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Pharmacokinetic interaction of ibuprofen enantiomers in rabbits

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Abstract

The potential interaction between two ibuprofen enantiomers was studied after intravenous administration of *R*-(–)-, *S*-(+)- and racemic ibuprofen to rabbits. The total body clearance values calculated by compartmental model analysis (0.65 ± 0.21 for *R*-(–)-ibuprofen and 0.63 ± 0.34 for *S*-(+)-ibuprofen) after intravenous administration of the racemate of ibuprofen were significantly smaller than those of individual enantiomers (0.95 ± 0.23 for *R*-(–)-ibuprofen and 1.03 ± 0.23 for *S*-(+)-ibuprofen), indicating that the enantiomer–enantiomer interaction results in a mutual inhibition. The enantiomeric interaction in the pharmacokinetic behaviour of ibuprofen after racemic administration is considered to be a result of an alteration in the metabolic or excretion phase (or both) rather than stereoselective protein binding in the systemic distribution.

Introduction

Ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), is widely used in its racemic form. It is well known that the pharmacological activity of the *S*-(+)-enantiomer, the eutomer of ibuprofen, is about 160 times more potent than that of *R*-(−)-ibuprofen, the distomer, as far as the in-vitro inhibition of prostaglandin synthesis is concerned (Neal et al 1998). Geisslinger et al (1990) recently reported that pure *S*-(+)-ibuprofen, marketed in Australia in 1994, may have a distinct advantage over the racemic form, because higher plasma concentrations of *S*-(+)-ibuprofen were rapidly observed after oral administration of *S*-(+)ibuprofen.

The inactive *R*-(−)-enantiomer has novel metabolic characteristics in that it undergoes a unidirectional chiral inversion to *S*-(+)-ibuprofen in-vivo (Lee et al 1985; Jamali et al 1992). It has been reported that the inversion fraction of *R*-(−)-ibuprofen to *S*-(+)-ibuprofen is 53–60% in man (Neal 1998). Thus, a dose of *S*-(+)-ibuprofen greater than half the dose of the racemate is needed to achieve the same pharmacological and clinical effect.

Enantiomeric interactions (Zhou et al 2002) have been reported for D-cefalexin (Tamai et al 1988) during the absorption phase, propranolol (Bode et al 1995) and disopyramide (Takahashi et al 1991) during the distribution phase, cisapride (Desta et al 2001), propafenone (Li et al 1998) and nitrendipine (Tokuma & Noguchi 1995) during the metabolic phase and sotalol (Carr et al 1994) during the excretion phase. As far as the 2-arylpropionic acids (2-APAs) of NSAIDs are concerned, an enantiomer–enantiomer interaction after intravenous administration of flurbiprofen has been demonstrated following displacement from plasma protein binding sites (Berry & Jamali 1989), but there was no evidence of a pharmacokinetic interaction between the enantiomers of etodolac (Brooks & Jamali 1990). Significant enantiomeric interactions have been reported in a protein binding study of ibuprofen enantiomers in-vitro (Itoh et al 1997). Only limited data are available about the interaction of individual enantiomers of ibuprofen in man because of ethical problems.

It is important to clarify the influence of such an interaction on the pharmacokinetic properties of ibuprofen enantiomers. This exploratory study is aimed at investigating the pharmacokinetic behaviour of ibuprofen enantiomers after intravenous administration of each enantiomer and the racemate to rabbits.

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Materials and Methods

Chemicals

Racemic ibuprofen was purchased from Wako Pure Chemicals (Osaka, Japan). *R*-(−)-ibuprofen, optical purity > 98%, was chromatographically purified from racemic ibuprofen and supplied by Daicel Chemical Industries (Tokyo, Japan). *S*-(+)-ibuprofen, optical purity > 98.64%, and ibufenac (internal standard) were kind gifts from Nagase & Co. (Osaka, Japan) and Kaken Pharmaceuticals (Tokyo, Japan), respectively.

Experimental design

Eighteen Japanese white male rabbits, 2.5–3.5 kg, were purchased from Kitayama (Nagano, Japan) and acclimatized in their cages for at least one week before any experiments were undertaken. Rabbits were fasted overnight and up to the end of the experiment with free access to water. They were divided into three groups, and each group received *S*-(+)-, *R*-(−)- or racemic ibuprofen by intravenous administration. There was no significant difference in body weight among the three groups. Ibuprofen was dissolved in ethanol–propylene glycol (3:4 v/v) then diluted with water to obtain a concentration of 50 mg mL^{-1} for single isomers of ibuprofen and 100 mg mL^{-1} for racemic ibuprofen. The subjects received 50 mg of each enantiomer and 100 mg of racemic ibuprofen. Blood samples (0.5 mL) were directly withdrawn from the marginal ear veins at pre-determined sampling times and these were centrifuged at 1520 *g* for 10 min. Serum specimens were stored at −20 °C until analysis.

