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A Pharmacokinetic-pharmacodynamic Linking Model for the α_2-Adrenergic Antagonism of Idazoxan on Clonidine-induced Mydriasis in the Rat

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Abstract

The relationship between concentration and inhibitory effect of the α_2-adrenoceptor antagonist idazoxan on clonidine-induced mydriasis has been studied in the rat using pharmacokinetic-pharmacodynamic simultaneous modelling.

Fifteen minutes after the anaesthesia of rats with sodium pentobarbitone (55 mg kg^{-1}, i.p.), and 5 min after the administration of clonidine (0·3 mg kg^{-1}, i.v.) to rats pretreated with idazoxan (3 mg kg^{-1}, i.v., and 3 and 10 mg kg^{-1}, orally) at different time intervals, pupil diameters were assessed. The pharmacokinetics of idazoxan were adequately described by a monoexponential equation. Using a pharmacokinetic-pharmacodynamic linking model, the concentration-effect relationships of idazoxan were derived, and were quantified by the inhibitory simple E_{max} model. At the effect compartment, the estimated apparent IC50 was 153·6 ng mL^{-1}. Values of clearance, volume of distribution and elimination half-life were 71·2 mL kg^{-1} min^{-1}, 3134 mL kg^{-1} and 30·5 min, respectively.

These results could contribute to better characterization of the pharmacodynamic and toxicological profiles of idazoxan in experimental models in which a different pharmacokinetic behaviour of the drug is presumed.

New therapeutic strategies in the field of endogenous depression have focused on inhibitory α_2-adrenoceptors that regulate the release of noradrenaline in the central nervous system (CNS) (Cohen et al 1980; Chapleo et al 1981; Smith & García-Sevilla 1982). Pupillary dilation as an index of CNS postsynaptic α_2-adrenoceptor activation in rat may provide a simple and effective model for quantifying CNS α_2-adrenoceptor activity (Koss 1986).

Idazoxan (2-(2-(1, 4-benzodioxanyl))-2-imidazoline) has been characterized as a potent α_2-antagonist in studies both in-vitro and in-vivo (Langer 1974; Chapleo et al 1981; Clifford et al 1982; Elliot et al 1984; Brown et al 1985), supporting the basis for a novel antidepressant strategy (Crossley 1984; Osman et al 1989). Idazoxan has also been described as a potent central α_2-antagonist when studied on the centrally-mediated responses in rat (Berridge et al 1983; Menargues et al 1990). The kinetic profile of the drug in rat and its bioavailability after oral, hepatoportal and intravenous administration were considered in a previous study (Vallès et al 1989). The range of doses administered was 1–10 mg kg^{-1}. Since the most striking findings were a linear kinetic profile after intravenous administration and a low bioavailability after oral administration, contrary to the bioavailability observed after the hepatoportal route, a first-pass effect in gut was argued.

Though the combined application of pharmacokinetics and pharmacodynamics allows prediction of the dose-concentration and the concentration-effect relationship (Holford & Sheiner 1981), the first prerequisite to obtain a quantitative measure of the effect of a drug in-vivo is not always available. This is particularly true for drugs acting on the CNS. Simultaneous modelling of pharmacokinetics and pharmacodynamics using a hypothetical effect compartment linked to the plasma compartment by a first-order process but receiving only a negligible amount of drug, allows the direct prediction of the concentration-effect relationship of a drug (Sheiner et al 1979). This modelling procedure is particularly useful in cases of delay in equilibrium with the receptor site, the presence of active metabolites or homeostatic responses (Holford & Sheiner 1981).

The purpose of our investigation was to quantify the concentration-inhibitory effect of intravenous idazoxan on clonidine-induced mydriasis in the anaesthetized rat.

Materials and Methods

Animals

Male Sprague-Dawley albino rats, 250–350 g, were housed in a temperature- and humidity-controlled room with a 12 h light-dark cycle. The animals were fasted 18 h before the experiment but had free access to water.

Drugs

The following drugs were used: clonidine (Sigma Chemical Co., St Louis, MO), idazoxan (synthesized at S.A. Lasa Laboratorios by Dr F. Geijo) and sodium pentobarbitone (Siegfried, Zöfingen). All drugs were dissolved in isotonic saline immediately before use. Drug concentrations were

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prepared in such a way that the necessary dose could be administered in volumes of $1\,mL\,kg^{-1}$ intravenously, $2\,mL\,kg^{-1}$ intraperitonealy and $10\,mL\,kg^{-1}$ by oral gavage.

