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SHORT COMMUNICATIONS

Excretion balance and urinary metabolism of indobufen in rats and mice

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Abstract—The excretion balance and the urinary metabolism of indobufen (±2-[*p*-(1-oxo-2-isoindolinyl)-phenyl] butyric acid), a platelet aggregation inhibitor, has been studied in rats and mice after oral administration. The urinary metabolic profile of indobufen exhibited a marked species difference. The major metabolic pathway in the mouse was acyl glucuronidation followed by renal excretion, whereas in rats, 5-hydroxylation and subsequent sulphation at the introduced hydroxyl group predominated. Comparison of these results with previous data obtained in humans indicates that the mouse, and not the rat, is the rodent species of choice to be considered in the study of this compound.

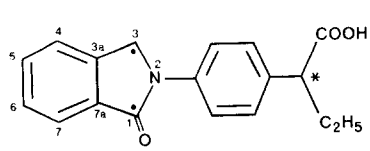
Indobufen (±2-[*p*-(1-oxo-2-isoindolinyl)-phenyl]butyric acid) (Fig. 1) is an inhibitor of platelet aggregation able to block various parameters of platelet function by reversible inhibition of the enzyme cyclooxygenase, thereby inhibiting production of thromboxane A_2 [1, 2]. Indobufen is used therapeutically in the prevention of venous thrombosis and in conditions such as peripheral vascular disease and cardiac ischaemic disease, and intermittent claudication where improvements in walking distances have been achieved [3–5]. A review of its properties and therapeutic efficacy in several diseases has appeared recently [6].

Although indobufen contains a chiral centre (Fig. 1), and thus exists as a pair of enantiomers, its activity *in vitro* and *in vivo* in animals and humans resides almost exclusively in the *S*-enantiomer, the *R*-enantiomer being essentially inactive, as with the closely structurally related profen (2-arylpropionic acid) non-steroidal anti-inflammatory drugs [7–9]. Indobufen possesses an ethyl group α- to the carboxyl group in place of the α-methyl group of the profens.

Metabolic chiral inversion from the less active *R*-enantiomer to the active *S*-enantiomer has been observed for several profens, both *in vivo* and *in vitro* [10]. Chiral inversion has, however, not been observed for indobufen after either single or repeated doses to animals or man [11–13], which makes it adequate in most studies for interpreting the pharmacokinetics of this compound without necessarily studying again the pharmacokinetics of the individual enantiomers. This report presents the results of the excretion balance and urinary metabolism of indobufen in rats and mice, two species used in its preclinical safety evaluation.

Materials and Methods

Chemicals. [^{14}C]Indobufen (±2-[*p*-(1-oxo-2-[1,3-^{14}C]iso-indolinyl)phenyl]butyric acid), sp. act. 38.07 mCi/mmol, radiochemical purity >98% by ratio TLC: batch No. RT 8545/39 was supplied by Farmitalia Carlo Erba (Milan, Italy), as were samples of unlabeled indobufen and 3-, 5- and 6-hydroxyindobufen. Other chemicals and reagents were obtained from usual commercial sources and were of the best available quality.



Indobufen

Fig. 1. The structure of indobufen. * Chiral centre, (•) ^{14}C label.

The acyl glucuronides of indobufen and the acyl and phenolic glucuronides of 5- and 6-hydroxyindobufen, for use as HPLC standards, were biosynthesized by incubation of the appropriate substrate with UDP-glucuronic acid and rabbit liver microsomes as described previously [14]. The acyl and phenolic glucuronides of 3-hydroxyindobufen were not detected after microsomal incubation of 3-hydroxyindobufen.

Administration of indobufen. Male Wistar rats (200–250 g) and female CD-1 mice (20–25 g) were housed in all glass metabolism cages (Metabowls for rats and Minimetabowls for mice, Jencons Ltd, Leighton Buzzard, U.K.) equipped for the separate collection of urine and faeces, with free access to food (GLP grade diet CRM, Special Diets Services, Cambridgeshire, U.K.) and water. [^{14}C]Indobufen (*ca.* 5 μCi/animal) was administered p.o. by gavage in propane-1,2-diol in volumes of 0.5 mL containing 20 mg/kg for rats and 0.2 mL containing 25 mg/kg for mice. Urine and faeces were collected daily for 4 days. Urine was collected in glass vessels containing glacial acetic acid (0.1 and 0.04 mL for rats and mice, respectively) cooled with dry ice.

