Pergamon

Biochemical Pharmacology, Vol. 47, No. 7, pp. 1267–1270, 1994.
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0006-2952/94 $6.00 + 0.00

0006-2952(93)E0081-H

Is the inversion from *R*- to *S*-ketoprofen concentration dependent? Investigations in rats *in vivo* and *in vitro*

(*Received* 26 *August* 1993; *accepted* 13 *November* 1993)

Abstract—The effects of dose on the pharmacokinetics of ketoprofen (KT) enantiomers were investigated in rats *in vivo* and in hepatoma cells in continuous culture *in vitro* following administration of the optically pure enantiomers and the racemate of KT. With the exception of AUC (area under the curve) no pharmacokinetic differences could be found following i.v. administration of various doses of KT enantiomers (2.5, 5 and 10 mg/kg) and of racemic KT (5, 10 and 20 mg/kg) and between single enantiomer and racemate administration in rats *in vivo*. Independent of the dose administered the fraction inverted was about 66%. In line with the findings *in vivo* good correlation between incubation concentration and AUC of *R*- and *S*-KT was found in the hepatoma cells *in vitro*. The ratios of AUC(*S*)/AUC(*R*) were not significantly affected by concentration after *R*-KT (2.5–20 μg/mL) and racemate incubation (5–40 μg/mL) in the concentration ranges investigated. However, unlike in rats *in vivo* enhanced inversion was observed following racemate as compared to single enantiomer incubation *in vitro*.

Key words: ketoprofen; inversion; dose effects; *in vivo*; *in vitro*

In the past 5 years much attention has been paid to the inversion of 2-arylpropionic acids in animal experiments *in vivo* and in freshly isolated cells or cell fractions *in vitro* [1–4]. The possible sites of inversion as well as factors influencing inversion or the enzymes involved have been the subject of many studies [5–8]. Recently, we were able to show that even liver tumor cell lines in continuous culture reflect the species and substance dependent inversion of 2-APAs* *in vivo* qualitatively, although they are known to be generally deficient of those xenobiotic metabolizing enzymes which represent differentiated functions of the liver [9].

The present study was done to examine whether the dose of KT enantiomers and racemic KT, a 2-APA-derivative which is known to be substantially inverted in rats [10], had an effect on inversion rate and other pharmacokinetic parameters *in vivo* and in rat hepatoma cells *in vitro*. Moreover, a comparison of the *in vivo* and *in vitro* findings should elucidate the hypothesis that tumor cells could serve as a useful *in vitro* model with respect to metabolic inversion of 2-APAs and thus help to reduce animal experiments.

Materials and Methods

Materials. *R*- and *S*-KT were kindly supplied by Bayer AG (Wuppertal, Germany). The optical purity of the enantiomers exceeded 98.5%. Racemic KT was obtained from Sigma Chemie (Deisenhofen, Germany). Dulbecco's modified Eagle's medium and calf serum was obtained from Gibco (Eggenstein-Leopoldshafen, Germany). All other reagents and solvents were of reagent or HPLC grade.

The H4IIE (rat hepatoma) cell line was kindly supplied by Prof. Dr C.-J. Estler (Department of Toxicology and Pharmacology, University of Erlangen-Nuernberg, Erlangen, Germany).

Animal experiments. Male Sprague–Dawley rats (250–300 g) were purchased from Savo Ivanovas (Kisslegg, Germany). The animals were catheterized with polyethylene tubing at the jugular vein during anaesthesia with diethylether. The rats (N = 3 each dose) were intravenously dosed with 5, 10 and 20 mg of racemic KT and with 2.5, 5 and 10 mg of *R*- and *S*-KT via the jugular vein after overnight recovery from surgery, respectively. After drug administration the catheter was flushed with 0.2 mL of saline. The rats were allowed free access to food until drug administration with free access to water during the experiments.

The KT enantiomers and KT racemate were dissolved in phosphate buffer (0.03 M) to yield concentrations of 2 mg/mL, respectively and pH was adjusted to 7.4 with NaOH (0.1 N). Blood samples (150 μL) were drawn from the vein cannulas at 0, 0.083, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hr post dosing. The catheter was flushed with 0.2 mL heparinized saline (50 U/mL).

