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Stereoselective Pharmacokinetics of Ibuprofen in Rats: Effect of Enantiomer-Enantiomer Interaction in Plasma Protein Binding

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ABSTRACT Stereoselective pharmacokinetics of ibuprofen (IB) enantiomers were studied in rats. Unidirectional conversion from R-ibuprofen (R-IB) to S-ibuprofen (S-IB) was observed following intravenous administration. S-IB concentrations in plasma following racemate administration were simulated according to a conventional compartmental model using the parameters obtained after the administration of individual enantiomers, and resulted in overestimation of S-IB concentrations.

Binding of IB enantiomers measured in rat plasma was stereoselective, the binding of R-IB being more favorable than that of S-IB. Moreover, there are interactions between IB enantiomers in binding, which may cause the increase of distribution volumes of IB enantiomers in the presence of their antipodes. Hence simulated S-IB concentrations according to a conventional compartment model were significantly greater than those observed. Indeed, when the enantiomer-enantiomer interactions were taken into account, simulation of S-IB concentrations in plasma following racemate administration was in good agreement with observed values. Therefore, interactions between stereoisomers as well as dispositional stereoselectivity have to be considered when pharmacokinetics of stereoisomers after administration of the racemate are compared to those after administration of individual isomers. *Chirality 9:354–361, 1997.*

KEY WORDS: ibuprofen; enantiomers; stereoselective; interactions; plasma protein binding; rat

Ibuprofen ((±)-α-methyl-4-(2-methylpropyl) benzeneacetic acid) is a nonsteroidal anti-inflammatory drug (NSAID) which possesses a chiral center in the propionic acid moiety and has been used clinically as the racemate. Its pharmacological activity mainly resides in the S-enantiomer,[1] and it is reported that unidirectional conversion from R-IB to S-IB takes place in vivo in humans,[2–5] rats,[6–9] and several other animal species.[10–12] Therefore, IB is one of the chiral drugs which show stereoselecctive pharmacological and pharmacokinetic characteristics.

Plasma protein binding of many chiral drugs, including IB, is stereoselective.[13–16] Binding of IB in human plasma and in aqueous solutions of human serum albumin (HSA) is stereoselective, the binding of R-IB being greater than that of S-IB.[17–20] Interactions between the binding of enantiomers are also reported for several NSAIDS,[21,22] including IB.[17,19] However, enantiomer-enantiomer interactions in the binding of IB in rat plasma and their effect on the pharmacokinetics of IB enantiomers have not yet been clarified. Since interactions between stereoisomers may occur in various pharmacokinetic processes in the body, the pharmacokinetic properties after racemate administration may differ from those following administration of the individual isomers. In the present study, pharmacokinetics of IB enantiomers following racemate administration were compared with those following the administration of individual enantiomers with consideration of enantiomer-enantiomer interactions in plasma protein binding as well as stereoselectivity.

MATERIALS AND METHODS

Materials

Ibuprofen was purchased from Tokyo Kasei (Tokyo, Japan). (+)R- and (–)S-α-Methylbenzylamine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Methanol was of HPLC grade purchased from Wako Pure Chemicals (Osaka, Japan). All other reagents were of analytical grade.

Resolution of IB Enantiomers

Racemic IB (2.5 g) and 1.5 ml α-methylbenzylamine (α-MBA) were dissolved in 400 ml ethyl acetate at 70°C. (+)R-α-MBA and (–)(S)-α-MBA were used to isolate R-IB and S-IB, respectively. The mixture was allowed to stand at

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room temperature overnight, and the precipitates were collected, dissolved in ethyl acetate (80 ml ethyl acetate for 1 g of the precipitate) at 70°C, and the mixture was left standing at room temperature to recrystallise. This procedure was repeated three times. Precipitates obtained after recrystallization were dissolved in 2 ml 1N HCl, and IB enantiomers were extracted with 5 ml of ether followed by evaporation under vacuum at 30°C. Specific rotations of the enantiomers in methanol were −57.1° and +53.2° for R-IB and S-IB, respectively. These values were similar to the reported values.[2] The enantiomeric purity determined with a chiral HPLC method (see below) was >98.4% for each enantiomer.

In Vivo Administration Study

Male Sprague-Dawley rats weighing 270–300 g (Nippon SLC Co., Hamamatsushi, Japan) were used in this study. Rats were anesthetized with ether during surgery. Rats were cannulated at the femoral vein and femoral artery with a polyethylene tubing (SP-45, 0.58 mm i.d., 0.96 mm o.d., Natsume Co., Tokyo, Japan) and at the common bile duct with a polyethylene tubing (SP-10, 0.28 mm i.d., 0.61 mm o.d., Natsume Co.). The bile duct was cannulated in order to avoid possible entero-hepatic circulation of IB,[23] although this may be unlikely. After surgery, rats were housed in restraining cages and allowed to recover from anesthesia before the administration of drugs. Rats had free access to water during the study. IB was administered through the femoral vein cannula and blood was sampled via the femoral artery cannula.

For administration of racemic IB, the racemate was dissolved in ethanol/0.1 M isotonic phosphate buffer (pH 7.4) (3/7 [v/v]) to obtain a 10 mg/ml solution. This was administered intravenously over 20 sec at a dose of 10 mg/kg. Blood was collected before the administration and at 1, 5, 10, 20, 30, 45, 60, 90 and 120 min after the administration and immediately centrifuged (1,000*g* for 5 min) and 0.1 ml aliquots of plasma were mixed with 1 ml of 0.2 M phosphate buffer (pH 2.0) to prevent possible hydrolysis of IB glucuronides. Plasma samples were stored frozen until they were analyzed.