Pharmacodynamic experiment

The rats were anaesthetized with sodium pentobarbitone ($55\,mg\,kg^{-1}$, i.p.). They were previously treated either with saline or with $3\,mg\,kg^{-1}$ idazoxan which was administered through the caudal vein at 5, 15, 30, 60, 90, 120, 180 and 240 min before clonidine administration ($0{\cdot}3\,mg\,kg^{-1}$, i.v.). At least seven rats were used for each experimental time. Just before and 5 min after clonidine, pupil diameters were measured at the point of greatest mydriasis by means of a monocular macroscope (10 × magnification) with an internal calibrated reticule (0·2 mm gradation). Pupil measurements were taken under a white light source of steady and soft intensity (18 W; 900 lumen) to avoid changes in background after induction of anaesthesia. Under these experimental conditions and 15 min after induction of anaesthesia, the resulting pupil diameter of control rats ranged between 0·2 and 0·4 mm and lasted for more than 60 min. Clonidine was administered intravenously at $0{\cdot}3\,mg\,kg^{-1}$, a dose which was selected on the basis of the relationship between doses of clonidine and the corresponding mydriatic measurements in rat (Menargues et al 1990). This dose of clonidine promotes a submaximal mydriatic effect before the assessment of the plateau in the mentioned relationship. Additionally, $0{\cdot}3\,mg\,kg^{-1}$ clonidine intravenously administered to the rat induces a rapid mydriatic response remaining stable for more than 60 min, and allows the measurement of the reversion of clonidine-induced mydriasis by the doses of idazoxan chosen in this study.

Idazoxan was also administered at 3 and $10\,mg\,kg^{-1}$ by oral gavage to two other groups of rats. In these rats, measurements of mydriasis after clonidine ($0{\cdot}3\,mg\,kg^{-1}$, i.v.) were obtained as described above. Rats had been pretreated orally either with saline or with idazoxan at 5, 10, 15, 20, 30, 45, 60, 120 and 180 min before clonidine administration. At least four rats were used for each experimental time.

Pharmacokinetic experiment

To characterize the kinetic profile of the drug, another group of rats was treated at $3\,mg\,kg^{-1}$ through the caudal vein. Blood samples were obtained by cardiac puncture before and 1, 3, 10, 20, 45, 60, 90, 120 and 180 min after the drug administration. Four rats were used for each blood withdrawal. Blood samples were centrifuged at 2000 *g* for 20 min, and plasma was removed and stored at −20°C for analysis.

Drug analysis

Idazoxan plasma levels were determined by a high performance liquid chromatography (HPLC) with UV detection as described elsewhere (Vallès et al 1989). 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 10% at the highest concentration ($1000\,ng\,mL^{-1}$). 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 4·4% at $1000\,ng\,mL^{-1}$. 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 0·7%. The limit of detection of idazoxan in plasma was $10\,ng\,mL^{-1}$, expressed as the concentration of compound that produced a peak height equal to the mean blank values plus 3 standard deviations.

Data analysis

A poly-exponential equation (eqn 1) was used to fit plasma levels of idazoxan. The selection between the monoexponential or the biexponential equation was carried out on the basis of the Schwarz criterion (Schwarz 1978), following the application of the MKMODEL program (Holford 1992).

$$C_t = \sum_{i=1}^{n} A_i e^{-\lambda_i t}) \qquad (1)$$

C_t is the plasma concentration of idazoxan at time t, and A_i and λ_i are, respectively, the coefficient and the exponent of the equation. Pharmacokinetic parameters such as total clearance (CL), volume of distribution at steady state (Vd_{ss}) and terminal half-life ($t_{\frac{1}{2}}$) were calculated according to standard procedures (Gibaldi & Perrier 1982).

Once the most probable pharmacokinetic model was selected, it was linked to two different pharmacodynamic models according to the scheme shown in Fig. 1 by means of the MKMODEL program (Holford 1992). The most suitable pharmacokinetic-pharmacodynamic linking model was also chosen on the basis of the Schwarz criterion (Schwarz 1978).