Treatment of rabbits complied with the guiding principles for the care and use of experimental animals and the study was approved by the Ethical Committee in Hokkaido College of Pharmacy.

Assay

Serum concentrations of *S*-(+)- and *R*-(−)-ibuprofen were analysed by a stereoselective HPLC method described in a previous report (Doki et al 2003). The assay involved extraction of ibuprofen from serum with cyclohexane and chiral separation of enantiomers using a chiral phase column (Chiralcel OD, 250 × 4.6 mm, particle size 10 μm; Daicel Chemical Industries, Tokyo, Japan). The mobile phase was hexane–2-propanol–trifluoroacetic acid (100:1:0.1), delivered at a rate of 1.0 mL min^{-1} and ibuprofen and ibufenac were detected at 225 nm. Linear calibration curves over the range 0.25–250 μg mL^{-1} with correlation coefficients of 0.999 were produced for each set of samples in the study.

Pharmacokinetic analysis

The elimination rate constant (k_e) and the volume of distribution (V_d) were calculated for each subject using a one-compartment open model with non-linear regression analysis. The total body clearance (CL_t) was calculated as $k_e \times V_d$. The area under the concentration–time curve (AUC) was determined by the trapezoidal rule up to the last measurable concentration followed by extrapolation to infinity by addition of the partial area, estimated by dividing the last measurable concentration by k_e. CL_t was also calculated by dividing the dose by the AUC. The mean fraction of inversion (f_i) from pooled data was derived from equation 1 (Lee et al 1985).

$$f_i = (AUC_{S\ \text{after}\ R} \times D_S)/(AUC_{S\ \text{after}\ S} \times D_R) \quad (1)$$

In this equation, $AUC_{S\ \text{after}\ R}$ and $AUC_{S\ \text{after}\ S}$ were the AUCs of *S*-(+)-ibuprofen after *R*-(−)-ibuprofen and *S*-(+)-ibuprofen administration, respectively. D_R and D_S are the doses of *R*-(−)-ibuprofen and *S*-(+)-ibuprofen, respectively.

Statistical analysis

Statistical evaluation of the pharmacokinetic parameters following administration of the racemate and each enantiomer were carried out by one-way analysis of variance. The differences between *R*-(−)- and *S*-(+)-ibuprofen at each time-point were evaluated using an unpaired Student's *t*-test. A value of $P < 0.05$ denoted statistical significance in all cases.

Results

The serum concentration–time profiles of ibuprofen enantiomers following intravenous administration of the racemate are shown in Figure 1. The initial *R*-(−)-ibuprofen serum concentrations were greater than those of *S*-(+)-ibuprofen, and then the order of serum concentrations of the ibuprofen enantiomers became gradually inverted with time.

Serum concentrations of *R*-(−)- and *S*-(+)-ibuprofen following intravenous administration of each enantiomer are shown in Figures 2 and 3. Although the initial concentrations of the enantiomers are almost identical to those following administration of racemic ibuprofen, significantly lower serum concentrations of both *R*-(−)- and *S*-(+)-ibuprofen after single isomer administration were

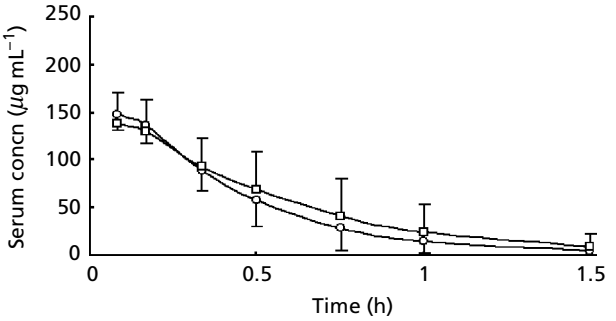

Figure 1 Serum concentration profiles of *R*-(−)- (○) and *S*-(+)- (□) ibuprofen following intravenous administration of racemic ibuprofen (100 mg) in 6 rabbits, mean ± s.d.

