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THE DISPOSITIONAL ENANTIOSELECTIVITY OF INDOBUFEN IN RAT AND MOUSE

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Abstract—The plasma pharmacokinetics and urinary elimination of the enantiomers of indobufen, a novel platelet aggregation inhibitor, have been studied in rats and mice given either the racemic compound or the individual enantiomers (rat 8 mg/kg racemate, 4 mg/kg enantiomers; mouse 25 mg/kg racemate, 12.5 mg/kg enantiomers). Enantiospecific analysis of indobufen in plasma and urine was achieved by HPLC of its L-leucinamide diastereoisomers. In rat, the two enantiomers have very different plasma elimination half lives (*S*, 3.9 hr; *R*, 12.2 hr), irrespective of the optical form administered. The plasma concentration–time curves of *S*-indobufen were identical after racemic or *S*-indobufen, but the plasma levels of *R*-indobufen were lower after the *R*-enantiomer than after the racemate. Urinary recovery of free and conjugated indobufen was less than 3% of the dose, independent of the optical form administered. In the mouse, *R*-indobufen was cleared from plasma more rapidly than its *S*-antipode (elimination $T_{1/2}$ *R*, 2.5 hr; *S*, 3.8 hr) but differences were smaller than those seen in the rat. The plasma concentration–time curves of the *S*-enantiomer were the same after racemic or *S*-indobufen, but levels of its *R*-antipode were much lower when it was given alone than after administration of the racemate. The urinary recovery of free and conjugated indobufen also exhibited enantioselectivity, with preferential elimination of the *S*-enantiomer.

Indobufen is a platelet aggregation inhibitor whose action arises from inhibition of thromboxane synthesis [1, 2]. It finds therapeutic application in the treatment of thromboembolytic disorders of various types [3]. Consideration of the structure of indobufen reveals that it contains in its structure a chiral carbon atom in the side chain, and the molecule thus exists as a pair of enantiomers. At the present time, the drug finds therapeutic use as a racemate. A variety of studies have shown that the antiplatelet activity of this drug resides in the *S*-enantiomer, the *R*-antipode being essentially inactive (C. Ferti, L. Pierucci, G. Corsi, R. Farrario, T. Mariotto and L. Romanzini, unpublished data).

There is currently a resurgence of interest in the stereochemical aspects of pharmacology, and in cases where there is steroselectivity of action of drugs, consideration is being given to the possibility that only the active enantiomer be used [4]. This is well exemplified by the profen (2-arylpropionic acid) non-steroidal anti-inflammatory drugs [5, 6] to which indobufen has a close structural similarity: it possesses an ethyl group α- to the carboxyl group in place of the α-methyl group of the profens. Although many factors bear upon such decisions, it is most frequently the case that metabolic and pharmacokinetic information are of value [7, 8].

This report presents details of the plasma pharmacokinetics of the indobufen enantiomers in the rat and mouse following administration of single doses of the racemate and of the individual isomers.

MATERIALS AND METHODS

Compounds. *RS*-Indobufen batch No. RR 16N024, (−)-(*R*)-indobufen, batch No. A02002 (optical purity = 97.3%). (+)-(*S*)indobufen, batch No. 16001 (optical purity = 98.8%) and (+)-(*S*)-indoprofen, batch No. RR 13E049 (optical purity = 98.5%), used as the internal standard, were all the products of Farmitalia Carlo Erba (Milan, Italy). L-Leucinamide hydrochloride (optical purity >99%) was purchased from the Aldrich Chemical Co. (Milwaukee, WA, U.S.A.). Other compounds were obtained from usual commercial sources and were of best available quality.

Animals and drug administration. Female Swiss mice (mean body wt 20 g) and female Sprague–Dawley rats (mean body wt 200 g), supplied by Charles River, Italy, were used. Mice received 25 mg/kg racemic indobufen or 12.5 mg/kg of the enantiomers, while rats received 8 mg/kg racemic indobufen or 4 mg/kg of the enantiomers. The animals were given the compounds in aqueous suspension in methocel (0.5% v/v) by stomach tube.

Groups of five mice and rats were exsanguinated from the abdominal aorta under ether anaesthesia at 1, 2, 4, 6, 8, 24 and 30 hr after dosing. Plasma was separated by centrifugation at 1200 *g* and stored at −20° prior to analysis.

Other animals were dosed with indobufen enantiomers (12.5 mg/kg) or racemate (25 mg/kg) as described, housed in metabolic cages and their 0–24 hr urine collected.