For administration of IB enantiomers, each enantiomer was dissolved at a concentration of 5 mg/ml. The solution used to dissolve IB enantiomers was the same as that used for racemic IB and administered intravenously at a dose of 5 mg/kg as for racemic IB.

Sample Preparation for HPLC Analysis

Thirty microliters of ketoprofen in methanol (0.1 mg/ml) was added as internal standard to mixtures of 0.1 ml of plasma and 1 ml of 0.2 M phosphate buffer (pH 2.0). Five milliliters of ether was added, and vortexed for 15 sec. The mixture was further shaken for 3 min, and centrifuged at 1,000*g* for 5 min. The ether layer was collected, and the aqueous layer extracted with 5 ml ether. The ether layer was added to that obtained previously. Two mililiters of 0.01 M phosphate buffer (pH 6.0) was added to the combined ether layers, the mixture vortexed for 15 sec, shaken for 3 min, and centrifuged at 1,000*g* for 5 min. This was necessary to eliminate interfering HPLC peaks. The ether was evaporated to dryness, and the residue was dissolved in methanol. The methanol solution was filtered through a ACRO2™ LC3A filter (0.45 µm in pore size, Gelman Sciences), and an aliquot (20–50 µl) was injected on to the HPLC.

HPLC Conditions

IB enantiomers were analyzed by HPLC on a chiral stationary phase (SUMICHIRAL OA-2500 or OA-2500S, 300 mm × 4 mm [i.d.], Sumika Chemical Analysis Service, Osaka, Japan). The mobile phase was 0.005 M ammonium acetate in methanol, flow rate of 0.7 ml/min. IB was detected at 220 nm.

Plasma Protein Binding Study

Binding of IB enantiomers in rat plasma was measured with equilibrium dialysis using a Spectra/Por® membrane (mw cutoff 12,000–14,000, Spectrum Co., TX, U.S.A.). Aliquots of racemic IB solution (10 mg/ml in ethanol) were added to rat plasma to obtain IB concentrations (as racemate) of 2–80 µg/ml. A volume of 1.8 ml of the plasma was dialyzed against an equal volume of isotonic phosphate buffer (pH 7.4) at 37°C for 16 hr. After equilibration, 0.1 ml of the plasma and 1.5 ml of the buffer were collected. The plasma was prepared for HPLC analysis as described above. To prepare the buffer sample for HPLC, 0.75 ml of 0.2 M phosphate buffer (pH 2.0) and 25 µl of ketoprofen solution (50 µg/ml in methanol) were added to 1.5 ml of buffer and IB extracted with ether as described for plasma samples.

In order to measure the binding of IB enantiomers in plasma in the absence of the antipode, aliquots of R-IB or S-IB in methanol were added to rat plasma to obtain enantiomer concentrations of 2–40 µg/ml. Binding was measured in the same manner as for racemic IB.

Binding of IB enantiomers was also measured in the presence of excess concentrations of the antipode, the concentrations of one enantiomer being varied between 5 and 25 µg/ml, while the concentration of its antipode was held at 100 µg/ml.

Binding parameters in the absence of the antipode were calculated according to a Langmuir equation (Eq. 1) by a non-linear least squares method (MULTI)[24]:

$$C_b = n \cdot P_t \cdot K \cdot C_u/(1 + K \cdot C_u) \qquad \text{(Eq. 1)}$$

where C_b is the bound concentration of the drug, n is the number of the binding sites, P_t is the total protein concentration, K is the binding constant, and C_u is the unbound concentration of the drug. Since it has been reported that IB binds to serum albumin, P_t was assumed to be the concentration of rat serum albumin (RtSA); i.e., $P_t = 5.7 \times 10^{-4}$ M, where RtSA concentration of 37 mg/ml and the molecular weight of RtSA of 64,600 were assumed. Also, a single class of binding sites was assumed for both enantiomers since Klotz plots showed straight lines for both R-IB and S-IB when the binding was measured in the absence of the antipode.

Binding in rat plasma in the presence of the antipode was analyzed assuming competitive interactions, which can be expressed with the following equations:

$$C_{b,R} = P_t \cdot K_R \cdot C_{u,R}/(1 + K_R \cdot C_{u,R} + K_S \cdot C_{u,S}) \quad \text{(Eq. 2)}$$

$$C_{b,S} = P_t \cdot K_S \cdot C_{u,S}/(1 + K_R \cdot C_{u,R} + K_S \cdot C_{u,S}) \quad \text{(Eq. 3)}$$

where $C_{b,R}$ and $C_{b,S}$ are the bound concentrations of R-IB and S-IB, $C_{u,R}$ and $C_{u,S}$ are the unbound concentrations of R-IB and S-IB, and K_R and K_S are the binding constants for R-IB and S-IB, respectively.

Since no significant changes of the volume of plasma samples were observed during dialysis, dilution of the protein concentration was not taken into consideration in the analysis of binding data.

