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METABOLIC CHIRAL INVERSION OF 2-ARYLPROPIONATES IN RAT H4IIE AND HUMAN HEP G2 HEPATOMA CELLS

RELATIONSHIP TO *IN VIVO* METABOLISM

S. MENZEL-SOGLOWEK,* G. GEISSLINGER, J. MOLLENHAUER and K. BRUNE

Department of Pharmacology and Toxicology, University of Erlangen-Nuernberg, Universitaetsstr. 22, D-8520 Erlangen, Germany

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Abstract—The inversion of 2-arylpropionic acids (2-APAs) has been investigated *in vitro* using rat H4IIE and human Hep G2 hepatoma cells in continuous culture. The effect of substrate concentration (15–150 μg/mL), cell density (1.5–12 × 10^6 cells/dish) and serum content of the culture medium (0–20%) on inversion was examined in rat H4IIE hepatoma cells using *R*-ibuprofen as model compound. Increasing *R*-ibuprofen concentrations and decreasing serum content of the medium resulted in increased inversion whereas variation of cell density had no effect. Furthermore, rat H4IIE and human Hep G2 hepatoma cells were incubated with the individual enantiomers of ibuprofen, ketoprofen and flurbiprofen under optimized culture conditions (serum-free culture medium). The elimination rate constants (k_{el}) and fractions inverted (F_i) were determined. Although inversion occurred slowly in the tumor cells and thus long incubation periods (120 hr) were required, the hepatoma cells were nevertheless able to mimic qualitatively the species and substance specificity of inversion of 2-APAs as observed *in vivo*.

2-Arylpropionic acids (2-APAs†) such as ibuprofen, ketoprofen and flurbiprofen, widely used anti-inflammatory agents, are marketed commercially as racemic compounds. An unusual feature of the biotransformation of this class of drugs is the unidirectional *R*- to *S*-inversion at the chiral centre. This metabolic process was demonstrated to be specie- and compound-dependent by numerous *in vivo* studies using various species and 2-APAs [1–3]. While *R*-ibuprofen was found to be inverted in all species tested [1] there was a lack of inversion of *R*-ketoprofen [4–6] and *R*-flurbiprofen [7, 8] in man and only negligible inversion of *R*-flurbiprofen in rats [7–10] (data based on plasma analysis). In order to gain detailed information about the possible site of inversion and the enzymes involved in the reaction, various *in vitro* experiments have been carried out recently. Inversion of *R*-ibuprofen occurred in the model of the *in situ* perfused rat liver [11], in crude rat liver homogenate [12] and freshly isolated rat hepatocytes [13, 14]. The results of experiments investigating inversion of 2-arylpropionates in subcellular fractions, however, are divergent. No inversion could be observed after incubation of subcellular hepatic fractions of the rat with ibuprofen racemate or of the guinea pig with both enantiomers of flurbiprofen [15], although *R*-ibuprofen is extensively inverted *in vivo* in the rat [9] and *R*-flurbiprofen in the guinea pig [10]. In contrast, a recent publication reports on stereoselective arylthioester formation of *R*-ibuprofen in mitochondria and microsomes of the rat liver with subsequent racemization [16]. The latter findings support the hypothesis of the molecular mechanism of inversion suggested by Nakamura *et al.* [17] who postulate a stereoselective activation of the *R*-enantiomer to its CoA thioester by an acyl-CoA synthetase, the thioester being racemized enzymatically to the optical antipode and cleaved by hydrolysis. Moreover, recent experiments have shown that addition of chemically synthesized CoA thioesters of both enantiomers of flurbiprofen to rat mitochondria resulted in inversion whereas addition of the *R*- and *S*-enantiomers of the free 2-APAs was without effect [18].

The *in vitro* experiments mentioned above require intricate preparation techniques, e.g. liver perfusion; they yield cells with only limited survival time and are subject to high inter-assay variability.

The main aim of our investigations was to determine whether human or rat hepatoma cells would be suitable for inversion experiments in spite of their generally low drug-metabolizing activity [19]. The advantages of hepatoma cell lines in continuous culture are evident: they are immortal and readily available and consist of rather homogeneous subpopulations, i.e. experiments are reproducible and comparable between one laboratory and another.

