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Pharmacokinetic Profile of Idazoxan in the Beagle Dog

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Abstract—The α_2-antagonist idazoxan (2- (2- (1,4-benzodioxanyl))-2-imidazoline) has been given intravenously and orally to five beagle dogs at 1, 3 and 10 mg kg^{-1} doses. Idazoxan plasma levels were determined by a HPLC method. After intravenous administration, a linear kinetic behaviour was obtained. Half-life and mean residence time values ranged 105·2–117·1 and 138·1–154·0 min, respectively. Total plasma clearance values and volume of distribution at steady state values ranged from 25·6–32·1 (mL kg^{-1}) min^{-1} and 3·60–4·36 L kg^{-1}, respectively. After oral administration, time to peak values averaged around 1 h. Dose normalized peak concentration values ranged 161–182 ng mL^{-1}. Bioavailability values ranged 60–88%. Low idazoxan bioavailability has been described in other animal species and attributed to a first-pass effect.

Idazoxan (2-(2-(1,4-benzodioxanyl)-2-imidazoline has been characterized as a selective and potent α_2-antagonist (Hannah et al 1983). In-vitro and in-vivo studies have demonstrated the activity of the drug to be by interfering in the noradrenaline feedback synaptic regulation at nerve terminals. These findings confer on idazoxan a possible therapeutic role, since drug administration to endogenous depressed patients could improve them by increasing noradrenaline levels at synaptic sites (Cohen et al 1980; Smith & Garcia Sevilla 1982; Crossley 1984).

Metabolism and drug disposition studies of idazoxan are scarce. At present, to our knowledge, there are only three publications taking into account kinetic and bioavailability information of the drug. Muir et al (1986) reported the kinetic profile of idazoxan after a single intravenous and oral administration of the drug to healthy volunteers. The mean plasma clearance value averaged $824·0 \pm 115·1$ mL min^{-1}. Idazoxan volume of distribution at steady state was $3·20 \pm 0·17$ L kg^{-1}, its half-life value averaged $4·20 \pm 1·04$ h. Bioavailability of idazoxan after an oral dose of 0·3 mg kg^{-1} was low and attributed to a first-pass metabolism; rather than to a poor absorption, since radiolabelled studies in man demonstrated extensive biotransformation and urinary recoveries >95% after oral dosing.

Lewis et al (1988) have published a metabolic study of [^{14}C]idazoxan in the rat. The drug was orally administered at doses ranging 10 to 100 mg kg^{-1}, which were very high compared with the dose used by Muir et al (1986) in humans (0·3 mg kg^{-1}). Though Lewis et al reported a complete absorption of the drug, poor bioavailability values were obtained (1 to 23%). Those authors observed high radioactivity levels at the hepatic site, so a low bioavailability could be explained by an hepatic first-pass effect. Metabolic pathways of idazoxan in the rat revealed hydroxylations at positions 6 and 7 to form phenolic metabolites, which were excreted as glucuronide and sulphate compounds in urine, but unconjugated in faeces (Lewis et al 1988).

In May 1988, we reported to the annual meeting of the Sociedad Española de Farmacologia a communication (Vallès et al 1988) in which the kinetic profile of idazoxan in rat and its bioavailability after oral, hepatoportal and intravenous administrations of the drug were considered. The range of administered doses was 1–10 mg kg^{-1}. Idazoxan plasma levels were determined by a HPLC method. The most striking findings obtained were a linear kinetic profile after the intravenous administration and a low bioavailability after the oral administration, contrary to the bioavailability observed after the hepatoportal route. In agreement with Muir et al (1986), we argued a possible gut wall metabolism in order to explain the bioavailability findings.

We report here the pharmacokinetic profile of idazoxan after intravenous and oral administrations to the beagle dog.

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Materials and Methods

Five male beagle dogs (9–17 kg) were used. Idazoxan (obtained from S.A.LASA Laboratorios) was administered intravenously and orally at 1, 3 and 10 mg kg^{-1} doses using capsules, manufactured in our laboratories for the purpose of this study. The time between both intravenous and oral doses was a week. Blood (4 mL) was withdrawn from a cephalic vein immediately before the administration of idazoxan (blank sample) and at different times up to 10 h after the drug administration.

The plasma was immediately separated and stored at −20°C until analysis.

Chemicals and reagents

Acetonitrile (HPLC grade) was purchased from Farmitalia Carlo Erba (Milan, Italy). Benzene, sulphuric acid, sodium carbonate, sodium bicarbonate (analytical reagents grade) and ethyl acetate ("Lichrosolv", used without further purification) were purchased from Merck (Darmstadt, FRG). Solvents for HPLC were filtered through a Millipore 0·5 μm filter and thoroughly degassed in an ultrasonic bath before use. The water used was double-distilled and purified through a Milli-Q system (18 MΩ.cm resistivity).