Pharmacokinetic Analysis

Total body clearance (CLtot) was calculated as D/AUC, where D is the intravenously administered dose and AUC is the area under the plasma concentration vs. time curve. Vd,β was calculated as CLtot/β, where β is the first-order rate constant of the terminal phase in the concentration profile of the drug.

Analysis with model A. Concentration profiles of IB in plasma were analyzed with a conventional two-compartment open model with a unidirectional conversion from R-IB to S-IB (Fig. 1A). Pharmacokinetic parameters were obtained by a non-linear least squares method (MULTI). S-IB concentration profiles following S-IB administration were analyzed with the following equation.

$$C_{S1}(D_S) = A_S \cdot \exp(-\alpha_S \cdot t) + B_S \cdot \exp(-\beta_S \cdot t) \quad \text{(Eq. 4-1)}$$

where C_{S1} (D_S) is the total (bound plus unbound) concentration of S-IB in plasma (i.e., S-IB concentration in the central compartment in Figure 1A) following S-IB administration, and A_S, B_S, α_S, and β_S are expressed by the following equations:

$$A_S = D_S \cdot (\alpha_S - k_{S21})/\{V_{S1} \cdot (\alpha_S - \beta_S)\} \quad \text{(Eq. 4-2)}$$

$$B_S = D_S \cdot (k_{S21} - \beta_S)/\{V_{S1} \cdot (\alpha_S - \beta_S)\} \quad \text{(Eq. 4-3)}$$

$$\alpha_S + \beta_S = k_{S10} + k_{S12} + k_{S21} \quad \text{(Eq. 4-4)}$$

$$\alpha_S \cdot \beta_S = k_{S10} \cdot k_{S21} \quad \text{(Eq. 4-5)}$$

where D_S is the administered dose of S-IB, V_{S1} is the volume of distribution of the central compartment, and k_{S10}, k_{S12} and k_{S21} are the first-order rate constants as shown in Figure 1A.

Concentration profiles of R-IB and S-IB following R-IB administration were analyzed by simultaneous fitting of R-IB and S-IB concentration profiles to Eqs. 5-1 and 6-1, respectively. R-IB concentrations in plasma following R-IB administration are expressed:

$$C_{R1}(D_R) = A_R \cdot \exp(-\alpha_R \cdot t) + \beta_R \cdot \exp(-\beta_R \cdot t) \quad \text{(Eq. 5-1)}$$

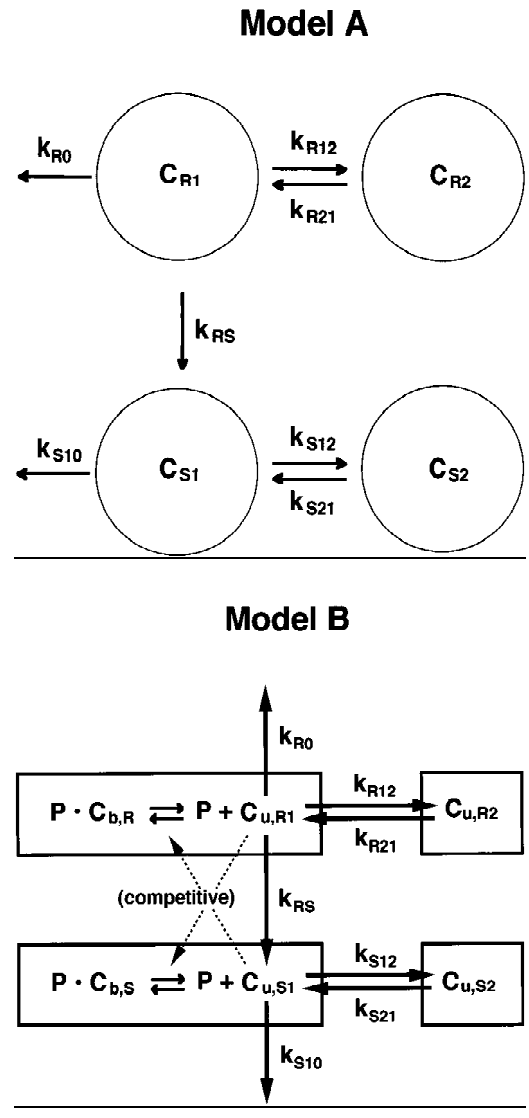

Fig. 1. A conventional pharmacokinetic model with unidirectional conversion from R-IB to S-IB (model A) and a pharmacokinetic model with the plasma protein binding and competitive interactions incorporated (model B).

where C_{R1} is the total (bound plus unbound) concentration of R-IB in plasma (i.e., R-IB concentration in the central compartment in Figure 1A), and A_R, B_R, α_R, and β_R are expressed by the following equations:

$$A_R = D_R \cdot (\alpha_R - k_{R21})/\{V_{R1} \cdot (\alpha_R - \beta_R)\} \quad \text{(Eq. 5-2)}$$

$$B_R = D_R \cdot (k_{R21} - \beta_R)/\{V_{R1} \cdot (\alpha_R - \beta_R)\} \quad \text{(Eq. 5-3)}$$

$$\alpha_R + \beta_R = k_{R10} + k_{R12} + k_{R21} \quad \text{(Eq. 5-4)}$$

$$\alpha_R \cdot \beta_R = k_{R10} \cdot k_{R21} \quad \text{(Eq. 5-5)}$$

where D_R is the administered dose of R-IB, V_{R1} is the vol-

ume of distribution of the central compartment, $k_{R10} = k_{RO} + k_{RS}$, and k_{RO}, k_{R12}, and k_{R21} are the first-order rate constants as shown in Figure 1A. S-IB concentrations in plasma following R-IB administration are expressed:

$$C_{S1}(D_R) = (k_{RS} \cdot D_R/V_{S1}) \cdot \{G_S \cdot \exp(-\alpha_R \cdot t) + H_S \cdot \exp(-\beta_R \cdot t) + I_S \cdot \exp(-\alpha_S \cdot t) + J_S \cdot \exp(-\beta_S \cdot t)\} \quad \text{(Eq. 6-1)}$$

where $C_{S1}(D_R)$ is the total (bound plus unbound) concentrations of S-IB in plasma (i.e., S-IB concentration in the central compartment in Figure 1A) following R-IB administration, k_{RS} is the rate constant for the conversion from R-IB to S-IB, and G_S, H_S, I_S, and J_S are expressed by the following equations:

$$G_S = (k_{R21} - \alpha_R) \cdot (k_{S21} - \alpha_R)/\{(\beta_R - \alpha_R) \cdot (\alpha_S - \alpha_R) \cdot (\beta_S - \alpha_R)\} \quad \text{(Eq. 6-2)}$$

$$H_S = (k_{R21} - \beta_R) \cdot (k_{S21} - \beta_R)/\{(\alpha_R - \beta_R) \cdot (\alpha_S - \beta_R) \cdot (\beta_S - \beta_R)\} \quad \text{(Eq. 6-3)}$$

$$I_S = (k_{R21} - \alpha_S) \cdot (k_{S21} - \alpha_S)/\{(\alpha_R - \alpha_S) \cdot (\beta_R - \alpha_S) \cdot (\beta_S - \alpha_S)\} \quad \text{(Eq. 6-4)}$$

$$J_S = (k_{R21} - \beta_S) \cdot (k_{S21} - \beta_S)/\{(\alpha_R - \beta_S) \cdot (\beta_R - \beta_S) \cdot (\alpha_S - \beta_S)\} \quad \text{(Eq. 6-5)}$$

For the fitting of S-IB concentration profile following R-IB administration, parameter values obtained for S-IB following R-IB administration, parameter values obtained for S-IB following S-IB administration were used for V_{S1}, k_{S21}, α_S and β_S. Therefore, k_{RS}, k_{R21}, α_R and β_R were estimated from the fitting.

Analysis with model B. Concentration profiles of IB enantiomers in plasma were also analyzed with competitive interactions in plasma protein binding being taken into account. The pharmacokinetic model used in the analysis is shown in Figure 1B, where all the rate constants represent those for the unbound drug. It is assumed that binding process is very rapid and is to complete instantaneously. Eqs. 7-1 and 7-2 were used for the analysis of the S-IB concentration profiles following S-IB administration.

$$C_{u,S1}(D_S) = A_{Su} \cdot \exp(-\alpha_{Su} \cdot t) + B_{Su} \cdot \exp(-\beta_{Su} \cdot t) \quad \text{(Eq. 7-1)}$$

$$C_{t,S1}(D_S) = P_t \cdot K_S \cdot C_{u,S1}(D_S)/(1 + K_S \cdot C_{u,S1}(D_S)) + C_{u,S1}(D_S) \quad \text{(Eq. 7-2)}$$

where $C_{u,S1}(D_S)$ is the unbound concentration of S-IB in plasma following S-IB administration, $C_{t,S1}(D_S)$ is the total (bound plus unbound) concentration of S-IB in plasma, P_t is the RtSA concentration in plasma, and K_S is the binding constant of S-IB as described above in the binding study. A_{Su}, B_{Su}, α_{Su}, and β_{Su} can be expressed in the same manner as for A_S, B_S, α_S, and β_S in Eqs. 4-2 to 4-5, except that all parameters represent those for the unbound drug (see Fig. 1B).

Concentration profiles of R-IB and S-IB in plasma following R-IB administration were analyzed by simultaneous fitting of R-IB concentration profile to Eqs. 8-1 and 8-2, and S-IB concentration profile to Eqs. 9-1 and 9-2.

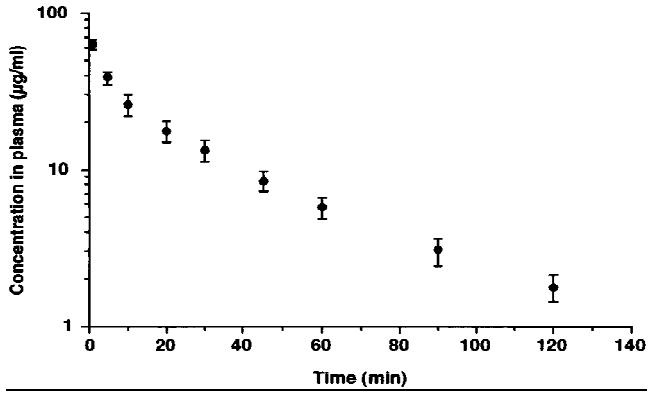

Fig. 2. S-IB concentration profile in plasma following intravenous administration of S-IB (5 mg/kg) to rats. Mean ± S.E., n = 4.