R-Ibuprofen, the best investigated 2-APA derivative with respect to *in vivo* as well as to *in vitro* studies of inversion, has proved to be inverted in the rat H4IIE hepatoma cell line in a preliminary experiment. In order to optimize culture conditions we examined the influence of incubation conditions on inversion of *R*-ibuprofen as model compound. Moreover, we used the optimized culture conditions for incubation of rat H4IIE and human Hep G2 hepatoma cells with the enantiomers of ibuprofen, flurbiprofen and ketoprofen to investigate whether

* Corresponding author.

† Abbreviations: 2-APA, 2-arylpropionic acid; DMEM, Dulbecco's modified Eagle's medium; AUD, area under the data points.

the cellular *in vitro* system would be able to reflect the inversion *in vivo* and thus serve as an *in vitro* model for detailed enzymatic studies of the inversion mechanism.

MATERIALS AND METHODS

The enantiomers of ibuprofen, ketoprofen and flurbiprofen were kindly supplied by Pharma Trans Sanaq AG (Basel, Switzerland), Bayer AG (Wuppertal, F.R.G.) and PAZ Arzneimittel-entwicklungsgesellschaft mbH (Frankfurt/Main, F.R.G.), respectively. The optical purity of the *R*- and *S*-enantiomers exceeded 98.5% in every case. Dulbecco's modified Eagle's medium (DMEM) and adult calf serum were obtained from Gibco (Eggenstein-Leopoldshafen, F.R.G.). All other reagents and solvents were of reagent or HPLC grade.

The H4IIE (rat hepatoma) cell line was kindly supplied by Professor Dr C.-J. Estler, Department of Toxicology and Pharmacology, University of Erlangen-Nuernberg (Erlangen, F.R.G.) and the Hep G2 (human hepatoma) cell line was a generous gift from Dr D. Schrenk, Institute of Toxicology, University of Tübingen (Tübingen, F.R.G.).

Cell culture. The H4IIE and Hep G2 cell lines were grown as monolayers in dishes of 8 cm diameter in DMEM (25 mL/dish) supplemented with 20% (H4IIE) adult calf serum, penicillin (100 U/L) and streptomycin (100 mg/L) at 37° in an atmosphere of 5% CO_2 and 95% humidified air (inoculation density 6.0×10^6 cells/dish). Cells were passaged by trypsination with 0.25% trypsin and counted in a Sysmex Microcellcounter F-300 (Digitana AG, Hamburg, F.R.G.).

Two days after passaging the cell cultures were incubated with the enantiomers of ibuprofen, ketoprofen and flurbiprofen to yield concentrations of 20 μg/mL. Drug-containing medium (0.5 mL) was removed at different time points (Figs 2–4) for stereoselective HPLC analysis.

The effect of substrate concentration, cell density, serum and glucose content of the medium on inversion was analysed using *R*-ibuprofen as model compound. Inversion was investigated at *R*-ibuprofen concentrations of 15, 30, 75 and 150 μg/mL, at seeding cell densities of 1.5, 3.0, 6.0, 9.0 and 12.0×10^6 cells/dish, at serum concentrations of 0, 5, 10, 15 and 20%, and at glucose concentrations of 1, 4, 8 and 12 g/L.

Analytical methods. The enantiomers of ibuprofen, ketoprofen and flurbiprofen were assayed according to a stereoselective HPLC method using a chiral α_1 acid glycoprotein column [6, 20]. The metabolites of ibuprofen were quantified by a non-stereoselective HPLC assay [21].

Data analysis. The area under the data points (AUD) was calculated by the linear trapezoidal rule. The elimination rate constants of the concentration–time curves were estimated using a regression equation [22]. The fraction inverted (F_i) during the experiment was calculated using the principles discussed by Pang and Kwan [23] fitted to the *in vitro* model as follows:

$$F_i = \frac{\text{AUDS}\,(R)}{\text{AUDS}\,(S)}$$

where AUDS (*R*) is the AUD of the *S*-enantiomer following incubation with the *R*-enantiomer and AUDS (*S*) is the AUD of the *S*-enantiomer following incubation with the *S*-enantiomer assuming incubation with equal concentrations of the individual enantiomers and equal incubation times.

Statistical analysis was carried out at 0.05 and 0.01 levels of significance using the Student's *t*-test (unpaired, two-tailed).

RESULTS

Effect of tissue culture conditions on the inversion of R-ibuprofen in H4IIE rat hepatoma cells

R-Ibuprofen was found to be inverted by the H4IIE rat hepatoma cell line in a preliminary experiment. Consequently, the effect of substrate concentration, cell density and serum and glucose concentration of the cell medium on inversion was investigated after incubation of rat hepatoma cells with this optical isomer.