Cell culture. The H4IIE cell line was grown as monolayer in Dulbecco's modified Eagle's medium supplemented with 20% 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, Germany). Two days after passaging the cell cultures were incubated with serum-free culture medium containing either the single enantiomers or KT racemate at concentrations that are also reached in rat blood *in vivo*, namely 2.5, 5, 10 and 20 μg/mL or 5, 10, 20 and 40 μg/mL, respectively. A 0.5-mL aliquot of the drug containing medium was removed at different time points for stereoselective analysis.

Analytical methods. The enantiomers of KT were assayed in plasma and culture medium according to a stereoselective HPLC method using a chiral α_1-acid glycoprotein column (Chrom, Herrenberg, Germany) described previously [11].

Data analysis. Pharmacokinetic parameters were calculated model independently using the TOPFIT® computer programme [12]. The area under the concentration–time curve from time zero to 24 hr $AUC_{0-24\,hr}$ and the area under the concentration–time curve to infinity $AUC_{0-\infty}$ was calculated by the linear trapezoidal rule. The elimination rate constants were estimated by regression analysis of the linear segment of the log plasma drug concentration–time data. In the animal experiments the total clearance (CL = dose/AUC) and the volume of distribution V_Z (V_Z = CL/λz) were calculated. The fraction inverted F_i was estimated

* Abbreviations: KT, ketoprofen; AUC, area under the curve; 2-APA, 2-arylpropionic acid; AUC *S*(*R*), AUC of *S*-KT after *R*-KT administration; AUC *S*(*S*), AUC of *S*-KT after *S*-KT administration.

Table 1. Pharmacokinetic parameters of *R*- and *S*-KT following intravenous administration of various doses of *R*-, *S*- and racemic KT to rats (means ± SD, N = 3)

		Racemic KT			*R*-KT			*S*-KT		
Dose, mg/kg		5	10	20	2.5	5	10	2.5	5	10
$AUC_{0-24\,hr}$	*R*	3.8 ± 1.1	9.7 ± 0.5	17.9 ± 1.0	4.7 ± 0.8*	8.3 ± 0.1†	18.5 ± 5.1*	—	—	—
(μg hr/mL)	*S*	37.1 ± 16.7	89.4 ± 9.0	201.6 ± 31.7	17.4 ± 1.6	31.3 ± 4.2	80.1 ± 22.9	25.3 ± 5.4	49.8 ± 18.9	129.9 ± 1.8§
$AUC_{0-\infty}$	*R*	5.3 ± 2.0	11.3 ± 0.4	20.3 ± 0.7	6.5 ± 2.6†	9.6 ± 0.4†	19.5 ± 7.1*	—	—	—
(μg hr/mL)	*S*	81.6 ± 38.6	126.6 ± 16.8	465.1 ± 40.4	27.0 ± 12.7	NC	179.3 ± 29.3	43.4 ± 16.7	63.9 ± 23.7§	198.6 ± 51.8‡
$T_{1/2}$ (hr)	*R*	4.3 ± 3.6	1.7 ± 0.2	1.7 ± 0.5	3.6 ± 4.5†	2.7 ± 1.6	2.9 ± 3.2†	—	—	—
	S	21.2 ± 7.8	12.6 ± 7.3	33.4 ± 0.3	15.6 ± 8.4	NC	33.2 ± 10.4	22.0 ± 11.1	10.1 ± 5.1	15.2 ± 7.8
CL	*R*	8.9 ± 3.9	7.4 ± 0.3	8.4 ± 0.5	7.0 ± 2.8*	8.8 ± 0.4†	8.8 ± 2.9*	—	—	—
(mL/min/kg)	*S*	—	—	—	—	—	—	1.1 ± 0.5	1.5 ± 0.6	0.8 ± 0.4
V_z (L/kg)	*R*	2.5 ± 1.9	1.1 ± 0.2	1.2 ± 0.4	1.7 ± 1.9	2.0 ± 1.1	1.8 ± 1.5	—	—	—
	S	—	—	—	—	—	—	1.8 ± 0.3	1.1 ± 0.4	0.9 ± 0.4
F_i (%)	*R*	—	—	—	68.8	62.9	67.7	—	—	—

NC not calculable due to insufficient data points.