Determination of the enantiomeric composition of indobufen. Indobufen enantiomers were determined in 0.1- or 0.2-mL aliquots of plasma by HPLC of

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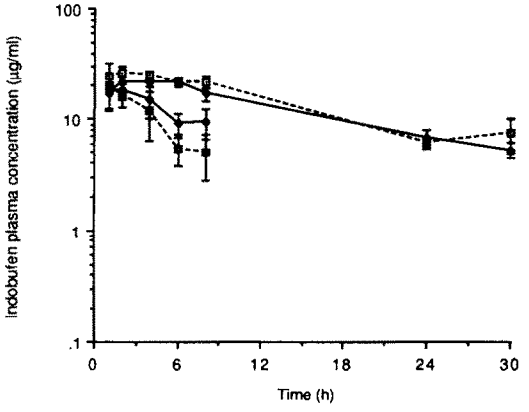

Fig. 1. Plasma concentration–time curves of indobufen enantiomers in the female rat. Data points are means ± SD of five animals. (□——□) (−)-(*R*)-Indobufen after 8 mg/kg racemic indobufen; (◆——◆) (+)-(*S*)indobufen after 8 mg/kg racemic indobufen; (◇——◇) (−)-*R*-indobufen after 4 mg/kg *R*-indobufen; (■- - - -■) (+)-(*S*)-indobufen after 4 mg/kg *S*-indobufen.

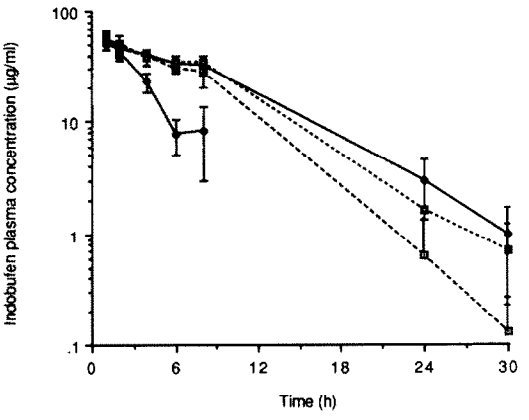

Fig. 2. Plasma concentration–time curves of indobufen enantiomers in female mice. Data points are means ± SD of five animals. (□——□) (−)-(*R*)-Indobufen after 25 mg/kg racemic indobufen; (◆——◆) (+)-(*S*)-indobufen after 25 mg/kg racemic indobufen; (◇——◇) (−)-(*R*)-indobufen after 12.5 mg/kg *R*-indobufen; (■- - - -■) (+)-(*S*)-indobufen after 12.5 mg/kg *S*-indobufen.

their L-leucinamide diastereoisomers, according to Bjorkman [9]. Concentrations were determined by reference to a calibration curve relating peak area ratio of the indobufen enantiomer derivatives to that of the internal standard with the concentration of the indobufen enantiomers. These curves were linear over the concentration range 0 to 13 μg analysed for each enantiomer, and were established on each occasion the assay was used. Intra-day variability was 5% for *R*- and 3% for *S*-indobufen and the interday variability was 2%. The limits of detection were 0.1 μg/mL in plasma and 1 μg/mL in urine (E. Moro and E. Frigerio, unpublished data).

Urinary concentration of indobufen enantiomers were determined by the above HPLC method after treatment of 0.2-mL aliquots of the urine with 0.05 mL (250 units) of β-glucuronidase (Glucurase, Sigma Chemical Co., St. Louis, MO, U.S.A.) for 16 hr at 37° at pH 4.5.

Pharmacokinetics. Plasma level–time curves for the individual enantiomers after the administration of the various forms were plotted on semilog paper and C_{max} and t_{max} obtained by inspection. The elimination rate constant β and the associated half-life $T_{1/2\beta}$ were calculated by the least-squares method applied to the linear terminal phase of the plasma level curve. The areas under the plasma level–time curves were calculated by the trapezoidal rule for the 0–8 hr time interval and after extrapolating to infinite time (AUC_∞).

Statistical analysis. To compare the pharmacokinetic behaviour of the indobufen enantiomers, the plasma concentrations measured after administration of the racemate and of the individual isomers (from 1 to 8 hr) were submitted to factorial analysis of variance with two fixed factors (times and treatments) [10]. Thereafter, using the mean plasma concentrations independent of time, the contrasts between the *S*-enantiomer (or its *R*-antipode) after racemic and *S*-indobufen (or *R*-indobufen) administration and the contrast between the two enantiomers after racemic indobufen were calculated adopting the Bonferroni criterion [11].