In order to quantify *S*-ibuprofen formed by inversion the AUD of its concentration–time course was determined. Moreover, the elimination rate constants of *R*-ibuprofen were calculated.

The AUD of *S*-ibuprofen increased almost linearly ($r = 0.95$) with increasing *R*-ibuprofen concentrations, all other variables of the experiment staying constant (Fig. 1A). The elimination rate constants of *R*-ibuprofen remained unaffected at the same time (data not shown).

Increasing the number of cells and thus the cell density did not result in significantly higher AUD values of the *S*-enantiomer at constant *R*-ibuprofen concentrations of 20 μg/mL as shown in Fig. 1B. Only with the highest cell density of 12×10^6 cells/dish could a slight increase in the AUD of *S*-ibuprofen be noted which reached, however, a level of no statistical significance. These findings may indicate that the inverting enzyme system was not saturated and that the amount of bioavailable *R*-ibuprofen is the limiting factor of inversion under the conditions of the *in vitro* experiment.

Reducing the serum concentration of the culture medium from 20% of the standard medium to 0% at constant cell densities and substrate concentrations increased the AUD of *S*-ibuprofen (Fig. 1C). The elimination rate constant of the *R*-enantiomer increased from $0.0046 \pm 0.0016\ h^{-1}$ (20% serum) to $0.0245 \pm 0.0014\ h^{-1}$ (0% serum) at the same time. The AUDS of the *S*-enantiomer formed by inversion in the experiment with 0 and 5% serum on the one hand and 10, 15 and 20% on the other hand differed significantly ($P < 0.01$). The differences between 0 and 5% and 10, 15 and 20% were not statistically significant.

Variation of the glucose concentration of the cell medium had no effect either on the AUD of *S*-ibuprofen or on the elimination rate constant of the *R*-enantiomer (data not shown).

In addition to inversion other metabolic elimination pathways of *R*-ibuprofen, i.e. hydroxylation and carboxylation could be observed after incubation

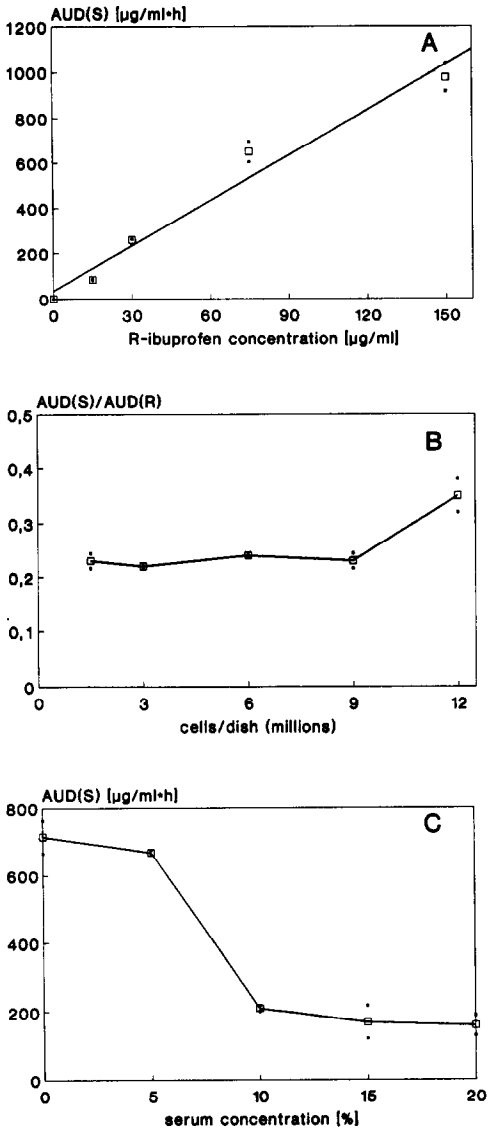

Fig. 1. The effect of substrate concentration, cell density and serum concentration of the medium on the metabolic chiral inversion of *R*-ibuprofen in H4IIE cells. (A) Effect of substrate concentration. Incubations were carried out with 6×10^6 cells/dish and at various substrate concentrations (15–150 µg/mL). (B) Effect of cell density. Incubations were carried out at substrate concentrations of 20 µg/mL with various inoculation cell densities (1.5–12.0 $\times$ 10^6 cells/dish). After incubation of ibuprofen cell division occurred every 4 days. (C) Effect of serum concentration of the medium. Incubations were carried out with 6×10^6 cells/dish and 20 µg/mL *R*-ibuprofen with various serum concentrations (0–20%) of the medium. Values are means of triplicate incubations ± SD.

of H4IIE rat hepatoma cells. The respective concentrations of the hydroxylated and carboxylated metabolites, however, were low as compared to the *S*-ibuprofen concentrations formed by inversion as illustrated in Fig. 2.