$$C_{u,R1}(D_R) = A_{Ru} \cdot \exp(-\alpha_{Ru} \cdot t) + B_{Ru} \cdot \exp(-\beta_{Ru} \cdot t) \quad \text{(Eq. 8-1)}$$

$$C_{t,R1}(D_R) = P_t \cdot K_R \cdot C_{u,R1}(D_R)/(1 + K_R \cdot C_{u,R1}(D_R) + K_S \cdot C_{u,S1}(D_R)) + C_{u,R1}(D_R) \quad \text{(Eq. 8-2)}$$

$$C_{u,S1}(D_R) = (k_{u,RS} \cdot D_R/V_{u,S1}) \cdot \{G_{Su} \cdot \exp(-\alpha_{Ru} \cdot t) + H_{Su} \cdot \exp(-\beta_{Ru} \cdot t) + I_{Su} \cdot \exp(-\alpha_{Su} \cdot t) + J_{Su} \cdot \exp(-\beta_{Su} \cdot t)\} \quad \text{(Eq. 9-1)}$$

$$C_{t,S1}(D_R) = P_t \cdot K_S \cdot C_{u,S1}(D_R)/(1 + K_R \cdot C_{u,R1}(D_R) + K_S \cdot C_{u,S1}(D_R)) + C_{u,S1}(D_R) \quad \text{(Eq. 9-2)}$$

where $C_{u,R1}(D_R)$ and $C_{u,S1}(D_R)$ are the unbound concentrations of R-IB and S-IB in plasma following R-IB administration, $C_{t,R1}(D_R)$ and $C_{t,S1}(D_R)$ are the total (bound plus unbound) concentrations of R-IB and S-IB in plasma, respectively, $k_{u,RS}$ is the rate constant for the conversion from unbound R-IB to unbound S-IB. A_{Ru}, B_{Ru}, α_{Ru}, β_{Ru} can be expressed in the same manner as for A_R, B_R, α_R and β_R in Eqs. 5-2 to 5-5, and G_{Su}, H_{Su}, I_{Su}, and J_{Su} can be expressed in the same manner as for G_S, H_S, I_S, and J_S in Eqs. 6-2 to 6-5, except that all parameters represent those for the unbound drug (see Fig. 1B). For the fitting of S-IB concentration profile following R-IB administration, parameter values obtained for S-IB following S-IB administration were used for $V_{u,S1}$, k_{S21}, α_{Su} and β_{Su}.

Statistical Analysis

Statistical analyses were conducted with paired or unpaired *t*-tests.

RESULTS AND DISCUSSION

The concentration profile of S-IB in plasma following intravenous administration of S-IB is shown in Figure 2. R-IB was not detected in plasma following S-IB administration, indicating that no conversion from S-IB to R-IB occurred in vivo in rats. The S-IB concentration profile was analyzed with a two-compartment open model (Eq. 4-1), and the parameters obtained are listed in Table 1.

Concentration profiles of R-IB and S-IB in plasma following intravenous administration of R-IB are shown in Figure 3. S-IB was observed in plasma following R-IB administration, showing the in vivo conversion of R-IB to S-IB. Shortly

TABLE 1. Pharmacokinetic parameters of IB enantiomers obtained following i.v. administration of a single enantiomer to rats

	S-IB	R-IB
V_1 (ml/kg)	67.4 ± 2.2	69.9 ± 7.3
$V_{d,\beta}$ (ml/kg)	157 ± 28	184 ± 20
CL_{tot} (ml/min/kg)	3.42 ± 0.17[a]	7.31 ± 0.52[a]
k_{10} (min^{-1})	0.051 ± 0.002[a]	0.106 ± 0.018[a,b]
k_{12} (min^{-1})	0.072 ± 0.008	0.081 ± 0.035
k_{21} (min^{-1})	0.080 ± 0.023	0.091 ± 0.029
k_{RS} (min^{-1})	—[c]	0.061 ± 0.005

Values are the means ± S.D. of three (for R-IB) or four (for S-IB) rats.
[a]Significantly different ($P < 0.05$) between R-IB and S-IB.
[b]$k_{10} = k_{RO} + k_{RS}$ (see model A).
[c]Not applicable.

after administration, S-IB concentration increased as the concentration of R-IB decreased. The concentration of S-IB exceeded that of R-IB at approximately 20 min after the administration and both R-IB and S-IB concentrations decreased afterwards, the concentration of S-IB being greater than that of R-IB. In order to calculate pharmacokinetic parameters for R-IB, concentration profiles of R-IB and S-IB were simultaneously fitted to Eqs. 5-1 and 6-1, respectively, with the conversion of R-IB to S-IB being taken into account (Fig. 1A). For fitting the concentration profile of S-IB, parameter values obtained following S-IB administration (Fig. 2 and Table 1) were used. Parameters thus obtained for R-IB are also listed in Table 1.