The simple and the sigmoidal inhibitory E_{max} models were selected as two of the possible pharmacodynamic models which could adequately describe the percentage idazoxan inhibitory response (IR) (eqn 2) over the whole range of concentrations of this drug (eqn 3):

$$IR(\%) = \frac{pd_{idaz} - pd_o}{pd_{saline} - pd_o}.100 \qquad (2)$$

$$IR(\%) = 100.\left(1 - \frac{C^n}{IC50_{app}{}^n + C^n}\right) \qquad (3)$$

where pd_{idaz} and pd_{saline} are the pupil diameters measured 5 min after clonidine in rats pretreated with idazoxan and saline, respectively, pd_o is the baseline pupil diameter measured just before clonidine administration, n is the Hill coefficient, C is the concentration of idazoxan and $IC50_{app}$ is the apparent concentration of this drug inducing a half maximal inhibitory response. When n = 1, the inhibitory

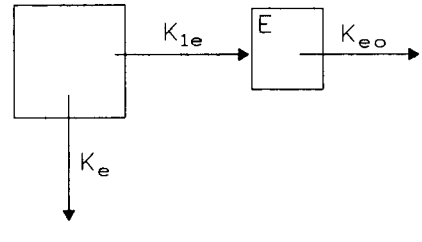

FIG. 1. Schematic representation of the pharmacokinetic-pharmacodynamic linking model with an effect compartment.

hyperbolic E_{max} model is selected, but when n is greater than 1, the constant expresses the sigmoidicity of the concentration-effect relationship, which characterizes the inhibitory sigmoidal E_{max} model. Equation 3 was selected because of the well-known competitive antagonism of idazoxan on the pupillary dilation induced by the α_2 agonists clonidine, guanoxabenz and UK-14304 (Berridge et al 1983).

At each experimental time, mean effective pupillary diameters after clonidine administration to rats pretreated with idazoxan ($pd_{idaz} - pd_o$) were compared with the corresponding control responses (mean effective pupillary diameter after clonidine treatment in rats pretreated with saline ($pd_{saline} - pd_o$)). Multiple statistical comparisons were performed by means of Dunnett's test. Differences were considered significant at $P < 0.05$.

In rats pretreated with idazoxan (3 mg kg^{-1}, i.v., or 3 and 10 mg kg^{-1}, orally) the time course of the inhibitory response on clonidine-induced mydriasis was studied.

Results

Plasma levels of idazoxan obtained in rats after the intravenous administration at 3 mg kg^{-1} are shown in Fig. 2. According to the Schwarz criterion, the monoexponential curve was selected as the most suitable pharmacokinetic function to describe the plasma concentration–time profile of the drug. Fig. 3 shows, at each experimental time, the effective pupillary diameters ($pd_{idaz} - pd_o$) obtained 5 min after clonidine in rats pretreated with idazoxan (3 mg kg^{-1}, i.v., or 3 and 10 mg kg^{-1}, orally).

In the group of animals which received 3 mg kg^{-1} idazoxan intravenously, as a pretreatment initiated from 5 to 240 min before clonidine, the effective pupillary diameters measured after clonidine administration were significantly lower than those obtained in control rats ($P < 0.01$). Moreover, in relation to the intravenous dose of idazoxan, the inhibitory simple E_{max} model was selected, according to the Schwarz criterion, as the most suitable dynamic function to describe the effect of the drug over the whole range of concentrations (Table 1). Fig. 4 shows the derived concentration-inhibitory effect relationship according to the pharmacokinetic-pharmacodynamic modelling procedure. The estimated values of

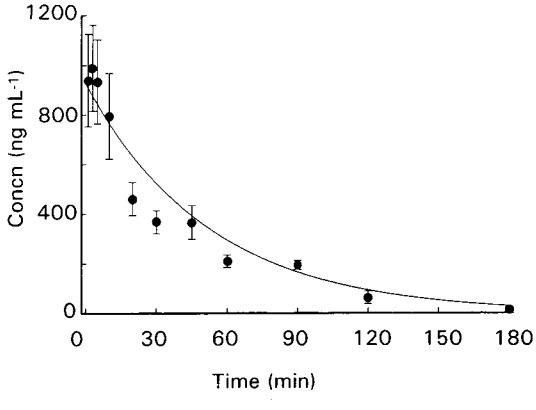

FIG. 2. Idazoxan plasma levels after the intravenous administration of 3 mg kg^{-1} to the rat. The values indicated are the mean ± s.e.m. of four rats.

