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The dispositional enantioselectivity of indobufen in man

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Abstract—The plasma pharmacokinetics and urinary elimination of the enantiomers of indobufen (2-[*p*-(1-oxo-2-isoindolinyl)-phenyl]butyric acid), a novel platelet aggregation inhibitor, have been studied in male healthy volunteers given either the racemic compound or the *S*-enantiomer (200 mg racemate, 100 mg *S*-enantiomer). Enantiospecific analysis of indobufen in plasma and urine was achieved by HPLC of its L-leucinamide diastereoisomers. After administration of the racemate, the pharmacokinetic behaviour of the *R*- and *S*-enantiomers differed, the plasma levels of the *S* form declining more rapidly [half-lives = 6.2 hr (*S*), 8.7 hr (*R*)]. No substantial differences were observed in terms of plasma level profile of *S*-indobufen when administered alone and in the racemic mixture. A statistically significant difference between the two enantiomers after administration of the racemate was found in the area under the curve (AUC), peak plasma levels (C_{max}) and elimination half-life ($t_{1/2\beta}$) whereas no statistically significant difference was detected in the time of peak (t_{max}). When the pharmacokinetic parameters C_{max}, AUC, $t_{1/2\beta}$ and t_{max} of S-indobufen administered alone or as racemate were compared, there were no statistically significant differences between treatments as well as between periods and sequences. The urinary excretion of total *S*-indobufen (free + glucuronide) and of total *R*-indobufen after administration of the racemate was essentially the same. No difference was observed either in the urinary excretion of total *S*-indobufen after administration of the racemate or of the *S*-enantiomer.

Indobufen (2-[*p*-(1-oxo-2-isoindolinyl)-phenyl]butyric acid*)(Fig. 1) is an inhibitor of platelet aggregation acting through cyclooxygenase inhibition [1, 2] but the inhibition is transient and reversible compared with the irreversible inhibition caused by aspirin [3, 4].

It would be of interest to know whether a relationship exists between thromboxane production and indobufen plasma concentrations in man. However, as indobufen possesses a chiral centre in its molecule and its activity resides principally in the *S*-enantiomer [2], it is important to know the plasma kinetics of each enantiomer following administration of the racemic compound before attempting to further correlate plasma levels and biochemical parameters.

The present study was thus designed to determine the plasma pharmacokinetics and urinary excretion of the enantiomers of indobufen after administration on separate occasions of racemic and *S*-indobufen to a group of healthy human volunteers.

Materials and Methods

Ethyl chloroformate, triethylamine and L-leucinamide hydrochloride were obtained from Fluka AG (Buchs, Switzerland) and β-glucuronidase was obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). All other solvents and reagents were of analytical grade (Farmitalia Carlo Erba, Milan, Italy) and were used without further purification.

The clinical part of the study was conducted under the direction of G. Noseda at the Beata Vergine Hospital, Mendrisio, Switzerland. The study was carried out according to local regulations and in compliance with the Helsinki Declaration. Each subject gave his written informed consent to participation in the study. After fasting overnight, 12 healthy male volunteers (aged 23–39 years, body wt 56–87 kg) received a single dose of 100 mg of *S*-indobufen or 200 mg of racemic indobufen, given as 5 mL of an aqueous suspension followed by 100 mL water, according to a crossover design with at least a 1-week period between studies. The volunteers did not eat any solid food until 4 hr after administration and they remained in hospital until the 12-hr blood sample was taken. Venous blood samples (10 mL) were taken immediately before and at 0.5, 1, 2, 4, 6, 8, 12, 24, 28, 32 and 48 hr after drug administration; the 0–12-, 12–24- and 24–48-hr urine samples were also collected. Plasma samples were obtained by centrifugation (1200 *g* for 10 min) and kept frozen at −20° until analysed. Urine samples were stored likewise.

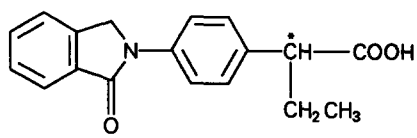

Fig. 1. Chemical structure of indobufen. * Chiral centre.

For the quantitative determination of the two enantiomers of indobufen in plasma, an HPLC method described previously [5] was used. Briefly, the drug was extracted from 1 mL plasma added with the internal standard (*S*-indoprofen) using diethyl ether. After evaporation of the solvent the two indobufen enantiomers were converted to their L-leucinamide diastereoisomers [6] and separated by reversed phase HPLC.