RESULTS

Rat

Plasma level–time curves for the enantiomers of

Table 1. Pharmacokinetic parameters of the indobufen enantiomers after oral administration of 8 mg/kg of racemic indobufen and 4 mg/kg of each enantiomer to female rats

Treatment	Compound determined	C_{max} (μg/mL)	t_{max} (hr)	$T_{1/2\beta}$ [4–30 hr] (hr)	AUC [0–8 hr] (μg × hr/mL)	AUC_∞ (μg × hr/mL)
8 mg/kg Racemic indobufen	*R*-Indobufen	26.84	2	12.6	181.25	573.98
	S-Indobufen	18.10	2	3.7	102.28	151.70
4 mg/kg *R*-Indobufen	*R*-Indobufen	21.6–21.8	2–6	12.2	153.25	456.76
4 mg/kg *S*-Indobufen	*S*-Indobufen	19.51	1	3.9*	84.38	112.32

* Evaluated considering the 24 hr concentration (not detected) equal to 0.1 μg/mL.

Table 2. Pharmacokinetic parameters of the indobufen enantiomers after oral administration of 25 mg/kg of racemic indobufen and 12.5 mg/kg of each enantiomer to female mice

Treatment	Compound determined	C_{max} (μg/mL)	t_{max} (hr)	$T_{1/2\beta}$ [8–30 hr] (hr)	AUC [0–8 hr) (μg × hr/mL)	AUC_∞ (μg × hr/mL)
25 mg/kg Racemic indobufen	*R*-Indobufen	61.77	1	2.9	300.53	416.19
	S-Indobufen	49.72	1	4.4	299.43	506.37
12.5 mg/kg *R*-Indobufen	*R*-Indobufen	56.73	1	2.5* [8–24 hr]	192.49	222.45
12.5 mg/kg *S*-Indobufen	*S*-Indobufen	54.26	1	3.8	312.36	505.73

* Evaluated considering the 24 hr concentration (not detected) equal to 0.1 μg/mL).

indobufen after the administration of racemic, *R*- and *S*-indobufen to rats are shown in Fig. 1 and selected pharmacokinetic parameters are presented in Table 1.

Statistically significant differences ($P < 0.01$) were detected between the mean plasma concentrations of the *S*- and *R*-enantiomers after racemic indobufen as well as between the mean plasma concentrations of the *R*-enantiomer after administration of racemic or *R*-indobufen. No statistically significant differences ($P > 0.05$) were detected between the mean plasma concentrations of the *S*-enantiomer after racemic and *S*-indobufen administration. The two enantiomers have different elimination half-lives, 3.9 hr for the *S*-enantiomer compared with 12.2 hr for its *R*-antipode. The elimination half-life values are essentially independent of the dose form given, with identical results obtained after administration of the enantiomers and the racemic drug.

Mouse

Figure 2 and Table 2 show mean plasma level–time curves and selected kinetic parameters for the indobufen enantiomers in the mouse after administration of the racemate and the individual isomers. Statistically significant differences ($P < 0.01$) were detected between the mean plasma concentrations of the *R*-enantiomer after racemic and *R*-indobufen administration, but there were no statistically significant differences ($P > 0.05$) in the mean plasma concentrations of the *S*- and *R*-enantiomers after racemic indobufen or in those of the *S*-enantiomer after racemic and *S*-indobufen administration.

R-Indobufen is cleared more rapidly and has a smaller AUC than its *S*-antipode when the isomers were given alone, but these differences are smaller than those seen in the rat. However, after racemic indobufen, the AUCs of the two enantiomers are essentially identical (Table 2).

Urinary elimination

The urinary elimination of indobufen enantiomers (free + glucuronide) by rats and mice given the individual enantiomers or racemic indobufen is shown in Table 3.

The urinary elimination of indobufen and its glucuronide is higher in the mouse than in the rat. However, in the mouse the elimination of the *S*-enantiomer is higher than that of the *R*-enantiomer, whereas in the rat the opposite is the case.

Table 3. Urinary elimination (0–24 hr) of indobufen enantiomers (free + conjugates) by rats and mice given the individual enantiomers or racemic indobufen

Form administered	Dose (mg/kg)	% Dose *S*	% Dose *R*
Rat			
Racemate	25	0.5	1.4
R	12.5	ND	2.7
S	12.5	0.5	ND
Mouse			
Racemate	25	13.0	4.8
R	12.5	0.4	11.2
S	12.5	29.5	ND

Values given are results from the pooled 0–24 hr urine of at least five animals in each case.
ND, not detected.

