J. Pharm. Pharmacol. 1985, 37: 367–369
Communicated July 30, 1984

Disposition of HI-6 oxime in rats after intravenous and intramuscular administration

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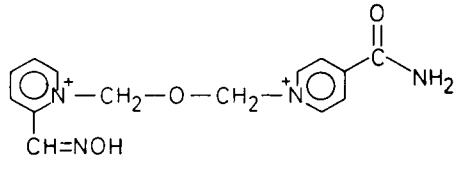
The pharmacokinetics of HI-6, a cholinesterase-reactivating oxime, were studied in rats, following intravenous or intramuscular administration. A two-compartment model was used to analyse the intravenous data and a one-compartment open model with first-order absorption was used for intramuscular data. Drug concentration had no influence on rate and extent of absorption of intramuscular injections, and bioavailability was 100%. Peak plasma concentrations of HI-6 occurred 15 min after intramuscular injection. No significant differences were found between mean values for half-life, plasma clearance, volume of distribution and area under the plasma concentration versus time curve for the two intramuscular doses and the intravenous dose used. Mean HI-6 plasma concentrations were 140·5 ± 4·2 μg ml^{-1} 3 min after 20 mg ml^{-1} i.v., with a mean elimination half-life of 65·2 ± 21 min. Plasma clearance rate was 3·95 ± 0·93 ml min^{-1} kg and the apparent volume of distribution was 0·38 ± 0·17 litre kg^{-1}. The oxime is rapidly distributed in and eliminated by rats when administered intravenously or intramuscularly.

HI-6 ((1-(2-hydroxyiminomethyl-pyridium)-2-4 carboxyamidopyridinium)-dimethyl ether dichloride) (I) is effective in the treatment of laboratory animals poisoned with the organophosphate anticholinesterase, soman (pinacolyl methylphosphonofluoridate) (Wolthuis & Kepner 1978; Lipp & Dola 1980; Clement & Lockwood 1982). The disposition of HI-6 in rats following intravenous administration has been examined by Maksimovic (1979). We have investigated the pharmacokinetics of HI-6 in rats following intravenous and intramuscular administration. We determined its bioavailability after intramuscular injection and evaluated the effect of concentration on the rate and extent of absorption of HI-6 given intramuscularly.



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I (HI-6 occurs as the dichloride salt with 1 molecule of water).

Methods

HI-6 was synthesized and purified at the Defence Research Establishment, Suffield, Alberta. Solutions, 25 and 125 mg ml^{-1} with 1·5% benzyl alcohol as preservative, were prepared in water for injection and sterilized by filtration through a Millipore 0·22 μm filter. Male out-bred Sprague-Dawley rats (200–250 g) from the University of Manitoba Central Animal Care Service (Gunton), were used in the pharmacokinetic studies (n = 35).

Before each study, two rats were anaesthetized with ether and the jugular vein cannulated and maintained patent with a Hepaleane (Harris Laboratories) heparin lock. A control blood sample (0·5 ml) was obtained, after which HI-6, 20 mg kg^{-1} was administered. Injection by the intravenous route (125 mg ml^{-1} solution) was into the dorsal vein of the penis, and by the intramuscular route (25 or 125 mg ml^{-1} solution) into the thigh muscle. One or other injection was given to each of a pair of rats according to a randomized dosing schedule. Blood samples (0·5 ml) from the first rat of the pair were withdrawn at 3, 7, 10, 15, 30 and 90 min after the dose. From the second rat, blood samples (0·5 ml) were withdrawn at 30, 45, 60, 90, 120 and 150 min after the dose. The volumes of blood withdrawn were replaced by an equal volume of 0·9% NaCl. The plasma was separated in capillary blood serum separator tubes and frozen (−10 °C) until analysis. Samples (0·2 ml) were treated with 0·2 ml 10% trichloroacetic acid, centrifuged and the supernatant, 0·2 ml, made alkaline with 0·2 ml 0·5 M ammonium hydroxide. Absorbance at 355 nm was measured immediately in 0·5 ml microcuvettes with a path length of 10 mm. The assay was specific and sufficiently sensitive to enable concentrations as low as 10 μg ml^{-1} in 0·2 ml plasma to be quantified.