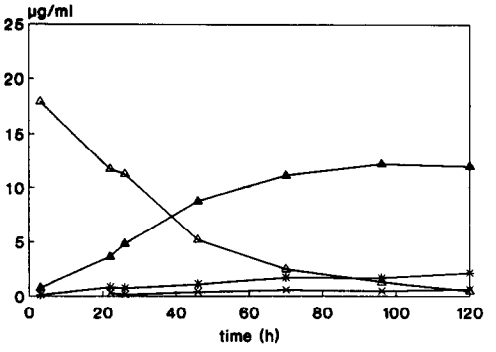

Fig. 2. Representative time courses of *R*-(–△–) and *S*-(–▲–)ibuprofen, hydroxy-(–*–) and carboxy-(–×–)-ibuprofen after incubation of H4IIE cells with *R*-ibuprofen to yield concentrations of 20 µg/mL. The cells were cultured in serum-free DMEM.

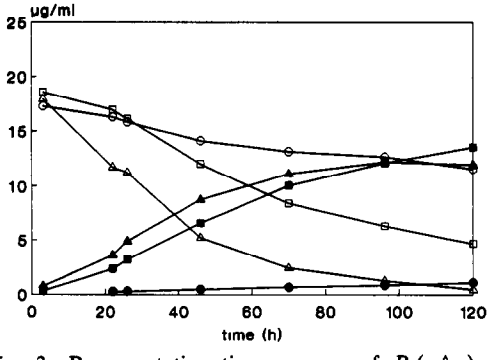

Fig. 3. Representative time courses of *R*-(–△–) and *S*-(–▲–)ibuprofen, *R*-(–□–) and *S*-(–■–)ketoprofen, *R*-(–○–) and *S*-(–●–)flurbiprofen after incubation of H4IIE cells with the respective *R*-enantiomers. The cells were cultured in serum-free DMEM.

Species and drug specificity of inversion in hepatoma cell lines

Figures 3 and 4 depict the concentration–time courses of the enantiomers of ibuprofen, ketoprofen and flurbiprofen after incubation of H4IIE and Hep G2 cells with the individual *R*-enantiomers. With respect to the results of the preceding experiments the cells were cultured in serum-free DMEM. As the optical purity of each enantiomer exceeded 98.5% and incubation of culture medium without cells with the *R*-enantiomers yielded no optical antipode, the *S*-enantiomers detected in the supernatant medium after incubation with the *R*-enantiomers could only be formed by chiral inversion in the hepatoma cells.

R-Ibuprofen and *R*-ketoprofen were inverted to their optical antipodes to a similar and considerable extent in H4IIE rat hepatoma cells. Only small amounts of the *S*-enantiomer could be quantified after incubation of *R*-flurbiprofen (Fig. 3). Regarding

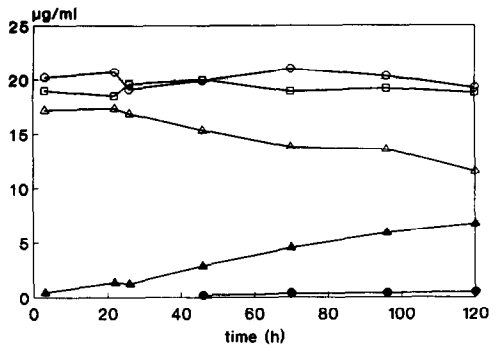

Fig. 4. Representative time courses of *R*-(–△–) and *S*-(–▲–)ibuprofen, *R*-(–□–) and *S*-(–■–)ketoprofen, *R*-(–○–) and *S*-(–●–)flurbiprofen after incubation of Hep G2 cells with the respective *R*-enantiomers. The cells were cultured in serum-free DMEM.

