PHARMACOKINETICS OF INDORAMIN IN MAN

G.H. DRAFFAN¹, P.J. LEWIS², J.L. FIRMIN, T.W. JORDAN & C.T. DOLLERY

Department of Clinical Pharmacology, Royal Postgraduate Medical School, Du Cane Road, London W12 0HS

1 The fate of oral and intravenous indoramin has been studied after single doses in man using the ¹⁴C labelled drug. Plasma concentrations of indoramin have been determined during chronic oral antihypertensive therapy employing a stable isotope dilution assay.

2 Following single oral dosing the drug is well absorbed and extensively metabolised. Faecal excretion in 72 h accounts for 46% of the administered radioactivity of which approximately 4% is associated with indoramin. Less than 2% of the radioactivity in urine is accountable as the parent drug. Concentrations of free drug in the plasma are exceeded ten fold by the concentration of total metabolites. Peak plasma levels both of drug and metabolites occur 1-2 h after dosing, the maximal lowering of blood pressure occurring at this time.

3 The clearance of indoramin, determined after intravenous administration is of the same order as liver blood flow. In the isolated perfused rat liver, the extraction ratio is 0.98.

Introduction

Indoramin (Wy 21901; 3-(2-(4-benzamidopiperid-1-yl) ethyl) indole hydrochloride) (Figure 1) is one of a series of benzamidopiperidylethylindoles (Archibald, 1968) with α -adrenoceptor antagonist and local anaesthetic properties. Indoramin lowers arterial pressure in anaesthetized animals by decreasing cardiac output with a concomitant reduction in peripheral resistance (Alps, Johnson & Wilson, 1970). This combination of pharmacological actions has been advocated on theoretical grounds for the treatment of high blood pressure (Zachest, Gilmore & Koch-Weser, 1972). In man indoramin has α -receptor blocking properties (Royds, Coltart & Lockhart, 1972) and lowers arterial pressure in hypertensive patients (Lewis, George & Dollery, 1973). In this paper we report the pharmacokinetics of the drug in man following single and continuous dosing. Since the drug is rapidly metabolized after oral administration, the possibility of extensive 'first-pass' hepatic metabolism was considered and extraction of indoramin by the isolated perfused rat liver was studied.

Figure 1 Structural formula of indoramin, WY 21901, with position of ¹⁴C label indicated (*).

Methods

Single doses

Indoramin was taken in single oral doses by seven subjects. Four were patients with hypertension, three were normal male volunteers. In all cases renal function was normal. Informed consent was obtained. Each subject took a single oral dose of either 40 or 60 mg in tablet form with water after an observation period of 90 minutes. Subjects remained supine throughout and blood was drawn at appropriate intervals via a venous cannula. Plasma from each sample was stored at -20°C for subsequent assay of indoramin and metabolites. Arterial pressure was measured every 30 min using the Hawksley juggled zero sphygmomanometer, the final set of observations being made 6 h after the dose. In experiments where ¹⁴C-labelled indoramin was used, stool and urine collections were carried out over 72 h after dosing. The dose

¹ Inveresk Research International, Inveresk Gate, Musselburgh, EH21 7UB, Scotland

² Clinical Pharmacology, Institute of Obstetrics and Gynaecology, Queen Charlotte's Hospital, London W6 0XG

of [^{14}C]-indoramin administered was 50 μCi in all cases. In three further patients a single intravenous injection of indoramin (15 mg) was given over a 2 min period in 10 ml 5% dextrose. The protocol was otherwise similar to the oral studies.

Continuous oral dosing

Eight patients were given indoramin in a double blind cross-over comparison of indoramin and placebo in hypertension (Lewis *et al.*, 1973). Subsequent development of an assay method for indoramin, enabled plasma concentrations to be determined after 2, 4 and 6 weeks on the drug, providing data unavailable in the original report. The doses of indoramin ranged from 60 to 150 mg daily in three daily divided doses. Subjects were selected from amongst newly referred patients with untreated hypertension. Patients with evidence of cardiac or hepatic disease were excluded and no patient had a plasma urea greater than 60 mg/100 ml.