Apparatus and chromatographic conditions

Chromatographic conditions were carried out on a Waters HPLC System (Millipore Waters, USA) equipped with two M-510 solvent delivery systems, a M-840 chromatographic

data station, a WISP 710 B automatic injector and a M-490 ultraviolet detector with a 10 μL-through cell. Peak heights were calculated with the M-840 chromatographic data processor.

The column was a μBondapak C Radial-Pak Cartridge (10 × 0·8 cm i.d., particle diameter 10 μm) obtained from Millipore Waters and used in conjunction with a Z module radical compression separation system. A Guard-Pak precolumn insert packed with μBondapak C_{18} was used to protect the column. The mobile phase consisted of acetonitrile–0·01% (v/v) H_2SO_4 aqueous solution, 20:80 (v/v) and the flow rate was 2 mL min^{-1}. The detection wavelength was set at 200 nm (0·04 AUFS sensitivity). Under these conditions the retention time of idazoxan was 3·6 min.

Standard and sample preparations

A stock solution of idazoxan hydrochloride (1·0 mg mL^{-1} of free idazoxan) was prepared in water. Aqueous standards in the concentration range 0·25–10 μg mL^{-1} were prepared by dilution of the stock solution with water. Aliquots (1·0 mL) of blank plasma from each beagle dog were transferred to 10 mL silanized glass centrifuge tubes fitted with PTFE-lined screw caps and 0·1 mL of one of the idazoxan aqueous standards added to obtain a calibration curve corresponding to 25, 50, 100, 250, 500, 1000 and 2000 ng idazoxan mL^{-1} plasma; the plasma blank was prepared by adding 0·1 mL water to 1·0 mL plasma. Then, 1·0 mL of a 0·4 M bicarbonate-carbonate buffered aqueous solution (pH = 10·3) and 4·0 mL of benzene–ethylacetate (1:1) were added. The tubes were shaken for 30 s in a vortex mixer and centrifuged at 3000 *g* for 5 min. Aliquots (3·0 mL) of the organic phases were transferred to 4 mL silanized conical glass reacti-vials. A re-extraction was made by adding 4·0 mL of ethyl acetate and then 4·0 mL of the organic phases were taken out and added to the previous separated organic phases. The organic extracts were evaporated to dryness with a stream of dry nitrogen at 40°C, the residue was redissolved in 200 μL of the mobile phase and 50 μL were injected into the column.

Samples from treated dogs were processed in parallel except for the standard addition. Samples in the concentration range 0·025–2 μg mL^{-1} were assayed by direct interpolation from the calibration curve; samples over 2 μg mL^{-1} were diluted conveniently using the mobile phase to reach a concentration level that could be interpolated from the curve.

The precision of the method, expressed as relative standard deviation calculated from nine calibration curves obtained on different days, ranged from 17% at the lowest concentration (25 ng mL^{-1}) to 11% at the highest concentration (2000 ng mL^{-1}). Variability of back-calculated values, i.e. concentration values estimated from the corresponding standard curve equations for each theoretical concentration level, ranged from 14·7% at 50 ng mL^{-1} to 1·3% at 2000 ng mL^{-1}. At 25 ng mL^{-1} this variability was 44%. The percentage difference between the calculated and the theoretical values (relative error) ranged from 10·6 to 0·025%. The limit of detection of idazoxan in plasma was ca. 25 ng mL^{-1}, expressed as the concentration of compound that produces a peak height equal to the mean blank value plus three standard deviations.

Pharmacokinetic treatment

After intravenous administration of idazoxan, a non-compartmental approach was used (Shumaker 1986) to estimate the following pharmacokinetic parameters: β-elimination half-life ($t_{\frac{1}{2}}$), total plasma clearance (CL), area under the plasma level curve versus time (AUC_0^∞) and mean residence time (MRT). The β-elimination constant was estimated by linear regression analysis of the log-transformed plasma level of the terminal disposition phase. The area under the curve (AUC_0^∞) was estimated by the trapezoidal-log trapezoidal rule.

After oral administration of idazoxan, experimental time to peak (t_{max}), peak concentration (C_{max}) and lag time (t_o) were estimated as the most characteristic absorption parameters of the drug.