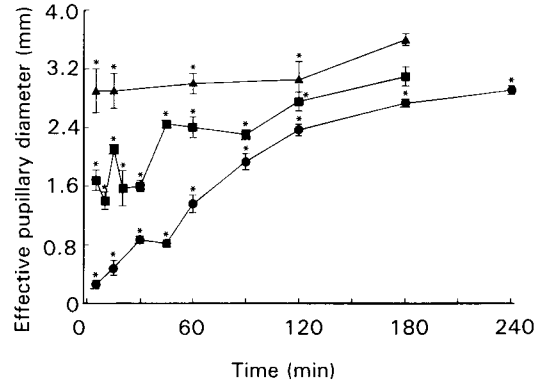

FIG. 3. Effective pupillary diameters obtained after clonidine in rats pretreated with idazoxan (3 mg kg^{-1}, i.v. (●), 3 mg kg^{-1}, orally (▲) and 10 mg kg^{-1}, orally (■)) at different time intervals. The values indicated are the means ± s.e.m. of at least four rats (oral) or seven rats (i.v.). In each rat, the effective pupillary diameter was obtained by substracting the pupillary diameter measured just before clonidine from the pupillary diameter measured 5 min after administration of this drug (0·3 mg kg^{-1}, i.v.). Mean ± s.e.m. effective pupillary diameters in control rats pretreated with saline (i.v. and oral) before clonidine were 3·21 ± 0·07 (●), 3·55 ± 0·17 (▲) and 3·10 ± 0·06 mm (■). (*$P < 0.01$; Dunnett's test.)

CL, Vd and $t_{\frac{1}{2}}$ were respectively 71·2 mL kg^{-1} min^{-1}, 3134 mL kg^{-1} and 30·5 min. The average $IC50_{app}$ of idazoxan was 153·6 ng mL^{-1}. No hysteresis between plasma concentration and the inhibitory response was observed. The value of the constant K_{eo} was large ($K_{eo} > 10^6$ min^{-1}), indicating that the effect compartment kinetics parallel those of drug in plasma.

From 5 to 120 min following the oral administration of 3 mg kg^{-1} idazoxan, mild but statistically significant inhibitory responses of idazoxan on clonidine-induced mydriasis were observed ($P < 0.01$). Higher inhibitory responses were obtained in rats which received 10 mg kg^{-1} oral idazoxan from 5 to 120 min before the agonist ($P < 0.01$).

Discussion

The purpose of the present investigation was to quantify the concentration-inhibitory response relationship of idazoxan on clonidine-induced mydriasis in rat. Clonidine produces its parasympatho-inhibitory effect by direct activation of CNS postsynaptic α_2-adrenoceptors, possibly acting on the pupilloconstrictor Edinger-Westphal neurons, resulting in reduction of parasympathetic tone and consequent pupillary

Table 1. Study on the inhibition by idazoxan of the clonidine-induced mydriasis in the rat, using pharmacokinetic-pharmacodynamic modelling. Parameter estimates were quantified by the inhibitory E_{max} models.

		Simple E_{max}	Sigmoidal E_{max}
Vd	(mL kg^{-1})	3134 ± 230	3134 ± 230
CL	(mL kg^{-1} min^{-1})	71·2 ± 5·5	71·2 ± 5·5
$IC50_{app}$	(ng mL^{-1})	153·6 ± 14·7	157·5 ± 15·6
K_{eo}	(min^{-1})	>10^6	>10^6
n		1	1·06 ± 0·11
Schwarz criterion		−94·22	−95·55

Mean ± s.e.m. values.

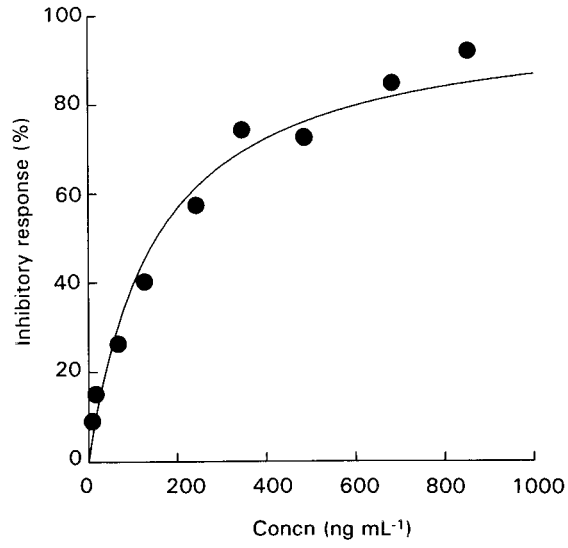

FIG. 4. Concentration-inhibitory response of idazoxan (3 mg kg^{-1}, i.v.) on mydriasis induced by clonidine (0·3 mg kg^{-1}, i.v.).

dilation (Koss 1986). Further, this mydriatic effect of clonidine has been shown to be dose-dependent in the rat (Gherezghiher & Koss 1979) and in the cat (Koss 1979).