The results in this study are consistent with the previously reported results where only R-IB was converted to the antipode in rats and humans.[2–9] The conversion rate constant obtained in this study was 0.061 hr^{-1}, which accounted for 58% of R-IB being converted to S-IB (Table 1). The extent of conversion from R-IB to S-IB was also estimated according to the following equation:

$$F_{RS} = AUC_S(D_R) \cdot CL_{tot,S}/D_R \qquad \text{(Eq. 12)}$$

where F_{RS} is the fraction of R-IB converted, $AUC_S(D_R)$ is the area under the plasma concentration vs. time curve of S-IB following R-IB administration, $CL_{tot,S}$ is the total body clearance of S-IB obtained following S-IB administration, and D_R is the dose of R-IB. The F_{RS} value obtained was 0.544, which was similar to that obtained by the simulation of S-IB concentration profiles described above. The extent of conversion observed in this study agrees with the previously reported value for the conversion of R-IB in rats.[18]

The CL_{tot} of R-IB was significantly greater than that of S-IB ($P < 0.05$) due to conversion of R-IB. However, when the inversion clearance was subtracted from the total body clearance of R-IB, the value obtained (3.08 ± 0.33 ml/min/kg; mean ± S.D., n = 4) was similar to the $CL_{tot,S}$ value (Table 1). Therefore, the metabolism of IB enantiomers in rats may not be stereoselective, except for the unidirectional conversion of R to S.

When the racemate was intravenously administered, R-IB and S-IB concentrations in plasma were almost identical at 1 min after the administration. However, the concentration of S-IB in plasma became greater than that of R-IB as time progressed, due to the conversion from R-IB to S-IB (data not shown). The concentration profile of R-IB following racemate administration was analyzed with a two-compartment open model, and the $V_{d,\beta}$ obtained for R-IB following racemate administration (223 ± 14 ml/kg, mean ± S.D., n = 4) was significantly greater ($P < 0.05$) than that following R-IB administration (184 ± 20 ml/kg, mean ± S.D., n = 3). Since the volume of distribution was greater when the racemate was administered, it is likely that there are interactions between the enantiomers in the plasma protein binding, which will be discussed below.

Total body clearance of R-IB was significantly greater ($P < 0.05$) following racemate administration (10.9 ± 2.0 ml/min/kg; mean ± S.D., n = 4) than that following R-IB administration (7.31 ± 0.52 ml/min/kg; mean ± S.D., n = 3).

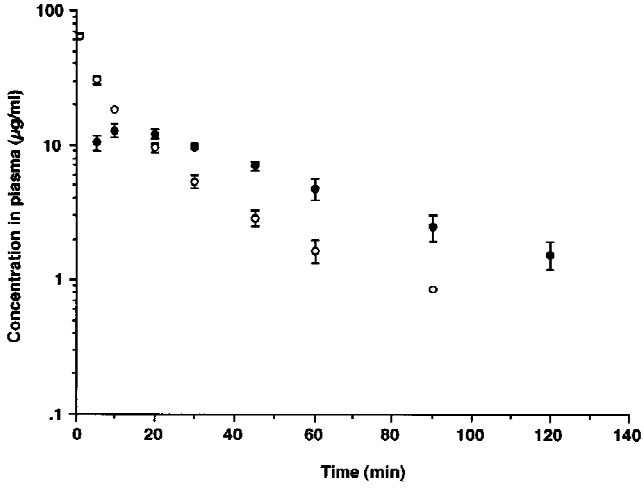

Fig. 3. Concentration profiles of R-IB (O) and S-2B (●) in plasma following intravenous administration of R-IB (5 mg/kg) to rats. Mean ± S.E., n = 3.

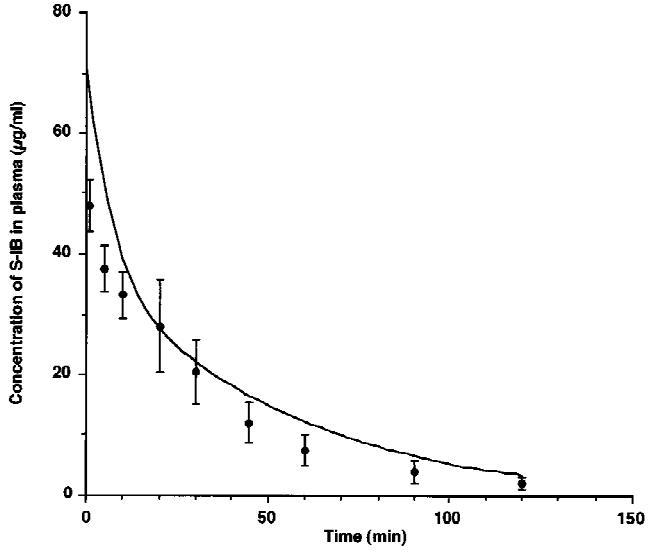

Fig. 4. Simulation of S-IB concentration profile according to model A. ——, simulated curve; ●, observed S-IB concentrations in plasma following racemate administration (10 mg/kg). Observed concentrations are shown as means ± S.D. of five rats.