Urine samples were analysed after treatment with β-glucuronidase. To 0.5 mL urine were added 50 μL β-glucuronidase solution (5000 Sigma U/mL) and 2 mL of 0.1 M acetate buffer, pH 5, and the whole incubated at 37° for 16 hr. After addition of the internal standard and 1 mL 1 M HCl, the samples were extracted with diethyl ether and the organic phase back extracted with 0.5 mL 1 M NaOH. One millilitre 1 M HCl was added to the aqueous phase and the whole extracted with diethyl ether. The organic layer was separated, evaporated to dryness and the residue derivatized as described for the plasma samples.

The chromatographic separation and quantitation of the enantiomers in the plasma and urine samples was performed by reversed phase HPLC analysis using a Lichrocart Superspher (4 μm) 100 RP-18 (125-4) column (Merck, Darmstadt, Germany) with an isocratic mixture of acetonitrile–10 mM phosphate buffer, pH 6.5 (35:65), as mobile phase and a UV detector set at 275 nm.

The analytical procedure used for this study allows the determination of each enantiomer down to 0.1 μg/mL plasma and 5 μg/mL in urine with good specificity, accuracy

* Abbreviations: indobufen, 2-[*p*-(1-oxo-2-isoindolinyl)-phenyl]butyric acid; c.v., coefficient of variation.

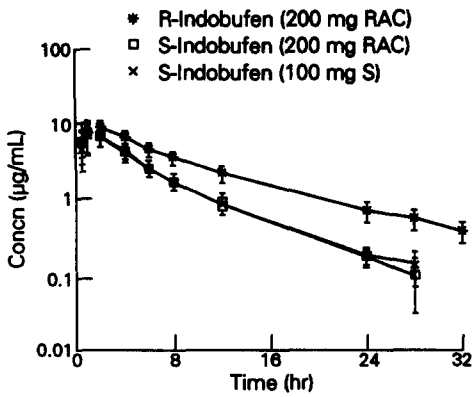

Fig. 2. Plasma levels (mean ± SD, N = 12) of the indobufen enantiomers in healthy volunteers after oral administration (as suspension) of 200 mg of racemate and 100 mg of *S*-indobufen.

(mean recovery values were 99.9 and 100.3% with coefficients of variation (c.v.s) of 4.6 and 3.5% for *R*- and *S*-indobufen, respectively; N = 15) and intra-day (c.v. 5.1 and 3.2%, N = 18) and inter-day precision (c.v. 1.7 and 1.8% for *R*- and *S*-indobufen, respectively; N = 54).

The plasma levels of both enantiomers after administration of the racemate and of the *S*-enantiomer after administration of the *S*-enantiomer were submitted to pharmacokinetic analysis for the evaluation of the following parameters: peak plasma levels (C_{max}) and time of peak (t_{max}) taken directly from the raw data; elimination rate constant (β) calculated by the least squares method applied to the terminal linear phase of the log plasma levels vs time curve; elimination $t_{1/2\beta}$ calculated as $0.693/\beta$; total area under plasma level curve (AUC) calculated by the trapezoidal rule up to the last measurable plasma sample $C(t')$ plus the extrapolated term to infinity $[C(t')/\beta]$.

Comparison of the *S*-enantiomer after racemic and *S*-indobufen administration: the parameters $t_{1/2\beta}$ (after reciprocal transformation), C_{max} and AUC (both after log transformation) were submitted to analysis of variance [7] for crossover design to evaluate the sequence, period and treatment effects. The parameter t_{max} was submitted to the non-parametric Koch's test for a two period crossover design [8]. For C_{max} and AUC, the relative bioavailability was also evaluated as ratio of geometric means and P = 0.95 symmetrical confidence intervals according to Westlake [9].

Comparison between the *R*- and *S*-enantiomer after administration of the racemate: the parameters $t_{1/2\beta}$, C_{max} and AUC were submitted to the Student's *t*-test for paired data. The non-parametric Wilcoxon's test for paired data was used for t_{max}.

Results and Discussion

The mean plasma levels of the two enantiomers of indobufen after single oral administration as suspension of the racemic drug (200 mg) and of the *S*-enantiomer (100 mg) to 12 healthy male volunteers are shown in Fig. 2. The cumulative urinary excretion of indobufen enantiomers (free + glucuronide) is presented in Table 1.

After the administration of the racemate, the pharmacokinetic behaviour of the *R*- and *S*-enantiomers differed, the plasma levels of the *S* form declining more rapidly. No substantial differences were observed in terms of plasma level profile and urinary excretion rate of *S*-indobufen when administered alone and in the racemic mixture. After administration of *S*-indobufen the *R*-enantiomer could generally not be detected in plasma or urine samples. Only very small amounts (about 0.1–0.15 μg/mL) of the *R*-enantiomer were found in some plasma samples, just accounting for the amount of the *R*-enantiomer (about 1%) present as impurity in the batch of *S*-indobufen used for preparing the administered suspension.