DISCUSSION

The results of this study show the occurrence of pharmacokinetic differences between the enantiomers of indobufen in rat and mouse. These differences, which are far more marked in the rat than in the mouse, are seen in the rat in terms of the slower disappearance of the pharmacologically inactive *R*-enantiomer. In both species the disposition of the *S*-enantiomer is the same whether it is given alone or as the racemic compound. However, the disposition of the *R*-enantiomer depends upon the optical form in which it is given, and this is especially noteworthy in the mouse. When given as the racemate, the AUC_∞ for *R*-indobufen is much greater than when it is given alone, suggesting that the *S*-enantiomer present in the racemate interferes with one or more processes determining the elimination of the *R*-isomer, most likely one for which the two enantiomers compete. The literature contains a number of examples of such interactions e.g. thalidomide, where the plasma pharmacokinetics and toxicity of the racemate differs greatly from its two constituent isomers [12, 13] and disopyramide [14].

No data are available in the literature on the stereochemical aspects of the fate of 2-arylbutyric acids, but as indobufen is closely related to the 2-arylpropionic acids, (the profens), it is relevant to consider the present results in the light of our knowledge of these drugs. The plasma elimination half-lives of the *S*-profens in the rat are always longer

than those of their *R*-antipodes [5, 15], the reverse of the situation seen here. It is particularly interesting to note that this is seen in the case of indoprofen, the profen analogue of indobufen, the plasma elimination of whose *S*-enantiomer in man is slower than that of its *R*-antipode [16]. Like the animal data reported here, *R*-indobufen has the longer half-life in man (E. Moro and E. Frigerio, unpublished data). These data illustrate the extreme sensitivity of the stereochemical features of drug disposition to very small changes in structure [5, 7, 17, 18].

There are a variety of possible origins for the observed differences in the plasma pharmacokinetic profiles of the indobufen enantiomers. Since the C_{max} and t_{max} values of the enantiomers are essentially identical, it is unlikely that there are differences in absorption. The processes of plasma protein binding, metabolism and urinary, biliary and faecal elimination may all be expected to exhibit enantioselectivity with respect to indobufen, since such selectivity is documented for profens [5, 14, 19, 20]. Indobufen is metabolized by oxidation of the indole nucleus and by glucuronidation of the carboxyl group, the latter process being known to exhibit considerable enantioselectivity [21, 22]. These glucuronides are diastereoisomers, since only β-D-glucopyranosiduronic acid is used, and this will increase the likelihood of isomeric discrimination in their renal or biliary elimination [14, 23, 24].

It is important to note that the present data strongly suggest that indobufen does not undergo metabolic chiral inversion in rat and mouse. Thus, the plasma concentrations of the *S*-enantiomer were the same independent of the optical form administered (*S*- or racemate), indicating that the R-form is not a precursor of its *S*-antipode. After administration of *S*-indobufen, no *R*-indobufen was recovered in rat and mouse urine (Table 3), while when the *R*-stereoisomer was given, no *S*-indobufen was excreted by rats and only very small quantities ($S/R = 0.036$) in mouse urine. This excretion in the mouse is of the order of the optical purity of the administered *R*-indobufen (enantiomeric excess 97.3%). This conclusion is further supported by studies of plasma pharmacokinetics in rats and mice after a range of doses given for up to 14 days (E. Frigerio, E. Moro, M. Strolin Benedetti, M. Brughera and R. Roncucci, unpublished data).

The urinary recovery of free indobufen and its ester glucuronide in the rat is very low (*ca.* 2% of dose) and thus this route of elimination does not influence the stereoselectivity of plasma disappearance appreciably. However, in the mouse, indobufen and its ester glucuronide account for some 20% of the dose and it is likely that there is stereoselectivity in the formation and/or urinary excretion of the glucuronide of the *S*-enantiomer (N. Grubb, J. Caldwell and M. Strolin Benedetti, unpublished data). This is in complete contrast to the slower elimination of *S*-indobufen from the plasma and the *in vitro* enantioselectivity of the glucuronidation of indobufen [21]. These data suggest that factors other than glucuronidation are responsible for the different pharmacokinetic behaviour of the indobufen enantiomers in the mouse.