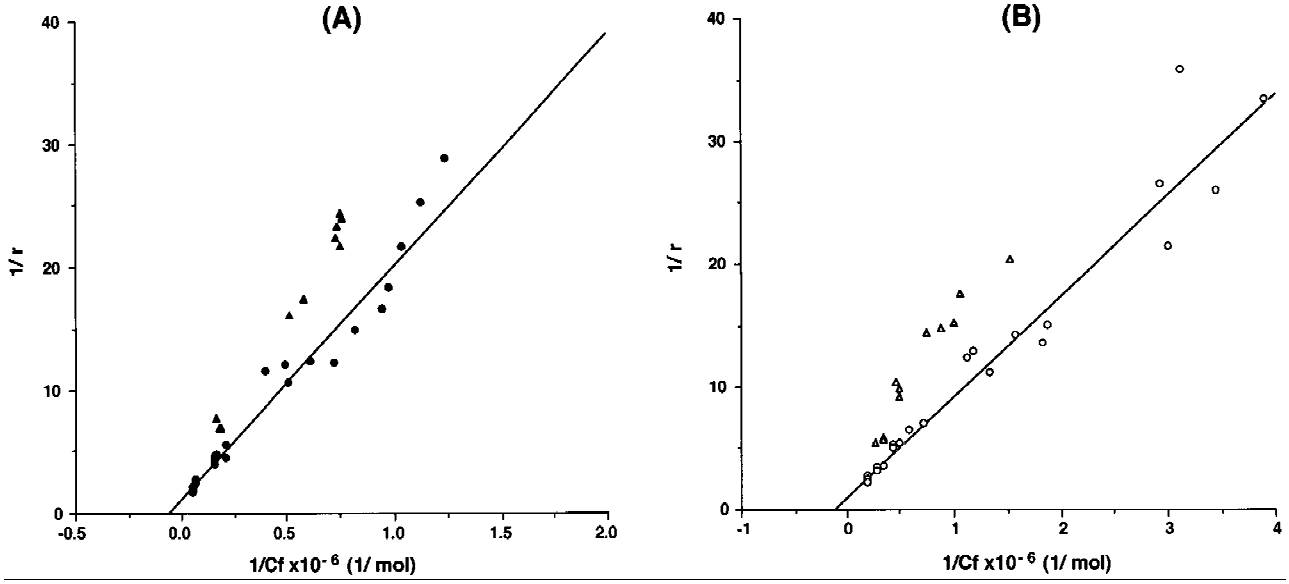

Fig. 5. Klotz plots for the binding of S-IB **(A)** and R-IB **(B)** in rat plasma. ●, S-IB alone; ▲, S-IB in the presence of excess R-IB; ○, R-IB alone; △, R-IB in the presence of excess S-IB.

This may also be due to the decreased plasma protein binding in the presence of the antipode.

S-IB concentrations in plasma following administration of the racemate were simulated according to model A (Fig. 1A) using Eqs. 4-1 and 6-1. S-IB concentration was calculated as C_{S1} (D_S) plus C_{S1} (D_R) using the parameter values listed in Table 1. The results are shown in Figure 4 together with the observed data. Simulated concentrations were significantly greater than the observed concentrations, suggesting the overestimation of S-IB concentrations due to the neglect of enantiomer-enantiomer interactions in plasma protein binding.

Binding of IB enantiomers in rat plasma was measured with the racemate at concentrations (as racemate) of 2–80 μg/ml. The unbound fraction (f_u) was in the range of 0.0179–0.0574 and 0.0278–0.0835 for S-IB and R-IB, respectively. When f_u was compared at equal concentrations, the f_u of S-IB ($f_u(S)$) was always greater than that of R-IB ($f_u(R)$), indicating that binding of IB in rat plasma is stereoselective with the binding of R-IB being more favorable than S-IB (data not shown). The $f_u(S)/f_u(R)$ ratio was 1.44 ± 0.18 (mean ± S.D., n = 16), significantly greater than unity (paired *t*-test, $P < 0.01$).

The binding of IB enantiomers in rat plasma was also measured in the absence of the antipode. The results for R-IB and S-IB are shown in Figures 5A and B, respectively. Klotz plots of R-IB and S-IB gave straight lines, indicating that a single class of binding sites is involved in the binding of both enantiomers. The number of binding sites (n) and the binding constants (K) for each enantiomer were: n = 1.00 and K = 1.21 × 10^5 M^{-1} for R-IB, and n = 0.91 and K = 5.76 × 10^4 M^{-1} for S-IB. The number of binding sites was almost equal to unity for both R-IB and S-IB, suggesting that there is a single binding site on RtSA for both enantiomers. The binding constant of R-IB was greater than that of S-IB, indicating that the stereoselectivity in the binding of IB enantiomers in rat plasma results from the difference in affinity between the enantiomers.

Stereoselectivity in plasma protein binding is similar in rat and human. Affinity of R-IB is reported to be greater than S-IB in human plasma.[17,19] Also, the affinity of the R-enantiomer is greater than that of its antipode for other 2-arylpropionic acids.[21,22,25] On the other hand, the binding constants of IB enantiomers obtained in the present study are slightly smaller than those reported in humans. It is reported that the binding constants of R-IB and S-IB in human plasma are 5.75 × 10^5 M^{-1} and 2.10 × 10^5 M^{-1}, respectively,[19] and that the binding constants of R-IB and S-IB to HSA are 5.3 × 10^5 M^{-1} and 1.1 × 10^5 M^{-1}, respectively.[20]

In order to clarify the interactions between IB enantiomers in plasma protein binding, the binding of each enantiomer was measured in the presence of excess concentrations of the antipode. When the binding of R-IB was measured in the presence of excess of S-IB, $f_u(R)$ values were significantly greater than those measured with R-IB alone at the same R-IB concentrations. The $f_u(S)$ values were also greater in the presence of R-IB than those in the absence of R-IB at equal S-IB concentrations. These observations are reflected in the Klotz plots as shown in Figures 5A and B. For each enantiomer, the slope of the Klotz plots was greater in the presence of the antipode than in its absence, suggesting that the binding of each enantiomer is inhibited by its antipode. Since only a single binding site was observed for both enantiomers and the enantiomers displaced each other from the binding site, it is very likely that IB enantiomers compete at the common binding site. Klotz plots of both enantiomers also supported competitive interactions (Figs. 5A and B). Moreover, it has been reported that the interactions between enantiomers in binding to HSA are competitive for other 2-arylpropionic acids.[21,22]