Following intravenous administration of idazoxan, the time-course of the plasma levels of the drug was described adequately by a monoexponential equation, with an estimated elimination half-life of 30 min, which allowed us to determine the complete concentration-response relationship. The concentration-inhibitory response of idazoxan did not show hysteresis, suggesting that no delay in onset of the effect was observed in the time frame of the experiment. In fact, intense inhibitory responses on clonidine-induced mydriasis were obtained in rats pretreated with idazoxan a few minutes before clonidine, indicating that a rapid equilibration takes place between the plasma and the effect compartment. This finding agrees with the results of autoradiographic studies of idazoxan in the rat (Lewis et al 1988). After 1 mg kg^{-1} [2′-^{14}C]idazoxan intravenously, there was a rapid uptake of radioactivity from blood to organs such as brain, kidney, liver, lung and salivary glands. The experiment clearly demonstrated that idazoxan has the capacity to penetrate through the blood-brain barrier. Lewis et al (1988) reported that the elimination curves for idazoxan from brain and plasma in rats dosed with 1 mg kg^{-1} [6, 7-H^{3}]idazoxan intravenously were parallel and, moreover, that quantitative determination of labelled idazoxan in tissues was followed by a decrease in all tissues to low levels, with a half-life value ranging from 0·5 to 3 h.

In addition to several potentially confounding factors which influence the relationship between concentration and pharmacological effect of drugs in-vivo, such as differences in the stereospecific disposition of enantiomers, changes in distribution at the site of action and development of acute tolerance, the potential contribution of biotransformation products to the measured response should be borne in mind (Dingemanse et al 1988). The major routes of idazoxan metabolism in rat are hydroxylation at positions 6 and 7 to form phenolic derivatives, which are excreted as glucuronide and sulphate metabolites in urine, but unconjugated in faeces. Other minor metabolic routes are 5-hydroxylation or oxidative degradation of the imidazoline ring (Lewis et al 1988). The formation of metabolites in the gut would be expected on the basis of previous bioavailability studies (Lewis et al 1988; Vallès et al 1989). Lewis et al (1988) reported that after intravenous and oral administrations of idazoxan to the rat, the formation of metabolites did not differ from a qualitative point of view. Nevertheless, from a quantitative point of view and in relation to intravenous administration, the oral route led to the appearance of higher levels of glucuronide conjugates.

In agreement with the low bioavailability obtained after the oral administration of 3 mg kg^{-1} idazoxan in rat (ca 10%) (Vallès et al 1989), the administration of clonidine to rats pretreated orally with 3 mg kg^{-1} idazoxan was accompanied by mild inhibitory responses. The intensity of the inhibitory response of idazoxan on mydriasis induced by the same dose of clonidine was higher in rats pretreated with oral idazoxan at 10 mg kg^{-1}. The absolute bioavailability of idazoxan following 10 mg kg^{-1} oral administration to the rat is ca 30% (Vallès et al 1989).

The results show that the relationship between concentration and the effect on the CNS of idazoxan can be characterized in individual rats using the clonidine-mydriatic model. This kinetic-dynamic modelling in rat allows us to quantify the effect of idazoxan on the CNS postsynaptic α_2-adrenoceptor by means of the inhibitory E_{max} model, demonstrating that idazoxan levels at the effect compartment of 154 ng mL^{-1} induce inhibitory responses close to 50% of maximal inhibition in this experimental model. Nevertheless, as these results have been obtained considering an average concentration-effect relationship in a population of animals, and not in individual rats, other possible pharmacodynamic models could not be discarded on the basis of individual relationships.

Kinetic-dynamic modelling of new drugs is strongly recommended by health authorities (Peck et al 1992). Thus, our results could contribute to better characterization of the pharmacodynamic and toxicological profiles of idazoxan in experimental models in which a different pharmacokinetic behaviour of the drug is presumed, such as in cases of renal and hepatic insufficiency and drug interactions.

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