Table 1 summarizes the main pharmacokinetic parameters of the two enantiomers. A statistically significant difference between the two enantiomers after administration of the racemate was found in the AUC ($P < 0.01$), C_{max} ($P < 0.01$) and $t_{1/2\beta}$ ($P < 0.01$) whereas no statistically significant difference was detected in the t_{max}.

When the pharmacokinetic parameters C_{max}, AUC, $t_{1/2\beta}$ and t_{max} of *S*-indobufen administered alone or as racemate were compared, there were no statistically significant differences between treatments as well as between periods and sequences. Moreover, the relative bioavailability (P = 0.95 symmetrical confidence intervals) (*S* after *S* vs *S* after racemate) was 102.3% (91–109%) for AUC and 100% (77%–123%) for C_{max}, respectively, which indicates bioequivalence of the two modalities of administration in terms of the *S*-enantiomer disposition.

Table 1. Main pharmacokinetic parameters and urinary excretion data of the enantiomers of indobufen after single administration (as oral suspension) of 200 mg of racemic indobufen and 100 mg of *S*-indobufen to 12 healthy volunteers

Treatment	Enantiomer	C_{max} (μg/mL)	t_{max} (hr)	β (hr^{-1})	$t_{1/2}$ (hr)	CL/F (L/hr)	V_d/F (L)	A_e (0–48 hr) (mg)
Racemic indobufen (200 mg)	*R*	9.73 (0.59)	1.87 (0.33)	0.080 (0.002)	8.68 (0.18)	1.21 (0.06)	15.12 (0.76)	82.67* (2.91)
	S	7.93 (0.53)	1.50 (0.28)	0.114 (0.005)	6.21 (0.27)	2.26 (0.09)	20.15 (1.02)	78.98* (2.37)
S-indobufen (100 mg)	*S*	8.10 (0.74)	1.62 (0.36)	0.119 (0.006)	6.00 (0.31)	2.23 (0.85)	19.14 (0.98)	77.47† (3.61)

Values are means (SEM).
* N = 9.
† N = 10.
CL, clearance; V_d, volume of distribution; *F*, bioavailability; A_e, urinary excretion.

Estimates of clearance and volume of distribution, assuming that the absolute bioavailability of indobufen is 1, give mean values of 1.2 L/hr and 15.1 L for the *R*-enantiomer and of 2.3 L/hr and 20.1 L for the *S*-enantiomer.

The urinary excretion of total *S*-indobufen and of total *R*-indobufen after administration of the racemate was essentially the same. No difference was observed either in the urinary excretion of total *S*-indobufen after administration of the racemate or of the *S*-enantiomer.

The results obtained in this study provide some clarification of the most important aspects of the disposition of the indobufen enantiomers in man. First, after administration of racemic indobufen, the plasma levels of the *S* form were markedly lower than those of the *R* form. Pharmacokinetic analysis suggests that this difference is due to faster elimination of the *S*-enantiomer. After administration of the *S*-enantiomer no detectable levels of the *R*-enantiomer were found either in plasma or in urine, showing that no chiral inversion of *S*-indobufen to its *R*-antipode occurs in man.

Plasma levels, urinary excretion and pharmacokinetic parameters of *S*-indobufen were the same after administration of the racemate or of the *S*-enantiomer when this was given at half of the dose, showing that in man the pharmacokinetic behaviour of the *S*-enantiomer is not affected by the presence of the *R*-enantiomer and that *R*-indobufen is not converted metabolically to the *S*-enantiomer. In man, the urinary excretion of total indobufen (free + glucuronide) is very important and this is in sharp contrast with data obtained in the rat [5]. In conclusion, as the pharmacokinetic behaviour of *S*-indobufen, which is the active enantiomer, in man showed some differences as compared with the *R*-enantiomer, any future work attempting to establish plasma concentration–response relationships should be done using the *S*-enantiomer values.

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Effect of ethanol on the Na^+- and the Na^+,K^+-ATPase activities of basolateral plasma membranes of kidney proximal tubular cells

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Abstract—The Na^+- and the Na^+,K^+-ATPase activities of basolateral plasma membranes from rat kidney proximal tubular cells were affected differentially by ethanol. Moreover, at concentrations of ethanol that can be reached *in vivo* in the blood plasma (50 mM) there was a significant effect on the Na^+-ATPase activity and practically no effect on the Na^+,K^+-ATPase activity.