Data analysis. The concentration of HI-6 versus time curves were analysed using a two-compartment model for the intravenous data and a one-compartment open model with first-order absorption for the intramuscular data. If the results from both rats of a study pair could be superimposed the data were analysed as one set of data, otherwise results from each rat were analysed separately. Experimental points were fitted by non-linear analysis using the University of California BMDP-77 Program on AMDAHL V/7 computer. The elimination half life ($t_{\frac{1}{2}}$) plasma clearance (Cl), and the apparent volume of distribution (Vd) were calculated from the formulae: $t_{\frac{1}{2}} = \ln 2/Ke$, Cl = (FD)/AUC and Vd = Cl/Ke (Gibaldi & Perrier 1982) where (F) is the fraction of the dose (D) absorbed, AUC is the area under the plasma concentration vs time curve calculated using the trapezoidal rule with extrapolation to infinity and Ke is the slope of the 'elimination' phase. If the mean AUC i.v. is equal to the mean AUC i.m., F = 1. Statistical analysis of the various pharmacokinetic

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parameters obtained from the three different doses was by the unpaired Student's *t*-test.

Results and discussion

The log mean ± s.d. HI-6 plasma concentration vs time plots from the intravenous and intramuscular doses (125 mg ml^{-1}) are shown in Figs 1 and 2 respectively. The mean ± s.d. pharmacokinetic parameters are shown in Table 1. Mean HI-6 plasma concentrations were 140·5 ± 4·2 μg ml^{-1} 3 min after the administration of the 20 mg kg^{-1} intravenous dose and these decreased to 14·9 ± 7·5 μg ml^{-1} after 2·5 h.

Table 1. Pharmacokinetic parameters[a] of HI-6 in rats given a 20 mg kg^{-1} dose.

Parameter (units)	i.v. (125 mg ml^{-1})	i.m. (125 mg ml^{-1})	i.m. (25 mg ml^{-1})
Absorption $t_{\frac{1}{2}}$ (min)	4·1 ± 1·3[b]	4·6 ± 3·1[c]	9·2 ± 5·3[c]
Elimination $t_{\frac{1}{2}}$ (min)	65·6 ± 30·0	59·5 ± 25·5	45·7 ± 16·8
AUC (μg ml^{-1} min)	5299 ± 1222	4937 ± 2549	4436 ± 1010
Cl (ml min^{-1} kg)	3·95 ± 0·93	4·95 ± 2·29	4·71 ± 1·07
Vd (litre kg^{-1})	0·38 ± 0·17	0·37 ± 0·12	0·30 ± 0·10

[a] Mean ± standard deviation.
[b] Distribution half-life (ln 2/α).
[c] Apparent absorption half-life (ln 2/α).

The mean elimination half-life of 65·6 ± 21·0 min was longer than the value of 30 min reported by Maksimovic (1979). The half-life after a 200 mg kg^{-1} dose of HI-6 was 101·9 min (Maksimovic 1979). The present experiments were terminated at 150 min, which is equivalent to 3 half-lives, when serum HI-6 concentrations were about 10% of initial values. Serum concentrations at 150 min were also approaching limits of assay reproducibility. The mean plasma clearance rate, 3·95 ± 0·93 ml min^{-1} kg^{-1} and apparent volume of distribution, 0·38 ± 0·17 litre kg^{-1} have not been reported previously.

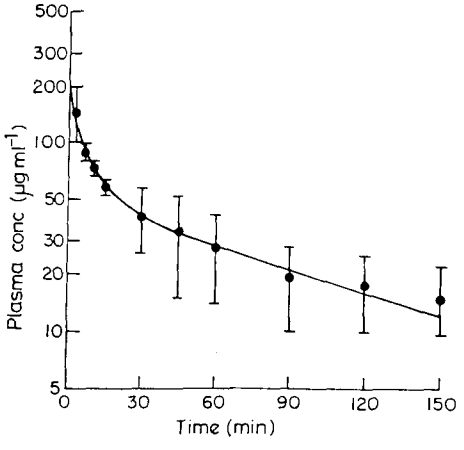

FIG. 1. Mean ± s.d. log plasma HI-6 concentrations versus time plot following an intravenous dose of 20 mg kg^{-1} to 10 rats (best computer-fitted line to mean data).