* *R*-KT after *R*-KT administration and *S*-KT after *S*-KT administration significantly different at $P < 0.01$.

† *R*-KT after *R*-KT administration and *S*-KT after *S*-KT administration significantly different at $P < 0.05$.

‡ *S*-KT after *S*-KT administration and *S*-KT after racemate administration significantly different at $P < 0.01$.

§ *S*-KT after *S*-KT administration and *S*-KT after racemate administration significantly different at $P < 0.05$.

Except the AUC values the pharmacokinetic parameters of *R*- and *S*-KT were not significantly different ($P > 0.05$) after administration of the various doses.

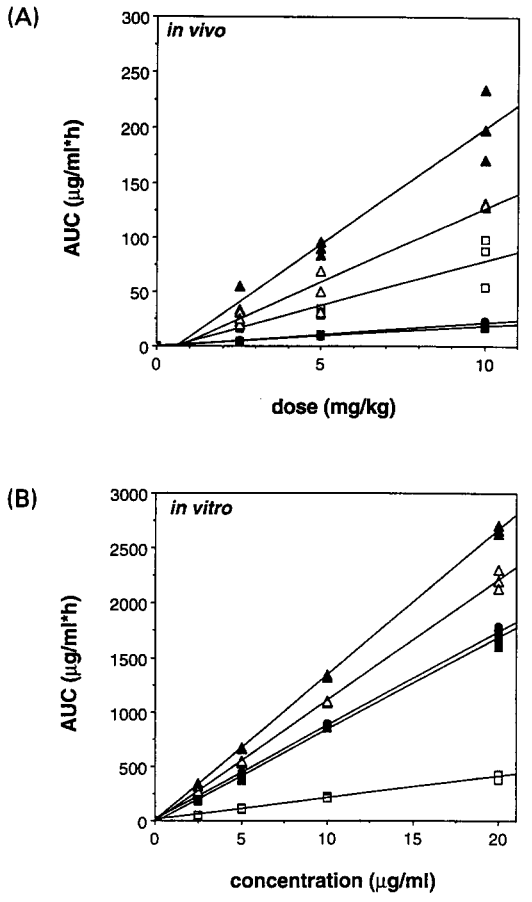

Fig. 1. AUC-dose and AUC-concentration relationships of *R*- and *S*-KT following oral administration of various doses of *R*-, *S*- and racemic KT to rats *in vivo* and rat hepatoma cells *in vitro*. For racemic KT the doses/concentrations of the individual enantiomers are given. *R*-KT after *R*-KT (●), *S*-KT after *R*-KT (□), *R*-KT after racemate (■), *S*-KT after racemate (▲), *S*-KT after *S*-KT (△).

using the principles discussed by Pang and Kwan [13]: F_i = AUC *S*(*R*)/AUC *S*(*S*) assuming administration of equal doses. In the animal experiments, however, the $AUC_{0-\infty}$ could not be calculated reliably in every case due to insufficient data points. Therefore, for AUC *S*(*R*) the $AUC_{0-24\,hr}$ of *S*-KT after *R*-KT administration and for AUC *S*(*S*) the $AUC_{0-24\,hr}$ of *S*-KT after *S*-KT was used.

In the cell culture experiments the extent of inversion was estimated as ratio of $AUC_{0-120\,hr}$ *S*(*R*)/$AUC_{0-120\,hr}$ *R*(*R*) as no other metabolization than inversion was measured.