Assay of indoramin and total metabolites in plasma, urine and faeces

Initial studies were undertaken using ^{14}C -labelled indoramin (Figure 1). The concentration of parent drug in plasma (3 ml) was determined by addition of unlabelled indoramin (50 μg) as carrier. The plasma was made basic with NaOH (1 ml, 1M) and extracted with heptane (20 ml) containing 2% isoamyl alcohol (v/v). Indoramin was recovered by back extraction into HCl (3 ml, 0.01M) and aliquot (1.5 ml) of the acid layer counted in Instagel scintillant (Packard). The recovery of indoramin was $39 \pm 7\%$ (s.d.). In checking the specificity of this procedure, the residual HCl extracts were adjusted to pH 12, the radioactivity quantitatively recovered into ether, concentrated and examined by thin layer chromatography. Greater than 90% of the radioactivity co-chromatographed with the indoramin band. Total radioactivity in plasma samples was determined by counting aliquots directly in scintillator.

Total radioactivity and [^{14}C]-indoramin in urine were determined by procedures similar to those used in plasma assay. Total radioactivity in homogenized faeces was determined in aliquots (circa 100 mg) by decolourization in Soluene (Packard) (1.5 ml) and 30% H_2O_2 (1 ml) at 60°C followed by scintillation counting. [^{14}C]Indoramin was measured in aliquots of faeces (circa 1 g) by addition of unlabelled carrier, suspension in NaOH (1 ml, 1M) and extraction in the manner described for plasma and urine.

Concentrations of non-radioactive indoramin in plasma were determined by stable isotope dilution

and selected ion monitoring gas chromatography-mass spectrometry (GC-MS) (Draffan, Clare, Williams, Emons & Jackson, 1974). Deuterium-labelled indoramin (indoramin d_5) was used as the carrier and internal standard, being added to plasma (30 μg in 3 ml). Extracts of plasma containing indoramin- d_5 and unlabelled indoramin (d_0) were assayed by GC-MS using a 1.0 m glass column of 3% OV-1 at 260°C. The mass spectrometer (AEI MS12: ionizing voltage 12 eV) was set to monitor, in electron impact mode, m/e 217, the base peak of indoramin- d_0 and m/e 223 as the reference signal from indoramin- d_5 . Response ratios were determined as peak heights from a potentiometric recorder. Calibration was linear for indoramin- d_0 added to plasma in the range 5 to 150 ng/ml. Precision was $\pm 14\%$ (s.d., $n = 6$) at 5 ng/ml and $\pm 5\%$ at 25 ng/ml.

Plasma protein binding

Plasma was obtained from healthy male volunteers who had not taken drugs for 7 days prior to the experiment. Aliquots (1 ml) containing either 54, 109, or 205 ng of [^{14}C]-indoramin were dialyzed against 1 ml of Krebs Ringer bicarbonate buffer, (1 ml, pH 7.4), for 7 h at 37°C. Dialysis was carried out in the Dianorm equilibrium dialysis system (Diachema A.G., Zurich) and the membrane used was Spectrapor, molecular weight cut off $12\text{--}14 \times 10^3$ (Spectrum Medical, Los Angeles). The radioactivity present in the plasma before dialysis and in the buffer after dialysis was measured by liquid scintillation counting. After dialysis the buffer was tested for the presence of protein. When this was detected, denoting membrane leakage, the results were discarded. Percentage binding of indoramin, y , was calculated thus:

$$y = 100 - \left(\frac{\text{d min}^{-1} \text{ ml}^{-1} \text{ buffer}}{\text{d min}^{-1} \text{ ml}^{-1} \text{ plasma}} \times 100 \right)$$

Drug extraction by the perfused rat liver

Four experiments were performed, two at a perfusion concentration indoramin (1 $\mu\text{g}/\text{ml}$) and two at 50 $\mu\text{g}/\text{ml}$. Rats were anaesthetized with ether and the liver perfused via the portal vein with a medium consisting of washed human erythrocytes in artificial plasma (Hems, Ross, Berry & Krebs, 1966). The liver was removed and mounted in a perfusion apparatus at 37°C (Cohen, Bakke & Davies, 1974). After 30 min perfusion with drug free medium, a medium equilibrated with [^{14}C]-indoramin was substituted, passing through the liver at 10 ml/minute. The effluent

was collected in 4 x 1 min samples and the concentration of parent drug measured as described above. The extraction ratio (ER) was expressed as the mean of four ER calculated thus:

$$ER = \frac{C_{in} - C_{out}}{C_{in}}$$

where C_{in} and C_{out} represent the concentration of unchanged drug in the inflow and outflow respectively.