Fig. 4 it becomes evident that the human hepatoma cell line was able to invert *R*-ibuprofen while the concentrations of *S*-flurbiprofen formed by inversion of the *R*-enantiomer were very low. No *S*-ketoprofen could be detected after incubation of Hep G2 cells with *R*-ketoprofen. In both human and rat hepatoma cell lines no *S*- to *R*-inversion occurred, i.e. the inversion process observed in the *in vitro* experiments proved to be unidirectional. The fractions inverted (F_i) as well as the elimination rate constants (k_{el}) of the *R*-enantiomers are listed in Table 1 for the three 2-APAs investigated.

DISCUSSION

This study has demonstrated that rat and human hepatoma cells in continuous culture are able to invert 2-APAs to their optical antipodes. The most significant finding with respect to the aims of the investigations was that the inversion reaction proved to be species, i.e. cell line and drug, dependent. This phenomenon is consistent with *in vivo* experiments. A comparison of the fraction inverted (F_i) between the three 2-APA derivatives in humans and rats *in vivo* and *in vitro* is shown in Table 1.

R-Ibuprofen and *R*-ketoprofen are reported to be inverted to a high extent *in vivo* in the rat [9, 24], but only small amounts of *S*-flurbiprofen can be detected in this species following administration of the *R*-enantiomer [9]. Similar to these *in vivo* experiments we found substantial inversion of *R*-ibuprofen and *R*-ketoprofen in the rat H4IIE cell line, while *R*-flurbiprofen has only been inverted to a negligible extent. *R*-Ibuprofen, a 2-APA derivative which is inverted in man [1, 25] has also been inverted by the human hepatoma cell line. Inversion of *R*-ketoprofen and *R*-flurbiprofen, however, occurred to only a small degree similar to the situation in man *in vivo*. Furthermore, this route of biotransformation was found to be unidirectional as is the case *in vivo*, as incubation of both cell lines with the *S*-enantiomers yielded no optical antipodes.

The elimination rate constants of *R*-ibuprofen which may serve as approximations of inversion rate constants, however, seem to differ extraordinarily between the *in vitro* experiments with tumor cells [k_{el}(H4IIE): 0.0311 ± 0.0008 hr^{-1}] on the one hand and *in vivo* or *in vitro* studies using freshly isolated rat hepatocytes (k_{el}: 0.0045 ± 0.0003 min^{-1}) on the other hand [13, 14].

Cells in continuous culture are known to contain a large number of enzymes whose activities represent functions of low tissue specificity. Specific functions, however, are no longer expressed in continuous culture [19]. Thus, it is not surprising that the activity of the enzymes of the inversion reaction is low. Consequently, long incubation times of up to 120 hr are required to achieve substantial inversion which can already be observed a few hours after drug administration *in vivo* and *in vitro* after incubation of freshly isolated rat hepatocytes with *R*-ibuprofen.

Table 1. Elimination rate constants and fractions inverted of *R*- and *S*-ibuprofen, *R*- and *S*-ketoprofen, and *R*- and *S*-flurbiprofen after incubation of H4IIE and Hep G2 cells with the respective enantiomer. k_{el} values are means ± SD of three experiments

	Rat H4IIE hepatoma cells		Rat *in vivo*	Human Hep G2 hepatoma cells		Human *in vivo*
	k_{el} (hr^{-1})	F_i	F_i	k_{el} (hr^{-1})	F_i	F_i
R-Ibuprofen	0.0311 ± 0.0008	0.49	0.68†	0.0035 ± 0.0001	0.26	0.52,§ 0.63‖
S-Ibuprofen	0.0015 ± 0.0001	—*	—	0.0013 ± 0.0001	—	—
R-Ketoprofen	0.0118 ± 0.0003	0.46	>0.56‡	0.0015 ± 0.0002	—	0.10¶
S-Ketoprofen	0.0020 ± 0.0001	—	—	<0.0010	—	—
R-Flurbiprofen	0.0034 ± 0.0001	0.03	0.045†	<0.0010	0.01	—**
S-Flurbiprofen	0.0020 ± 0.0006	—	—	<0.0010	—	—

* No inversion detectable.
† Calculated from plasma concentrations using area analysis [9].
‡ Calculated by means of the cumulative amount of recovery of the *S*-enantiomer in bile [25].
§ Calculated from plasma concentrations using area analysis [26].
‖ Calculated from plasma concentrations using area analysis [27].
¶ Calculated by means of cumulative urinary excretion of the conjugated *S*-enantiomers [5].
** [7, 20].