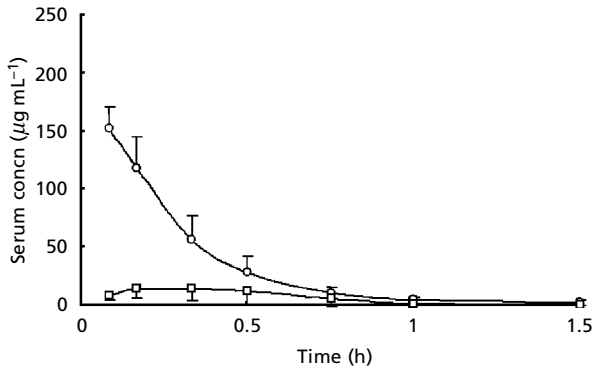

Figure 2 Serum concentration profiles of *R*-(−)- (○) and *S*-(+)- (□) ibuprofen following intravenous administration of *R*-(−)- ibuprofen (50 mg) in 6 rabbits, mean ± s.d.

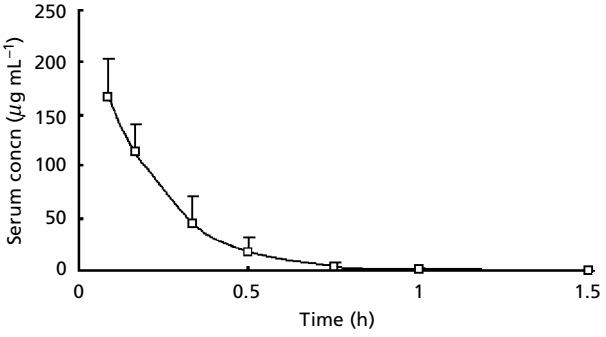

Figure 3 Serum concentration profiles of *S*-(+)-ibuprofen following intravenous administration of *S*-(+)-ibuprofen (50 mg) in 6 rabbits, mean ± s.d.

noted compared with those at 20 and 30 min after racemic ibuprofen administration.

Pharmacokinetic parameters of individual enantiomers after administration of the racemate and individual enantiomers were evaluated using a one-compartment model and the results are shown in Table 1. The total body clearance values calculated by compartmental analysis were also significantly smaller in the presence of the antipode ($P < 0.05$), but those calculated by moment analysis were not significantly different ($P = 0.07$ for *R*-(−)-ibuprofen and $P = 0.08$ for *S*-(+)-ibuprofen). The V_d value of *S*-(+)-ibuprofen after single *S*-(+)-ibuprofen administration was significantly smaller than that after administration of racemic ibuprofen. The AUC values presented no significant difference from one dose to another ($P = 0.07$ for *R*-(−)-ibuprofen and $P = 0.07$ for *S*-(+)-ibuprofen). The mean fraction of chiral inversion was calculated to be 16.1% after intravenous administration.

Discussion

The f_i value after intravenous administration was calculated to be 16.1%, which was lower than that reported in rabbits after intravenous infusion (Williams et al 1991) and that in other species (e.g. 53∼60% in man (Neal 1998); 70% in dogs, independent of the route of administration (Ahn et al 1991; Beck et al 1991)). The species-dependent chiral inversion of ibuprofen, which was also proposed by Chen et al (1991), is confirmed in this study.

Earlier reports have shown that the protein binding of ibuprofen enantiomers is competitive and stereoselective (Lee et al 1985; Evans et al 1989; Paliwal et al 1993; Smith et al 1994; Hage et al 1995; Itoh et al 1997). *R*-(−)- and *S*-(+)-ibuprofen have a common binding site in human and rat serum albumin, and the binding favoured the *R*-enantiomer. The binding constants of *R*-(−)- and *S*-(+)- ibuprofen have been shown to be $5.3 \times 10^5\ \text{M}^{-1}$ and $1.1 \times 10^5\ \text{M}^{-1}$, respectively, in human serum albumin (Hage et al 1995). The initial serum concentration of *S*-(+)-ibuprofen was considered to be lower than that of the antipode by the higher affinity of the *R*-enantiomer for serum protein, when administered as racemic ibuprofen (Figure 1). Paliwal et al (1993) and Smith et al (1994) reported the affinity constants and competitive inhibition parameters that were used in estimating the interaction of ibuprofen enantiomers. They found that the

Table 1 Pharmacokinetic parameters of *R*-(−)-ibuprofen and *S*-(+)-ibuprofen following intravenous administration in rabbits.