Comparison of plasma and blood concentrations of [14 C]-indoramin

[14 C]-indoramin was added to aliquots (4 ml) of heparinized, freshly-drawn human blood to provide concentrations of 100 ng and 500 ng/ml. Blood samples were equilibrated for 10 min or 30 min at 37°C. Plasma was separated and concentrations of indoramin in plasma compared with the original blood concentration.

Results

Recovery of indoramin and total radioactivity

In four experiments after an oral dose of 50 μ Ci [14 C]-indoramin, complete collections of urine for 48 h and faeces for 72 h were obtained. The mean ($\pm$ s.e. mean) recovery of radioactivity was $81.9 \pm 7.9\%$, $35.4 \pm 3.6\%$ of the dose being recovered in the urine and $46.5 \pm 5.6\%$ in the faeces. In one experiment where a satisfactory

collection was made after an intravenous dose, the recovery was 60%, 27% in the urine and 33% in the faeces.

The excretion of unchanged drug was determined in two subjects after oral administration and accounted for a mean of 3.8% of the dose in faeces and 0.6% in urine. The fraction of the total radioactivity in urine eliminated as unchanged drug was constant in individuals in 0-6 h, 6-24 h and 24-48 h collection periods being 2% throughout in one subject and 1.5% in the other. In one subject the free drug fraction in urine after intravenous administration declined from 25% in the 6 h post dose to 5% in 6-24 h and 2.5% in the 24-48 h periods.

Single oral dosing

Seven studies were carried out, six with 40 mg indoramin and one with 60 mg. The peak plasma levels and the ratios of parent drug to residual radioactivity (total metabolites) are shown in Table 1. The highest plasma concentrations of indoramin were observed between 1 and 2 h after dosing except in subject D.M., in whom the peak was delayed until 4 hours. This subject had eaten a heavy meal prior to the study. Figure 2 shows the plasma concentration-time curves in one subject, R.P., after 60 mg orally. In every experiment using 14 C tracer, the plasma concentration of total metabolites exceeded that associated with the parent drug, the profiles being essentially parallel throughout (Figure 2).

Figure 3 shows the plasma concentrations of indoramin and estimated elimination half-life in three subjects. Indoramin half-lives in the seven

Table 1 Plasma concentrations of indoramin and ratio of indoramin to residual radioactivity in plasma (total metabolites) following a single oral dose.

Subject	Sex	Dose (mg)	Time of peak indoramin concentration (h)	Peak concentration (ng/ml)	Ratio of [14 C]-indoramin to residual radioactivity in plasma:	
					2 h post dose	6 h post dose
R.P.	M	60*	2	40	0.14	0.10
J.R.**	M	40*	2	14	0.03	0.10
R.S.	M	40*	1	21	0.06	0.05
D.M.	M	40*	4	13	0.10	0.07
P.L.**	M	40	2	15	—	—
G.D.**	M	40	1	45	—	—
M.M.	F	40	2	40	—	—
Mean					0.095	0.08

* 14 C-labelled dose

**Normotensive volunteer

Figure 2 Plasma concentrations of indoramin (○) and total metabolites (●) in subject R.P. after ingestion of a 60 mg oral dose. Total metabolite concentration is expressed as equivalents of parent drug based on the specific activity of the dose.

Figure 3 Plasma concentrations and elimination half-life of indoramin ($h \pm 95\%$ confidence interval) in three subjects after a 40 mg oral dose (●, M.M., $T_{1/2} = 4.8 \pm 0.9$ h; ○, G.D., $T_{1/2} = 1.7 \pm 0.4$ h; ■, P.L., $T_{1/2} = 2.1 \pm 0.6$ h).

subjects ranged between 1.7 and 8.6 h (mean 5.5 ± 3.3 h, s.d.).