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REFERENCES

1. Bergamaschi M, Ceserani R, Pierucci L, Pogliani E and Spelta A, Indobufen, a new inhibitor of platelet aggregation. Studies in guinea pigs. *Pharmacol Res Commun* **11**: 279–294, 1979.
2. Cattaneo M, Bevilacqua C, Lecchi A and Mannucci PM, *In vitro* and *ex vivo* effects of indobufen on human platelet aggregation, the release reaction and thromboxane B_2 production. *Haemostasis* **17**: 293–300, 1987.
3. Crow HJ, Salter MCP, Donaldson DR and Rajah SM, Inhibition of platelet function with indobufen: correlation with plasma drug level. *Thromb Res* **38**: 303–306, 1985.
4. Ariens EJ, Wuis EW and Veringa EJ, Stereoselectivity of bioactive xenobiotics: a pre-Pasteur attitude in medical chemistry, pharmacokinetics and clinical pharmacology. *Biochem Pharmacol* **37**: 9–18, 1988.
5. 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.
6. Hutt AJ and Caldwell J, The importance of stereochemistry in the clinical pharmacokinetics of the 2-arylpropionic acid non-steroidal anti-inflammatory drugs. *Clin Pharmacokinet* **9**: 371–373, 1984.
7. Caldwell J, Winter SM and Hutt AJ, The pharmacological and toxicological significance of the stereochemistry of drug disposition. *Xenobiotica* **18**: 59–70, 1988.
8. Smith RL and Caldwell J, Racemates: towards a New Year resolution? *TIPS* **9**: 75–77, 1988.
9. Bjorkman S, Determination of the enantiomers of indoprofen in blood plasma by high-performance liquid chromatography after rapid derivatization by means of ethyl chloroformate. *J Chromatogr* **339**: 339–346, 1985.
10. Huitson A, *The Analysis of Variance*. Griffin, London, 1966.
11. Bailey BJR, *J Am Statistical Assoc* Vol. 72, 469–478, 1977.
12. Fabro S, Smith RL and Williams RT, Toxicity and teratogenicity of optical isomers of thalidomide. *Nature* **215**: 269, 1967.
13. Hague DE, *Some Biochemical Aspects of Thalidomide Toxicity*. Ph.D. Thesis, University of London, 1969.
14. Giacomini KM, Nelson WL, Pershe RA, Valdevieso L, Turner-Tamiyasu K and Blaschke TF, *In vivo* interactions of the enantiomers of disopyramide in human subjects. *J Pharmacokinet Biopharm* **14**: 335–356, 1986.
15. Fournel S and Caldwell J, The metabolic chiral inversion of 2-phenylpropionic acid in rat, mouse and rabbit. *Biochem Pharmacol* **35**: 4153–4159, 1986.
16. Tamassia V, Jannuzzo MG, Moro E, Stegnjaich S, Groppi W and Nicolis FB, Pharmacokinetics of the enantiomers of indoprofen in man. *Int J Clin Pharm Res* **IV** 3: 223–230, 1984.
17. 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.
18. Jenner P and Testa B, The influence of stereochemical factors on drug disposition. *Drug Metab Rev* **2**: 117–184, 1973.
19. Nakamura Y and Yamaguchi T, Stereoselective metabolism of 2-phenylpropionic acid in rat. I. *In vitro* studies on the stereoselective isomerization and glucuronidation of 2-phenylpropionic acid. *Drug Metab Dispos* **15**: 529–534, 1987.
20. El Mouelhi M, Ruelius HW, Fenselau C and Dulik DM,

Species-dependent enantioselective glucuronidation of three 2-arylpropionic acids: Naproxen, ibuprofen and benoxaprofen. *Drug Metab Dispos* **15**: 767–772, 1987.

21. Nicholls S, Grubb N, Caldwell J, Strolin-Benedetti M and Roncucci R, Studies on the enantioselectivity of the microsomal glucuronidation of indobufen. In: *Chirality and Biological Activity* (Eds. Frank H, Holmstedt B and Testa B), pp. 145–151. Alan R. Liss Inc., New York, 1990.
22. Tonani R, Pianezzola E and Pella E, GCMS and radio-GLC in the structural determination of indobufen metabolites in rat urine. In: *Advances in Mass Spectrometry, Proceedings of the 8th International Mass Spectrometry Conference, Oslo, 1979* (Ed. Quayle A), pp. 1274–1279. Heyden and Son Ltd, London, 1980.
23. Spahn H, Iwakawa S, Benet LZ and Liu ET, Influence of probenecid on the urinary excretion rates of the diastereoisomeric benoxaprofen glucuronides. *Eur J Drug Metab Pharmacokinet* **12**: 233–237, 1987.
24. Caldwell J, Grubb N, Sinclair AJ, Hutt AJ, Weil A and Fournel-Gigleux S, Structural and stereochemical aspects of glucuronide formation and reactivity. In: *Cellular and Molecular Aspects of Glucuronidation* (Eds. Siest G, Magdalou J and Burchell B), pp. 185–192. INSERM/John Libbey Eurotext Ltd, 1988.