Radiochemical techniques. ^{14}C in urine and HPLC eluents was assayed by liquid scintillation counting using Packard TriCarb instruments Models 4640 or 4450 (Canberra-Packard, Pangbourne, U.K.). Aliquots (0.05–0.5 mL) were counted in triplicate in minivials using a water-miscible biodegradable scintillation cocktail (Ecoscint, National Diagnostics, Watford, U.K.). Quench correction was achieved by reference to an external standard using a standard curve stored in the instrument which was established at regular intervals. The ^{14}C content of faeces was determined after alkali digestion and bleaching. The faeces from each 24-hr collection were homogenized in water (30 and 15 mL for rats and mice, respectively) using a Stomacher Lab-Blender (Seward Medical, London, U.K.) for 1 min. Two millilitres of the homogenate was mixed with 1 mL 5 M NaOH, 2 mL H_2O_2 and five drops of iso-amyl alcohol to control foaming and left overnight. The solution was neutralized with 260 μL glacial acetic acid and the volume made up to 10 mL with ethanol. The solution was then heated at 70° for 30 min to remove excess H_2O_2. The ethanol lost through evaporation was replaced and the solution clarified by centrifugation. One-half-millilitre aliquots were counted in triplicate in 10 mL of Ecoscint. A quench correlation curve was established using faeces from untreated animals.

HPLC. All HPLC assays were performed using a

Table 1. Retention time of indobufen and of the synthetic and biosynthetic standards available in the HPLC system used

Compound	t_r (min)
5-Hydroxyindobufen glucuronide (phenolic)	3.8
6-Hydroxyindobufen glucuronide (phenolic)	5.1
5-Hydroxyindobufen glucuronide (acyl)	6.4
6-Hydroxyindobufen glucuronide (acyl)	8.0
3-Hydroxyindobufen	10.4
5-Hydroxyindobufen	15.1
6-Hydroxyindobufen	16.6
Indobufen glucuronide	18.0
Indobufen	36.8

Shimadzu LC-6A pump, RF-535 fluorescence detector and C-R6A Chromatopac computing integrator (supplied by Dyson Instruments Ltd, Houghton-le-Spring, Tyne and Wear, U.K.). Samples were introduced on to the column with a Waters Associates (Northwich, U.K.) WISP Model 710B autoinjector. The fluorescence detector was set at excitation 290 nm, emission 440 nm. The column was a Merck LiChrosorb C18 cartridge, 250 × 4 mm i.d., and mobile phase was a step gradient of acetonitrile:water, 25:75 (v/v) containing 0.1% trifluoroacetic acid from 0 to 30 min, then acetonitrile:water, 40:60 (v/v) containing 0.1% trifluoroacetic acid from 30 to 50 min, flow rate 2 mL/min throughout at room temperature. Fluorescence detection was used to characterize the chromatographic behaviour of indobufen and the synthetic and biosynthetic standards available. After injection of urine, fractions of column eluent were collected every 30 sec in mini-scintillation vials, using an LKB 2112 RadiRac fraction collector (LKB instruments Ltd, Croydon, U.K.) and counted for ^{14}C as above. Urine was examined by HPLC before and after treatment with β-glucuronidase, sulphatase or mild alkali, the ester glucuronides being cleaved by mild alkali or β-glucuronidase treatment, whereas the phenolic glucuronides are hydrolysed only by β-glucuronidase [15].

Analysis of indobufen metabolites in rat and mouse urine. The metabolites of [^{14}C]indobufen in rat and mouse urine were analysed using HPLC. Indobufen and 5- and 6-hydroxyindobufen were characterized by coelution with authentic standards. Indobufen glucuronide was characterized by coelution with biosynthetic indobufen glucuronide and by selective hydrolysis of urine with β-glucuronidase (Glucurase, 5000 U/mL β-glucuronidase *ex* bovine liver, Sigma Chemical Co., Poole, U.K.) or mild alkali (pH 10, 1 hr, room temperature).