Falls in blood pressure from the pre-dose level were observed only in subjects R.P. (60 mg), M.M. and G.D. In these subjects plasma concentrations of indoramin 40–50 ng/ml were achieved. Figure 4 shows the inverse relationship noted between mean supine arterial pressure and the level of parent drug in plasma in one of the subjects.

Intravenous dosing

Two studies were undertaken with [^{14}C]-indoramin (15 mg). Figure 5 shows the plasma concentration-time curves for one subject following rapid intravenous administration. Parent drug concentration data could be fitted to a two compartment open model using a computer programme providing a late phase $T_{1/2}$ of 2.6 hours. In the second subject the late phase $T_{1/2}$ was 4.6 hours. Both an intravenous and oral dose were administered on different occasions to a third patient using 15 mg and 40 mg of unlabelled drug respectively. Elimination half-lives were 3.2 h (i.v.) and 4.8 h (p.o.). A comparison of the areas under the concentration-time curves using the trapezoid rule, showed a relatively greater availability of the drug in the systemic circulation when it was

administered by intravenous injection. After correction for dosage, the availability, calculated as the ratio of the area under the oral curve to the area under the intravenous curve was 0.31.

The apparent volumes of distribution of indoramin, ($V_{D\beta}$), were 7.7, 7.7 and 6.8 litres/kg (mean 7.4 litres/kg) for the three subjects and the clearances 1300, 1900 and 1100 ml/min respectively (mean, 1433 ml/min).

Chronic oral dosing

The relationship between the plasma concentration of indoramin and fall in systolic and diastolic blood pressure was investigated using the data from a double blind cross-over comparison of indoramin and placebo in patients with essential hypertension (Lewis *et al.*, 1973). Twenty-one plasma samples were available from this study of eight patients whose blood pressure was measured on three 2-weekly visits while taking indoramin.

In each case blood was drawn at 17.00–18.00 h, i.e. 4–6 h after the last dose of indoramin. The mean plasma concentrations ($\pm$ s.e. mean) were for 150 mg daily, 126 ± 18 ng/ml (five patients, range 78–218 ng/ml), for 120 mg daily, 51 ± 4 ng/ml (one patient) and for 60 mg daily, 23 ± 5 ng/ml (two patients). The fall in blood pressure on

Figure 4 Plasma concentrations of indoramin (○) and mean arterial blood pressure (●) in subject M.M. after ingestion of a 40 mg oral dose.

indoramin was taken as the difference between the blood pressure on each occasion as a percentage. The mean fall in supine diastolic pressure on the twenty-one occasions when plasma concentrations were measured was $6.2\% \pm 9.9\%$ (mean and s.d.) and the correlation coefficient between blood pressure fall and log plasma concentration was 0.22 (NS). The mean fall in supine systolic pressure was $6.3\% \pm 13.9\%$ (mean and s.d.) and the correlation coefficient with log plasma concentration, 0.46 (NS).

Table 2 shows the plasma concentrations of indoramin on three consecutive visits in four patients who commenced indoramin after taking placebo for 6 weeks.

Plasma protein binding

The mean plasma protein binding of indoramin for the four individuals' plasma was $91.7 \pm 0.6\%$

Figure 5 Plasma concentrations of indoramin (○) and total metabolites (●) in subject F.Y. after intravenous injection of indoramin (15 mg). Total metabolite concentration is expressed as equivalents of parent drug based on the specific activity of the dose.

($\pm$ s.e. mean). There was no change in the amount bound over the concentration range 54–205 ng/ml which was chosen to span the values seen in chronic oral dosing.

Drug extraction by the perfused rat liver

The mean extraction ratios from the two experiments at $1 \mu\text{g/ml}$ were 0.98 and 0.97 and for the two at $50 \mu\text{g/ml}$ 0.99 and 0.98. The mean ratios of parent drug to total metabolite concentrations in the outflow were 0.22 at $1 \mu\text{g/ml}$ and 0.18 at $50 \mu\text{g/ml}$.