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

Results and Discussion

The pharmacokinetic parameters of *R*- and *S*-KT in rats are summarized in Table 1. As illustrated in Fig. 1A the $AUC_{0-24\,hr}$ of *R*-KT increased almost linearly with dose after administration of the racemate ($r^2 = 0.982$) and after administration of the *R*-enantiomer ($r^2 = 0.935$). The $AUC_{0-24\,hr}$ values of *R*-KT after administration of *R*-KT and of the racemate (double dose) were not different. An enantiomeric interaction as it could be shown, e.g. for flurbiprofen in rats, could not be detected with this 2-APA derivative. Due to the high extent of inversion in rats high concentrations of the *S*-enantiomer appeared immediately after *R*-KT administration similarly to the administration of the racemate. The terminal half-life of *R*-KT was independent of dose and the presence of *S*-KT in the racemic compound. The terminal half-life of *S*-KT after administration of the racemate as well as after *S*-KT administration showed high interindividual variability, probably due to the high extent of enterohepatic circulation in rats [14]. However, short blood sampling periods (24 hr) as compared to the half-life of the drug may further contribute to variability. The terminal half-life of *R*-KT after *R*-KT or racemate administration was at least 5-fold shorter as compared to that of the *S*-enantiomer after *S*-KT or racemate administration. In line with the terminal half-life also the clearance values differed significantly ($P < 0.05$) between *R*-KT after *R*-KT administration and *S*-KT after *S*-KT administration (Table 1) for each dose but not between doses. The fraction inverted was found to be independent of dose (about 66%) in the dose range evaluated in this study.

In line with previous investigations the elimination rate i.e. inversion rate of *R*-KT was slow in hepatoma cells in continuous culture [9]. Comparing the elimination rate constants of *R*-KT in the tumor cells with the inversion rate constants of e.g. *R*-ibuprofen in experiments using freshly isolated rat hepatocytes a 30-to 40-fold decrease has to be noted [7]. Nevertheless, in spite of the long incubation periods required (up to 120 hr) the data obtained in the hepatoma cell line *in vitro* were in good agreement with the results *in vivo* with respect to dose–AUC linearity. As illustrated in Fig. 1B the $AUC_{0-120\,hr}$ of *R*-KT ($r^2 = 0.996$) and *S*-KT ($r^2 = 0.986$) after *R*-KT administration

Table 2. Elimination rate constants of *R*-KT and AUC(*S*)/AUC(*R*) ratios after incubation of H4IIE cells with various concentrations of *R*-KT and of KT racemate. k_{el} values are means ± SD of three experiments

	k_{el} (hr^{-1})	AUC(*S*)/AUC(*R*)
R-KT after *R*-KT (μg/mL)		
2.5	0.0064 ± 0.0004	0.25 ± 0.01
5	0.0072 ± 0.0007	0.25 ± 0.03
10	0.0068 ± 0.0006	0.24 ± 0.02
20	0.0064 ± 0.0011	0.24 ± 0.02
R-KT after racemic KT (μg/mL)		
5	0.0113 ± 0.0011	1.73 ± 0.15
10	0.0108 ± 0.0010	1.63 ± 0.06
20	0.0099 ± 0.0010	1.68 ± 0.11
40	0.0081 ± 0.0009	1.54 ± 0.06

increased linearly with increasing concentration and the same holds true for the incubation of the racemate. The AUC(*S*)/AUC(*R*) ratio remained nearly unaffected by various incubation concentrations. Unlike in rats in the hepatoma cells the AUC(*S*)/AUC(*R*) ratios and the elimination rate constants of the *R*-enantiomer were found to be higher ($P < 0.05$) after racemate incubation [AUC(*S*)/AUC(*R*): 0.5 ± 0.1, starting at a ratio of 1 in the racemic compound] as compared to *R*-KT incubation [AUC(*S*)/AUC(*R*): 0.26 ± 0.2] as summarized in Table 2. These findings are surprising as an inhibition of inversion of *R*-KT by the optical antipode was expected rather than enhanced inversion, e.g. Tracy *et al.* could show a diminished coenzyme A thioester formation of *R*-ibuprofen—a prerequisite for inversion—in rat liver homogenate in the presence of the *S*-enantiomer [15]. A possible reason for the increased inversion after racemate incubation in the tumor cells may be enhanced penetration of the *R*-enantiomer through cell membranes by passive diffusion, a mechanism unable to distinguish between enantiomers, and by that enhanced "bioavailability" of the *R*-enantiomer. Attempts to demonstrate inversion of either *R*-KT or *R*-ibuprofen in homogenates of hepatoma cells or hepatoma cells with disrupted cell membranes made by (a) using a mechanically driven homogenizer or (b) freezing and (c) addition of 0.1% Triton X 100 were not successful.