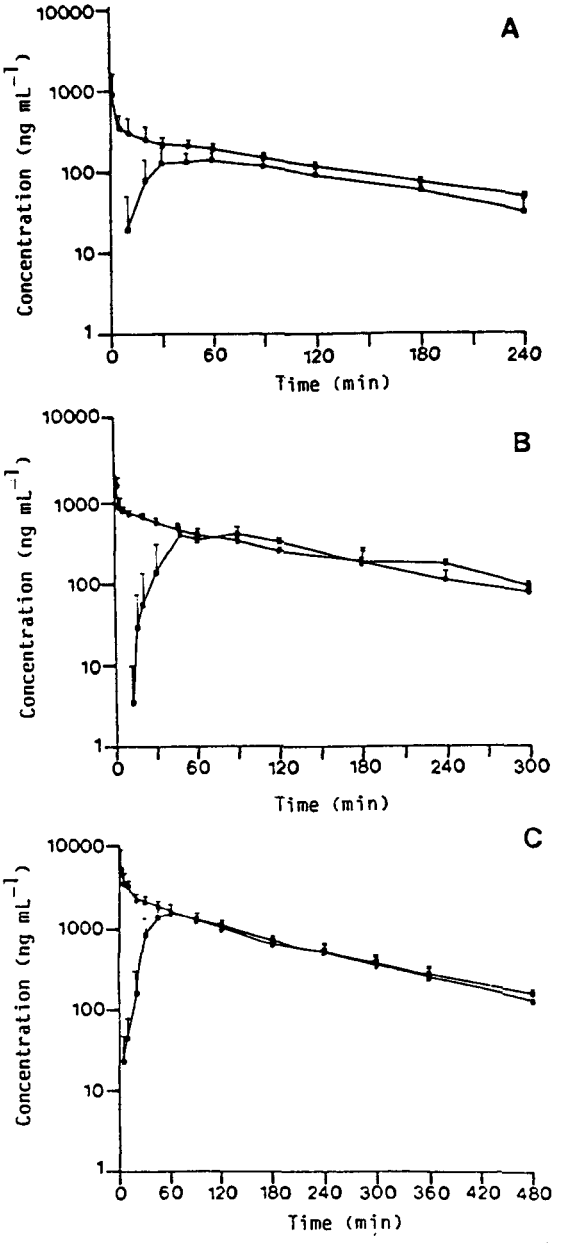

FIG. 1. Mean ± s.d. idazoxan plasma levels after intravenous (●) and oral (■) administration of 1 (A), 3 (B) and 10 (C) mg kg^{-1} to the beagle dog.

Table 1. Mean pharmacokinetic parameters of idazoxan after i.v. administrations of 1, 3 and 10 mg kg^{-1} to the beagle dog.

	Idazoxan (mg kg^{-1})			
Parameters	(n = 5)	3 (n = 5)	10 (n = 5)	Units
Half-life ($t_{\frac{1}{2}}\beta$)	105·5 ± 17·9	105·2 ± 23·0	117·1 ± 10·5	min
Mean residence time (MRT)	142·2 ± 26·8	138·1 ± 24·6	154·0 ± 19·0	min
Area under curve (AUC^{∞}_{0})	40147 ± 6655	95406 ± 14734	401086 ± 107900	(ng min^{-1} mL^{-1})
Total plasma clearance (Clp)	25·6 ± 5·1	32·1 ± 5·1	26·7 ± 8·0	(mL kg^{-1} min^{-1})
Volume of distribution at steady state (Vd_{ss})	3·60 ± 0·72	4·36 ± 0·54	4·01 ± 0·87	L kg^{-1}

Values are expressed as mean ± s.d.

Table 2. Mean pharmacokinetic parameters of idazoxan after oral administration of 1, 3 and 10 mg kg^{-1} to the beagle dog.

	Idazoxan (mg kg^{-1})			
Parameters	1 (n = 5)	3 (n = 5)	10 (n = 5)	Units
Area under curve (AUC^{∞}_{0})	24253 ± 5481	84132 ± 15866	303572 ± 112873	ng min^{-1} mL^{-1}
Half-life ($t_{\frac{1}{2}}\beta$)	86·6 ± 31·5	105·2 ± 17·9	103·8 ± 33·1	min
Bioavailability (F%)	60·2 ± 8·9	88·4 ± 12·7	76·8 ± 25·7	
Peak concentration (C_{max})	182·0 ± 18·1	482·8 ± 142·0	1727 ± 336	ng mL^{-1}
Time to peak (T_{max})	42·8 ± 12·7	84·8 ± 36·7	66·0 ± 31·1	min
Lag time (t_o)	13·4 ± 7·8	21·5 ± 6·9	11·1 ± 5·8	min

Values are expressed as mean ± s.d.