Table 2 Serial plasma concentrations of indoramin in four patients who commenced indoramin after being on placebo.

Patient	Daily dose (mg)	Plasma concentrations (ng/ml) on indoramin for:		
		2 weeks	4 weeks	6 weeks
D.M.	150	186	215	135
P.R.	60	42	30	30
M.R.	60	15	20	11
R.S.	120	52	65	55
Mean $\pm$ s.e. mean		77 $\pm$ 39	83 $\pm$ 45	58 $\pm$ 27

Comparison of plasma and blood concentrations

Following additions of [^{14}C]-indoramin to fresh heparinized blood, the resulting plasma concentrations did not differ from the original blood concentrations, being within the standard deviation of the assay technique. Distribution was unaffected by blood concentration (100 ng/ml or 500 ng/ml) and equilibration time at 37°C (10 min or 30 min).

Discussion

Indoramin is a basic (pK_a 7.7), lipid soluble drug which is well absorbed after oral dosing and almost completely metabolized. Less than 4% of an oral dose of [^{14}C]-indoramin is recovered unchanged in the faeces and only 2% of the radioactivity present in urine is accountable as the parent drug. After a single oral dose, the concentration of total metabolites in plasma is high even initially (Figure 2) and exceeds indoramin concentration approximately ten-fold (Table 1). The systemic-bioavailability of the drug is greater after intravenous than after oral dosing. These data suggest that the drug undergoes significant metabolism at the first pass either in the gut wall or in the liver.

The kinetics of indoramin studied after intravenous administration indicate a high apparent volume of distribution, mean 7.4 litres/kg, and a mean clearance of 1.43 litres/min, which is of the same order as liver blood flow (Bradley, 1963). A high first pass hepatic metabolism has been described for propranolol (Shand, Nuckolls & Oates, 1970) and other compounds and while our data do not exclude an extrahepatic component to the metabolism, the kinetics of indoramin are similar. The very high extraction of indoramin in the isolated perfused rat liver is consistent with this hypothesis. Extraction is mainly attributable to extensive metabolism, the ratio of drug to metabolites in the outflow being 0.2. Extrapolation from these observations in the animal to those in man depends upon a correlation of human and rat metabolite pattern. Preliminary data indicate that the principal primary oxidation product of indoramin is identical in rat and man (Draffan, Firmin & Jordan, unpublished observations).

Indoramin is 92% bound to human plasma proteins and binding is constant in the range of concentrations encountered clinically.

There is a range in plasma concentrations of 13-45 ng/ml after an oral dose of 40 mg, a less marked variation than that encountered with propranolol (Shand *et al.*, 1970). A 2.5 fold increase in dose, from 60 to 150 mg/day, resulted in an increase of 5.5 times in steady state plasma level. However, it is of interest that extraction by the isolated rat liver was not diminished by raising the inflow concentration from 1 to 50 $\mu\text{g}/\text{ml}$. It was previously suggested (Lewis *et al.*, 1973) that tolerance to the effect of the drug developed on chronic oral therapy and that this might have a metabolic explanation, the drug inducing its own metabolism. However, when several plasma samples from patients on chronic treatment were analyzed, there was no trend to a fall in plasma concentration (Table 2) and this suggestion may be discounted.

In a double-blind cross over comparison with placebo, indoramin produced modest falls in blood pressure, the dose limiting side effect being sedation (Lewis *et al.*, 1973). However, since indoramin was not detected in the plasma of several of the trial patients on one occasion, non-compliance possibly influenced these results. The correlation between pressure reduction and log plasma indoramin concentration is not significant but where there is an effect on arterial pressure of single oral doses this is seen at the time of peak drug concentration.

The most significant consequence of the first-pass effect for indoramin is the high concentration of metabolites in plasma on oral dosing; because of the near parallel relationship between the drug and metabolite concentration (Figure 2 and Table 1) no distinction can be made between the pharmacological activity of the drug and its metabolites. The fall in arterial pressure in the three patients given the drug by the intravenous route was not large enough to provide such a distinction. Present studies are, therefore, directed towards the characterization and synthesis of the major metabolite of indoramin.

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