The observed dependence of inversion on the serum content of the medium may be explained by the high binding rate of ibuprofen to serum albumin which may reduce the bioavailability of this compound in the *in vitro* experiments.

The metabolites of ibuprofen could not be determined stereoselectively and the inversion rate constants of *R*-ibuprofen were therefore not calculable. Data in the literature with respect to enantioselective metabolism of ibuprofen in freshly isolated rat hepatocytes are divergent. While Müller *et al.* [14] assumed that no biotransformation of *R*-ibuprofen except inversion occurred using this *in vitro* model, Sanins *et al.* [13] could quantify *R*-hydroxyibuprofen following incubation of freshly isolated rat hepatocytes with *R*-ibuprofen by means of GC-MS analysis. Consequently, the elimination rate constants of *R*-ibuprofen calculated in this study have to be considered as the products of both the inversion reaction and other metabolic elimination pathways of *R*-ibuprofen and may therefore only serve as approximations of inversion rate constants. In the experiments where inversion occurred, i.e. after incubation of H4IIE cells with ibuprofen or ketoprofen and of Hep G2 cells with ibuprofen, the elimination rate constants of the *S*-enantiomers were small compared with those of the *R*-enantiomers. In contrast, the difference in the elimination rate constants between *R*- and *S*-flurbiprofen after incubation of H4IIE cells with the respective enantiomers is negligible. At the same time the rate constants of the poorly inverted *R*-profens are low in comparison with 2-APAs which are substantially inverted. These data indicate that inversion represents the major metabolic pathway of 2-arylpropionates in cultured hepatoma cells.

No *S*-ketoprofen could be detected after incubation of Hep G2 cells with the *R*-enantiomer. Consequently, the observed elimination of *R*-ketoprofen has to be due to metabolic routes other than inversion. No metabolic elimination of *S*-ketoprofen and the flurbiprofen enantiomers could be noted in the Hep G2 cell line (Table 1). Slight decline of their concentration–time course is caused by sample drawing for the enantioselective HPLC analysis.

In conclusion, these experiments have shown that hepatoma cell lines in continuous culture comprise a practical *in vitro* system to investigate the metabolic chiral inversion of 2-arylpropionates. The long incubation periods required did not hinder the applicability of this *in vitro* model. From a practical standpoint, the use of tumor cell lines in continuous culture does not require the time-consuming and technically complicated procedures involved in the preparation of isolated rat hepatocytes or of subcellular fractions, and numerous parallel experiments can be performed with a homogeneous cell population. The good correlation observed between inversion of 2-APAs *in vitro* and published data on the metabolic chiral inversion of these drugs *in vivo* lends support to this conclusion.

