme kinetic data based on the disappearance of parent compounds. On the other hand, the disadvantages of the method are as follows: (1) it is difficult to measure very low CL_{int} in vitro values, (2) cannot obtain individual metabolite information and (3) cannot obtain exact K_m and V_{max} parameters. In particular, the experimental error increases in the high substrate concentration range because of slight concentration differences between pre- and post-incubation. Several studies have also calculated CL_{int} in vitro from substrate disappearance (Lave et al., 1997; Obach, 1999; Naritomi et al., 2001). It is reported that the metabolic parameter values provided from the substrate decrease assay were comparable with those from the metabolite formation assay of propranolol (Komura et al., 2005).

It has been reported that first-pass metabolism in the human small intestine is not negligible for some drugs which were metabolized mainly by CYP3A4, such as cyclosporine (Benet et al., 1996). In contrast, propranolol is known to be metabolized mainly by 2C19 and 2D6 (Perkinson, 1996). We, therefore, assumed that extrahepatic clearances could be negligible in this study.

To evaluate the suitability of the PBPK model, the simulated propranolol levels were compared with the reported concentrations. In the model, k_a was based on reported T_{max} and k_{el} values. The k_a generally changes with individual experimental conditions, and T_{max} was obtained as an intermittent actual measurement. In this study, the k_a for PBPK simulation was $0.009\,min^{-1}$, which was the average calculated from the reference received as conventional tablets (Yasuhara et al., 1990). In addition, simulations were performed using $F_a = 0.65$. This F_a value was designed from a comparison between observed and simulated plasma concentrations to obtain a reasonable approximation; therefore, the F_a value affected not only absorption but also many factors such as the employed scaling factor and reported parameters, other pharmacokinetic mechanisms, etc. When the F_a value increased, the plasma concentration proportionately increased but k_{el} and T_{max} were not affected. In this study, we used a whole body PBPK model, enabling investigation of the effects of various factors on propranolol pharmacokinetics. For example, variations of physiological and biochemical parameters can be incorporated into this model, which are caused by aging and the disease condition.

Although other reported plasma concentration–time curves at 40, 80 and 160 mg doses (Kopitar et al., 1986) were close to the simulation curves, as shown in Fig. 4, the observed curve at 10 mg dose decreased more rapidly than both predicted slopes by the PBPK model and observed points at other doses. The authors considered that the non-linear propranolol pharmacokinetics between 10 and 40 mg doses was probably caused by factors such as intestinal and hepatic metabolism, distribution of the drug in body tissues and changes of blood supply to

organs due to the administered drug. They proposed two reasons for the non-linear pharmacokinetics. The first was saturated metabolic enzymes in the liver when a larger amount of the drug passes through the liver. It appeared that saturation of the hepatic enzyme was achieved with a dose between 20 and 30 mg. The second was the existence of two different enzymes for propranolol metabolism, one of which is saturable at doses of propranolol higher than 10 mg, whereas the other is less saturable or non-saturable in the liver. Additionally, they mentioned the possibility that relatively high intraindividual differences of the blood levels, which are well known for beta-blockers, could influence the relationship between the AUC and dose. In this study, we investigated the propranolol hepatic metabolism, and then simulated propranolol plasma kinetics. Although the results showed that propranolol was metabolized in human liver microsomes by two components, the high capacity non-saturable component mainly affected propranolol plasma kinetics and the low capacity-saturable component had hardly any effect within the clinical dose range unlike the report. Although we did not examine other factors affecting propranolol pharmacokinetics including intestinal metabolism, the absorption rate and protein bindings, other factors which facilitate propranolol disappearance from the systemic circulation in a lower dose range might be possible.

When the k_a value decreased as with a sustained release formulation, C_{max} decreased and T_{max} was delayed. A repeated dosing study was designed to mimic clinical situations where propranolol is administered at a 160 mg daily dose, namely four times of 40 mg, twice of 80 mg or once of 160 mg. The simulation was repeated until the plasma propranolol concentration reached the steady-state; however, the k_a value did not affect the propranolol AUC value. Although the metabolic parameters decreased to one tenth, as did k_a, the AUC increased almost proportionally with the propranolol dose. Consequently, the pharmacokinetics of propranolol was hardly affected by the dosage form.