As a general conclusion it appears that studies of inversion in rat hepatoma cells may be used as an *in vitro* model for determining the inversion phenomena. In particular, the experiments in rats *in vivo* and in rat hepatoma cells *in vitro* are consistent with respect to dose–AUC-linearity but differ with respect to pharmacokinetics comparing single *R*-enantiomer and racemate administration. Whether these *in vitro* findings of KT are a common feature of cellular systems or a characteristic only of tumor cells remains to be investigated. Attempts to improve the *in vitro* system appear worthwhile as they contribute to reduce animal experiments as compared to e.g. studies in freshly isolated hepatocytes.

Acknowledgement—This work was supported in part by a grant of ZEBET, Fo 2.1–1328–211.

Department of Experimental and Clinical Pharmacology and Toxicology
University of Erlangen-Nuernberg
Universitaetsstr. 22
D-91054 Erlangen
Germany

S. MENZEL*
C. SAUERNHEIMER
K. BRUNE
G. GEISSLINGER

REFERENCES

1. 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.
2. 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.
3. Knihinicki RD, Williams KM and Day RO, Inversion of 2-arylpropionic acid non-steroidal anti-inflammatory drugs. I. *In vitro* studies of ibuprofen and flurbiprofen. *Biochem Pharmacol* **38**: 4389–4395, 1989.
4. Chen CS, Shieh WR, Lu PH, Harriman S and Chen CY, Metabolic stereoisomeric inversion of ibuprofen in mammals. *Biochim Biophys Acta* **1078**: 411–417, 1991.
5. Berry BW and Jamali F, Presystemic and systemic chiral inversion of *R*-(−)-fenoprofen in the rat. *J Pharmacol Exp Ther* **258**: 695–701, 1991.
6. Knights KM, Addinall TF and Roberts BJ, Enhanced chiral inversion of *R*-ibuprofen in liver from rats treated with clofibric acid. *Biochem Pharmacol* **41**: 1775–1777, 1991.
7. Müller S, Mayer JM, Etter JC and Testa B, Influence of palmitate and benzoate on the unidirectional chiral inversion of ibuprofen in isolated rat hepatocytes. *Biochem Pharmacol* **44**: 1468–1470, 1992.
8. Shieh WR and Chen CS, Purification and characterization of novel "2-arylpropionyl-CoA epimerases" from rat liver cytosol and mitochondria. *J Biol Chem* **268**: 3487–3493, 1993.
9. Menzel-Soglowek S, Geisslinger G, Mollenhauer J and Brune K, Metabolic chiral inversion of 2-arylpropionates in rat H4IIE and human HepG2 hepatoma cells. *Biochem Pharmacol* **43**: 1487–1492, 1992.
10. Iwakawa S, He X, Hashimoto S, Volland C, Benet LZ and Lin ET, Stereoselective disposition of ketoprofen in rats. *Drug Metab Dispos* **19**: 717–718, 1991.
11. Menzel-Soglowek S, Geisslinger G and Brune K, Stereoselective high-performance liquid chromatographic determination of ketoprofen, ibuprofen and fenoprofen in plasma using a chiral α_1-acid glycoprotein column. *J Chromatogr* **532**: 295–303, 1990.
12. Heinzel G, Wolosczcak R and Thomann P, *Topfit 2.0: Pharmacokinetic and Pharmacodynamic Data Analysis System for the PC.* Fischer, Stuttgart, 1993.
13. Pang KS and Kwan KC, A commentary: methods and assumptions in the kinetic estimation of metabolite formation. *Drug Metab Dispos* **11**: 79–84, 1983.
14. Menzel S, Beck WS, Brune K and Geisslinger G, Stereoselectivity of biliary excretion of 2-arylpropionates in rats. *Chirality* **5**: 422–427, 1993.
15. Tracy TS, Wirthwein DP and Hall SD, Metabolic inversion of (*R*)-ibuprofen: formation of ibuprofenyl-coenzyme A. *Drug Metab Dispos* **21**: 114–120, 1993.

* Corresponding author.