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REFERENCES

1. Hutt AJ and Caldwell J, The metabolic chiral inversion of 2-arylpropionic acids—a novel route with pharmacological consequences. *J Pharm Pharmacol* **35**: 693–704, 1983.
2. Jamali F, Mehvar R and Pasutto FM, Enantioselective aspects of drug action and disposition: therapeutic pitfalls. *J Pharm Sci* **78**: 695–715, 1989.
3. Caldwell J, Hutt AJ and Fournel-Gigleux S, The metabolic chiral inversion and dispositional enantioselectivity of the 2-arylpropionic acids and their biological consequences. *Biochem Pharmacol* **37**: 105–114, 1988.
4. Foster RT, Jamali F, Russell AS and Alballa SR, Pharmacokinetics of ketoprofen enantiomers in healthy subjects following single and multiple doses. *J Pharm Sci* **77**: 70–73, 1988.
5. Jamali F, Russell AS, Foster RT and Lemko C, Ketoprofen pharmacokinetics in humans: evidence of enantiomeric inversion and lack of interaction. *J Pharm Sci* **79**: 460–461, 1990.
6. Menzel-Soglowek S, Geisslinger G and Brune K, Stereoselective high-performance liquid chromatographic determination of ketoprofen, ibuprofen and fenoprofen in plasma using a chiral alpha$_1$-acid glycoprotein column. *J Chromatogr* **532**: 295–303, 1990.
7. Jamali F, Berry BW, Tehrani MR and Russell AS, Stereoselective pharmacokinetics of flurbiprofen in humans and rats. *J Pharm Sci* **77**: 666–669, 1988.
8. Mayer JM, Stereoselective metabolism of anti-inflammatory 2-arylpropionates. *Acta Pharm Nord* **2**: 197–216, 1990.
9. Knihinicki RD, Day RO, Graham GG and Williams KM, Stereoselective disposition of ibuprofen and flurbiprofen in rats. *Chirality* **2**: 134–140, 1990.
10. Menzel-Soglowek S, Geisslinger G, Beck W and Brune K, Variability of inversion of *R*-flurbiprofen in different species. *J Pharm Sci*, in press.
11. Cox JW, Cox SR, VanGiessen G and Ruwart MJ, Ibuprofen stereoisomer hepatic clearance and distribution in normal and fatty in situ perfused rat liver. *J Pharmacol Exp Ther* **232**: 636–643, 1985.
12. Knihinicki RD, Williams KM and Day RO, Chiral inversion of 2-arylpropionic acid non-steroidal anti-inflammatory drugs. I. *In vitro* studies of ibuprofen and flurbiprofen. *Biochem Pharmacol* **38**: 4389–4395, 1989.
13. Sanins SM, Adams WJ, Kaiser DG, Halstead GW and Baillie TA, Studies on the metabolism and chiral inversion of ibuprofen in isolated rat hepatocytes. *Drug Metab Dispos* **18**: 527–533, 1990.
14. Müller S, Mayer JM, Etter JC and Testa B, Metabolic chiral inversion of ibuprofen in isolated rat hepatocytes. *Chirality* **2**: 74–78, 1990.
15. Mayer JM, Bartolucci C, Maitre JM and Testa B, Metabolic chiral inversion of anti-inflammatory 2-arylpropionates: lack of reaction in liver homogenates, and study of methine proton acidity. *Xenobiotica* **18**: 533–543, 1988.
16. Knadler MP and Hall SD, Stereoselective arylpropionyl-CoA thioester formation in vitro. *Chirality* **2**: 67–73, 1990.
17. Nakamura Y, Yamaguchi T, Takahashi S, Hashimoto S, Iwatani K and Nakagawa Y, Optical isomerization mechanism of *R*(−)-hydratropic acid derivatives. *J Pharmacobiodyn* **4**: s-1, 1981.
18. Porubek DJ, Sanins SM, Stephens JR, Grillo MP, Kaiser DG, Halstead GW, Adams WJ and Baillie TA, Metabolic chiral inversion of flurbiprofen-CoA in vitro. *Biochem Pharmacol* **42**: R1–R4, 1991.
19. Wiebel FJ, Roscher E and Hesse S, Metabolism of xenobiotics in cellular test systems: problems and

prospects. In: *Alternatives to Animal Experiments in Risk Assessment* (Eds. Günzel P, Reinhardt C and Schiffmann D), pp. 85–92. Sala Druck, 1988.
20. Geisslinger G, Menzel-Soglowek S, Schuster O and Brune K, Stereoselective high-performance liquid chromatographic determination of flurbiprofen in human plasma. *J Chromatogr*, **573**: 163–167, 1992.
21. Geisslinger G, Dietzel K, Loew D, Schuster O, Rau G, Lachmann G and Brune K, High-performance liquid chromatographic determination of ibuprofen, its metabolites and enantiomers in biological fluids. *J Chromatogr* **491**: 139–149, 1989.
22. Gibaldi M and Perrier D, *Pharmacokinetics*. Marcel Dekker, New York, 1982.
23. Pang KS and Kwan KC, A commentary: methods and assumptions in the kinetic estimation of metabolite formation. *Drug Metab Dispos* **11**: 79–84, 1983.
24. Kaiser DG, VanGiessen GJ, Reischer RJ and Wechter WJ, Isomeric inversion of ibuprofen (*R*)-enantiomer in humans. *J Pharm Sci* **65**: 269–273, 1976.
25. Foster RT and Jamali F, Stereoselective pharmacokinetics of ketoprofen in the rat. Influence of route of administration. *Drug Metab Dispos* **16**: 623–626, 1988.
26. Geisslinger G, Schuster O, Stock KP, Loew D, Bach GL and Brune K, Pharmacokinetics of *S*(+)- and *R*(−)-ibuprofen in volunteers and first clinical experience of *S*(+)-ibuprofen in rheumatoid arthritis. *Eur J Clin Pharmacol* **38**: 493–497, 1990.
27. Lee EJD, Williams K, Day R, Graham G and Champion D, Stereoselective disposition of ibuprofen enantiomers in man. *Br J Clin Pharmacol* **19**: 669–674, 1985.