When the dose increased from 10^3 to 10^6 mg, the AUC/dose value increased to 1.4 times and approached the value when CL_{int1} is assumed to be negligible. Suzuki et al. (1974) previously reported the non-linearity of propranolol pharmacokinetics in rats. Their examined dose range was between 1 and 12 mg/kg, and the estimated AUC value when it was presumed to be dose-linear was 1.4–1.7 times larger than the observed values. In this study, the AUC/dose values increased about 1.4 times from 10^3 to 10^6 mg dosing, namely the 16 mg/kg to 16 g/kg dose range in humans. From the metabolic parameters in rat microsomes, the contribution of the CL_{int1} component is larger and propranolol more rapidly metabolizes than in humans. From these results, it is considered that the reported non-linear pharmacokinetics of propranolol was investigated in such a dose range. However, the dose range between 10^3 and 10^6 mg is far higher than both reported and clinical doses. In this study, we incorporated Michaelis–Menten type metabolism into the PBPK model and other kinetic parameters were presumed to be constant. Hence, these parameters are possible to change practically with the dose and the obtained results should be treated as a hypothetical dose range of reference.

In addition, Agoram et al. (2001) reported metabolic parameter values predicted using an advanced compartmental absorption and transit model. The optimized V_{max} value was 0.045 mg/s and K_m was 0.05 μg/mL based on the plasma unbound fraction (9%), the values of which were optimized to fit the plasma concentration. The CL_{int} was calculated to be about 1.84×10^5 mL/min. On the other hand, our sum human CL_{int} value (in vitro CL_{int1} plus CL_{int2}) was calculated to be 1.43×10^4 mL/min. The CL_{int} value corrected by the plasma unbound fraction was obtained as 1.37×10^5 mL/min. These two CL_{int} values are relatively similar, although their metabolism process was presumed to be a saturable process and propranolol pharmacokinetics was considered to have a non-linear dose-response relationship.

In consideration of the K_p value of propranolol in the liver (5.67) and the examined propranolol concentration range in vitro, propranolol concentrations in the liver after clinical dosing were covered in our in vitro experiments as a rough estimate; therefore, propranolol pharmacokinetics was considered to be controlled mainly by the CL_{int2} component and to be dose-independent within the clinical dose range under these hypotheses of the PBPK model (40–160 mg dose, $C_{max} < 0.5$ μg/mL).

Appendix

Arterial blood (compartment 1)

$$V_1 \frac{dC_1}{dt} = Q_{tot}\left(\frac{C_3 \times R_B}{K_{p,3}} - C_1\right)$$

where Q_{tot} is total blood flow through the body.

Venous blood (compartment 2)

$$V_2 \frac{dC_2}{dt} = \sum Q_i \frac{C_i \times R_B}{K_{p,i}} + (Q_6 + Q_7 + Q_8)\frac{C_6 \times R_B}{K_{p,6}} - Q_{tot}C_2$$

where i is compartments 4, 5, 9–13.

Lung (compartment 3)

$$V_3 \frac{dC_3}{dt} = Q_{tot}\left(C_2 - \frac{C_3 \times R_B}{K_{p,3}}\right)$$

Liver (compartment 6)

$$V_6 \frac{dC_6}{dt} = Q_6 C_1 + \sum Q_i \frac{C_i \times R_B}{K_{p,i}} - (Q_6 + Q_7 + Q_8) \times \frac{C_6 \times R_B}{K_{p,6}} - \frac{C_6 \times f_B \times R_B}{K_{p,6}} \times \left(\frac{V_{max\,1}}{K_{m1} + C_6 \times f_B(R_B/K_{p,6})} + CL_{int2}\right)$$

where i is compartments 7 and 8.

Absorption site = gut (compartment 8)

$$V_8 \frac{dC8}{dt} = Q_8\left(C_1 + k_a \times \text{Dose} \times F_a \times e^{-k_a \times t} - \frac{C_8 \times R_B}{K_{p,8}}\right)$$

where F_a is fraction absorbed from the intestinal tract.

Non-eliminating tissues (compartments 4, 5, 7, 9–13)

$$V_i \frac{dC_i}{dt} = Q_i \left(C_1 - \frac{C_i \times R_B}{K_{p,i}} \right)$$

where i is compartments 4, 5, 7, 9–13.

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