Since it has been shown that there are interactions be-

TABLE 2. Pharmacokinetic parameters of unbound IB enantiomers obtained following i.v. administration of a single enantiomer to rats

	S-IB	R-IB
V_1 (L/kg)	1.06 ± 0.26[a]	2.11 ± 0.32[a]
k_{10} (min^{-1})	0.095 ± 0.009[a]	0.192 ± 0.032[a,b]
k_{12} (min^{-1})	0.124 ± 0.019	0.184 ± 0.052
k_{21} (min^{-1})	0.066 ± 0.014	0.142 ± 0.013
k_{RS} (min^{-1})	—[c]	0.095 ± 0.016

Values are the means ± S.D. of three (for R-IB) or four (for S-IB) rats.
[a]Significantly different ($P < 0.05$) between R-IB and S-IB.
[b]$k_{10} = k_{RO} + k_{RS}$ (see model B).
[c]Not applicable.

tween IB enantiomers in the plasma protein binding, plasma concentration profiles were analyzed according to model B (Fig. 1B). In this model, all the parameters represent those for the unbound drug, assuming instantaneous attainment of the equilibrium and competitive interactions between IB enantiomers. Parameter values for unbound S-IB were obtained by fitting the plasma concentration profile following S-IB administration (Fig. 2) to Eqs. 7-1 and 7-2. Parameters were obtained by simultaneous fitting of the plasma concentration profiles of R-IB and S-IB including the conversion rate constant, for unbound R-IB (Fig. 3) to Eqs. 8-1 to 8-2 and 9-1 to 9-2, respectively. Parameters obtained for unbound S-IB and R-IB are listed in Table 2. Using these values, the concentration profile of S-IB in plasma following racemate administration was simulated using Eqs. 7-1 to 7-2 and 9-1 to 9-2, i.e. S-IB concentration following racemate administration was calculated as $C_{t,S1}(D_S)$ in Eq. 7-2 plus $C_{t,S1}(D_R)$ in Eq. 9-2. The simulated curve is compared with the observed concentrations in Figure 6 and the simulated concentrations of S-IB were in good agreement with the observed concentrations, indicating the validity of model B. It is therefore suggested that the interactions between IB enantiomers in plasma protein binding play a significant role in the disposition of IB following racemate administration, although the concentrations of IB in plasma observed in the present study were higher than the therapeutic concentrations.

The f_u value in the presence of equal concentration of the antipode was calculated using the binding parameter values and was compared with the f_u value calculated in the presence of the antipode. The f_u values were slightly (1.1-fold) greater in the presence than those in the absence of the antipode at relatively lower concentrations (10 μg/ml in plasma). However, at higher concentrations (50 μg/ml in plasma), both $f_u(R)$ and $f_u(S)$ values were significantly (approximately 2.5-fold) greater in the presence than those in the absence of the antipode. Therefore, the increase in f_u may cause an increase in the distribution volume and total body clearance following administration of the racemate.

In the present study, the pharmacokinetic characteristics of IB enantiomers were studied in rats. A unidirectional conversion from R-IB to S-IB was observed in vivo, the extent of conversion being 54–58%. Enantiomer-enantiomer interactions as well as stereoselectivity was observed in the binding of IB enantiomers in rat plasma.

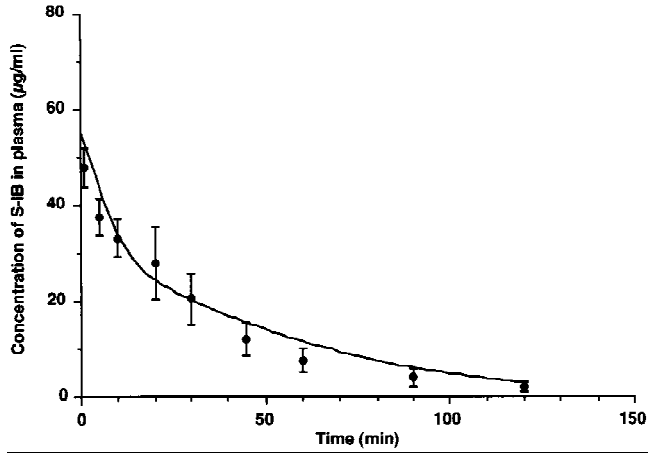

Fig. 6. Simulation of S-IB concentration profile according to model B. ——, simulated curve; ●, observed S-IB concentrations in plasma following racemate administration (10 mg/kg). Observed concentrations are shown as means ± S.D. of five rats.

Pharmacokinetic characteristics following racemate administration were different from those following the administration of individual enantiomers, due to enantiomer-enantiomer interactions in plasma protein binding. Prediction of the concentration profile following racemate administration according to the conventional compartment model (model A) gave poor agreement with the observed data. When the interactions between the enantiomers in plasma protein binding were taken into account, the prediction was closer to the observed data. Therefore, interactions between the isomers as well as stereoselectivity should be taken into consideration in analyzing the data obtained following racemate administration.

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