Parameter	Racemate 100 mg		*R*-ibuprofen 50 mg		*S*-ibuprofen 50 mg
	R	*S*	*R*	*S*	*S*
CL_t by $k_e \times V_d$ ($L\,h^{-1}$)	0.65 ± 0.21	0.63 ± 0.34	0.95 ± 0.23*	—	1.03 ± 0.23*
CL_t by D/AUC ($L\,h^{-1}$)	0.67 ± 0.20	0.68 ± 0.30	0.94 ± 0.25	—	0.99 ± 0.25
k_e (h^{-1})	2.53 ± 0.81	2.16 ± 1.01	4.23 ± 1.19*	—	5.39 ± 1.01*
V_d (L)	0.26 ± 0.03	0.30 ± 0.06	0.23 ± 0.03	—	0.19 ± 0.03*
AUC ($\mu g\,/\,mL^{-1}$)	79.5 ± 23.0	87.9 ± 39.8	56.8 ± 16.2	8.7 ± 7.2	53.8 ± 16.8
f_i (%)			16.1		

CL_t, total body clearance; k_e, elimination rate constant; V_d, volume of distribution; AUC, area under the concentration–time curve; f_i, fraction of inversion was obtained from the mean value of AUC. Each value is the mean ± s.d. of results from 6 rabbits. *$P < 0.05$ vs value for corresponding enantiomer after administration of racemate.

fraction of unbound *R*-(−)-ibuprofen increased 38% after oral administration of 300 mg *R*-(−)-ibuprofen and 600 mg *S*-(+)-ibuprofen, rather than 300 mg *R*-(−)-ibuprofen, while unbound *S*-(+)-ibuprofen increased 29% at a dose of 300 mg *R*-(−)-ibuprofen and 600 mg *S*-(+)-ibuprofen rather than 300 mg *S*-(+)-ibuprofen.

As shown in Table 1, the V_d value of *S*-(+)-ibuprofen after pure *S*-(+)-ibuprofen administration was calculated to be lower than that after administration of the racemate. Lower serum concentrations in the initial phase and larger V_d values were demonstrated after racemate administration despite the apparent amount of *S*-(+)-ibuprofen being increased by unidirectional chiral inversion, which indicates that the replacement effect of the protein binding of *S*-(+)-ibuprofen by *R*-(−)-ibuprofen was greater than the effect of unidirectional chiral inversion. No replacement effect on the protein binding of *R*-(−)-ibuprofen resulted in no change in the volume of distribution after administration of the racemate and single isomer.

The k_e and CL_t values obtained by compartmental analysis after single isomer administration were higher than those after racemate administration. The CL_t value obtained by moment analysis was directly dependent on the AUC value for which no significant difference was found because of the high degree of inter-rabbit variability. The CL_t values of *S*-(+)-ibuprofen after administration of the racemate should be increased in the presence of the antipode because of the large V_d value due to the increased free concentration of *S*-(+)-ibuprofen. Contrary to expectation, these values were smaller than those after single isomer administration in this study, indicating that the k_e value plays an important role in providing a CL_t value that is more consistent with a mutual inhibition of metabolism or excretion of the enantiomers. There is a need to evaluate this mechanism using authentic metabolite(s) of ibuprofen enantiomers.

Evans et al (1989) demonstrated that there is a degree of variability in the extent of inversion between individuals and that the kinetics of inversion may differ depending on the dosing situation after oral administration of *S*-(+)-ibuprofen and racemate in patients. For example, the clinical effect of *S*-(+)-ibuprofen at half the dose of the racemate was found to be more potent than, or at least equivalent to, the racemate in terms of relief from dental pain (Evans 2001). *S*-(+)-ibuprofen was also reported to produce less gastric damage and exhibit improved analgesic and anti-inflammatory effects in rodents after intravenous and oral administration of *S*-(+)-ibuprofen (Bonabello et al 2003; White 2003).

The clearance value of the pharmacologically active *S*-enantiomer decreased in the presence of the optical antipode and *S*-(+)-ibuprofen maintained a certain therapeutic concentration for a longer period. This means that racemic ibuprofen might be more valuable than pure *S*-(+)-ibuprofen as far as pharmacokinetic findings in this study are concerned.

Conclusion

Different pharmacokinetic behaviour of ibuprofen was observed following administration of the racemate and individual enantiomers in this study. The CL_t value of each enantiomer increased in the absence of the antipode while the V_d value decreased, suggesting that the interaction between enantiomers depended on a mutual inhibition of the metabolic or excretion phase rather than the competitive protein binding of the enantiomers.

Administration of the racemate and single *S*-(+)-enantiomer has its advantages. Simultaneous study of the pharmacokinetics and pharmacodynamics is essential to evaluate the suitability of particular ibuprofen preparations.

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