Bioavailability of idazoxan after oral administration was determined from equation 1:

$$F = (AUC^{\infty}_{0\ p.o.})/(AUC^{\infty}_{0\ i.v.}) \quad (1)$$

Statistical methods

Multiple comparisons of mean pharmacokinetic parameter values were performed using the Peritz' F test (Harper 1984). The level of significance adopted was $P = 0{\cdot}05$.

Results

The mean plasma levels after intravenous and oral administrations of idazoxan are plotted in Fig. 1.

The limit of the detection of the assay allowed us to follow accurately the time course of plasma levels during 240, 300 and 480 min after the administration of 1, 3 and 10 mg kg^{-1}, respectively.

The relevant intravenous pharmacokinetic parameters are summarized in Table 1. Range values of both half-life ($t_{\frac{1}{2}}$) and mean residence time (MRT) were 105·2–1117·1 and 138·1–154·0 min, respectively. No differences between doses were obtained (($P > 0{\cdot}05$), Peritz' F test). Total plasma clearance (CL) and volume of distribution at steady state (Vd_{ss}) have shown respective range values of 25·6–32·1 (mL kg^{-1})min^{-1} and 3·60–4·36 L kg^{-1}. In each case, when the parameter values were checked by Peritz' F test, no differences between doses were denoted ($P > 0{\cdot}05$). Area under curve values after the intravenous administration showed a linear relationship with doses ($r = 0{\cdot}9975$, $P < 0{\cdot}05$) with an intercept term not significantly different from zero. The corresponding range of intravenous AUC^{∞}_{0} were 40147·0–401086·0 ng min^{-1} mL^{-1}.

The pharmacokinetic parameters obtained after the idazoxan oral administration are shown in Table 2. Area under curve levels (AUC^{∞}_{0}) after the oral administration showed a linear relationship with doses ($r = 0{\cdot}9999$, $P < 0{\cdot}01$). The absorption of idazoxan was almost complete at 3 and 10 mg kg^{-1}. Bioavailabilities (F%) obtained were 60·2, 88·4 and 76·8%.

Mean peak concentration (C_{max}) values (± s.d.) were 182·0 ± 18·1, 482·8 ± 142·0 and 1727·6 ± 336·3 ng mL^{-1}, with their corresponding time to peak (T_{max}) 42·8 ± 12·7, 84·8 ± 36·7 and 66·0 ± 31·1 min. A linear correlation was observed between peak concentration values and doses ($r = 0{\cdot}9996$, $P < 0{\cdot}05$). When mean time to peak values were compared between groups, no significant difference was observed ($P > 0{\cdot}05$, Peritz' F test). Lag time values averaged 13·4 ± 7·8, 21·5 ± 6·9 and 11·1 ± 5·8 min. No differences between groups were observed ($P > 0{\cdot}05$, Peritz' F test).

Discussion

After idazoxan intravenous administration to the beagle dog, a linear kinetic profile was seen. Total plasma clearance, mean residence time and half-life values ranged similarly at each level of dose. $AUC^{\infty}_{0\ i.v.}$ values and doses correlated significantly ($P < 0{\cdot}05$).

Also when idazoxan was administered intravenously to the Sprague-Dawley rats, results showed a linear kinetic behaviour of the drug (Vallès et al 1988).

In the range of administered doses, total plasma clearance values estimated in dogs (around 0·03 L kg^{-1} min^{-1}) were three-fold higher than the corresponding value estimated in man (0·01 L kg^{-1} min^{-1}) and lower than that obtained in rat (0·06 L kg^{-1} min^{-1}).

Also the elimination half-life values in dogs (1·5–2 h) were between those obtained in the other two species (0·5 h in rat and 4 h in man). The respective volumes of distribution at

steady state values ranged 3·2 L kg^{-1} in man, 1·95–3·18 L kg^{-1} in rat and 3·6–4·4 L kg^{-1} in dog.

After idazoxan oral administration in the dog, the absorption rate obtained was similar to that reported in man by Muir et al (1986). (Time to peak observed in man ranged between 1 and 2 h. Time to peak in the dog was ca 1 h). In rat the absorption rate was high (time to peak between 5–10 min).

In those species in which idazoxan has been studied, rat, dog and man, the drug has shown a low bioavailability when low oral doses were administered (between 0·3 and 1 mg kg^{-1}). Complete idazoxan absorption was demonstrated with [^{14}C]idazoxan by Lewis et al (1988), a first-pass effect has been postulated in both man and rat to explain the low bioavailability values.

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