Table 2. Cumulative urinary and faecal excretion of ^{14}C after the oral administration of [^{14}C]indobufen to rats (20 mg/kg) and mice (25 mg/kg)

Time (hr)	Urine	Faeces
Rat		
0–24	16.9 ± 4.7	35.6 ± 7.5
24–48	9.6 ± 0.9	18.3 ± 4.6
48–72	3.2 ± 1.2	4.2 ± 2.3
72–96	1.0 ± 0.8	1.1 ± 1.1
Total	30.7 ± 4.2	59.1 ± 7.3
Mouse		
0–24	18.0 ± 6.7	11.3 ± 3.6
24–48	19.1 ± 6.5	21.8 ± 11.0
48–72	7.8 ± 4.6	8.1 ± 3.8
72–96	2.2 ± 2.4	1.2 ± 1.0
Total	47.1 ± 5.4	42.4 ± 10.2

Values are mean % ^{14}C dose excreted ± SD, N = 6.

5-Hydroxyindobufen sulphate was characterized by hydrolysis of urine with sulphatase (Type H-1, *ex Helix pomatia*, sp. act. 18,000 U/g, Sigma). Aliquots of urine (0.5 mL rats and 0.2 mL mice) were diluted 2:1 with 0.2 M acetate buffer pH 5.0 and incubated at 37° overnight with either 0.3 vol. glucurase or 0.64 mg sulphatase. Positive and negative control incubations to confirm enzymic activity were performed according to Hutt and Caldwell [10].

Results and Discussion

The retention times of indobufen and of the synthetic and biosynthetic standards in the HPLC used in the present study are listed in Table 1.

The excretion balance and urinary metabolite profile of [^{14}C]indobufen in rats and mice are presented in Tables 2 and 3, respectively. The recovery of ^{14}C was essentially quantitative with some 90% of the dose being recovered in urine and faeces over 4 days. Faecal ^{14}C recovery (59% in 0–96 hr) was higher than the urinary recovery (31% in 0–96 hr) in the rat, while in the mouse the reverse was true, with 47 and 42% of the dose being recovered in the urine and faeces in 0–96 hr, respectively. The total elimination of ^{14}C in rats was more rapid than in mice with 53% of the dose being recovered in 0–24 hr compared with 29% over the same period in mice.

The major metabolic transformation of indobufen in rats was 5-hydroxylation and subsequent sulphation of the 5-hydroxyl group (6.0 and 12.1% of the dose as free and sulphated 5-hydroxy-indobufen, respectively, in urine, 0–

Table 3. Urinary metabolites of [^{14}C]indobufen after oral administration to rats (20 mg/kg) and mice (25 mg/kg)

	Urine collection			
	Rats		Mice	
Metabolite	0–24 hr	24–48 hr	0–24 hr	24–48 hr
Indobufen				
Free	0.8	0.9	0.3	0.5
Glucuronide	0.3	0.2	12.8	13.1
5-Hydroxyindobufen				
Free	2.3	3.7	0.4	0.4
Sulphate	8.4	3.7	ND	ND
6-Hydroxyindobufen	ND	ND	0.4	0.6
Unknown(s)	5.1	1.1	4.1	4.5
Total	16.9	9.6	18.1	19.1

Figures are mean % ^{14}C dose excreted as that metabolite, N = 6.
ND, not detected.

48 hr) (Table 3). In the mouse, however, the major urinary metabolite was indobufen glucuronide (25.9% of dose, 0–48 hr) (Table 3).

These results show a marked species difference in the metabolism of indobufen in the rat and mouse. The major metabolic pathway in the rat is 5-hydroxylation and subsequent sulphation of the introduced 5-hydroxyl group followed by urinary excretion. It appears that hydroxylation is regioselective for position 5 and that hydroxylation in position 6 is at most a very minor reaction. Moreover, no hydroxylation occurs at position 3. Similar data with different methodologies were obtained by Tonani *et al.* after oral administration of indobufen to rats [16]. Hydroxylation of moieties remote from the carboxyl group and chiral centre can be an important metabolic transformation of certain 2-phenylpropionic acid non-steroidal anti-inflammatory drugs and has been demonstrated to show regioselectivity [17, 18]. Unlike the situation with many profens, indobufen acyl glucuronide was essentially absent from rat urine, although indobufen glucuronide is a major biliary metabolite in the rat [19].

In humans about 80% of the administered dose is recovered in the 0–48 hr urine mostly as indobufen acyl glucuronide [13, 20, 21], which suggests that biliary elimination of the drug and/or its metabolites in humans, if any, occurs only to a very limited extent.

The disposition and urinary metabolic profile in the mouse resemble man [13, 20, 21] in that not only is urine a preferential route of excretion of radioactivity, although not as important as in man, but also indobufen acyl glucuronide is the major urinary metabolite. The mouse, therefore, represents a better model for disposition and metabolism of indobufen in man than does the rat.

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