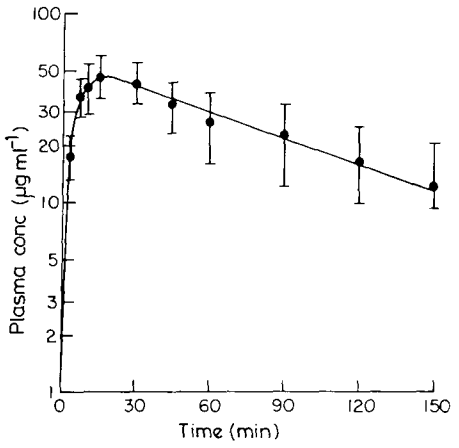

FIG. 2. Mean ± s.d. log plasma HI-6 concentrations versus time plot following a concentrated (125 mg ml^{-1}) intramuscular dose of 20 mg kg^{-1} to 10 rats (best computer-fitted line to mean data). The curve for the 25 mg ml^{-1} dose is almost superimposable.

Mean peak HI-6 plasma concentrations of 46·1 ± 8·7 and 46·8 ± 7·9 μg ml^{-1} occurred at 15 min after the 125 and 25 mg ml^{-1} intramuscular doses respectively. These were not significantly different ($P = 0·05$). There was no significant difference ($P = 0·05$) between the mean values obtained for half-life, plasma clearance and volume of distribution obtained from the intravenous dose and the two intramuscular doses. There was no significant difference ($P = 0·05$) between the mean values for absorption and elimination half-lives, plasma clearance and volume of distribution obtained with both intramuscular doses. The mean values for the AUC were not significantly different ($P = 0·05$) from each other or from the value obtained from the intravenous dose.

From these results it can be concluded that HI-6 when administered intravenously or intramuscularly is rapidly eliminated by rats. The intramuscular route has 100% bioavailability and the concentration of solutions 25 or 125 mg ml^{-1} as 20 mg kg^{-1} doses by this route had no effect on the rate or extent of absorption. In man, if HI-6 elimination half-lives are found to be similar to values of 73·7 ± 15·2 min found with pralidoxime (Sidell & Groff 1971), parenteral drug administration will need to be repeated every 3–4 h to maintain effective serum concentrations, but the suitability of the rat model can only be evaluated when HI-6 is approved for administration to man.

This research was supported by the Defence Research Establishment, Suffield, Ralston, Alberta T0J 2NO and by the US Army Medical Research Development Corps. We would like to acknowledge the technical assistance of Debrah Keling and Cathy Keller.

REFERENCES

Clement, J. G., Lockwood, P. A. (1982) Toxicol. Appl. Pharmacol. 64: 140–146

Gibaldi, M., Perrier, D. (1982) Pharmacokinetics, Marcel Dekker, Inc NY, pp 1–5, 33, 40, 145–149, 211–219, 319–322

Lipp, J., Dola, T. (1980) Arch. Int. Pharmacodyn. 246: 138–148

Maksimovic, M. (1979) Arh. Hig. Rada. Toksikol. 30: 227–239

Sidell, F. R., Groff, W. A. (1971) J. Pharm. Sci. 60: 1224–1228

Wolthuis, O. L., Kepner, L. A. (1978) Eur. J. Pharmacol. 49: 415–425

J. Pharm. Pharmacol. 1985, 37: 369–372
Communicated September, 1984

Cocaine potentiates the responses to methacholine and noradrenaline in the rat vas deferens

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The ability of cocaine (10 μM) to potentiate the contractile responses of the epididymal half of the rat vas deferens to methacholine was reversed by prazosin. Prazosin also partially reversed the ability of cocaine to increase the spontaneous overflow of ^3H following loading of the tissue with [^3H]noradrenaline. We suggest that cocaine potentiated the responses to methacholine by stimulating, directly or indirectly, α_1-adrenoceptors.

In many studies assessing the potency of agonists or antagonists at adrenoceptors, cocaine has been routinely added to inhibit neuronal uptake. Cocaine will only be useful in such studies if it has no additional actions (discussed by Furchgott 1972). However cocaine (i) is a general depressant of cardiac tissue (Trendelenburg 1968; Doggrell & Vincent 1982; Lew & Angus 1983), (ii) has a postjunctional action whereby some blood vessels become supersensitive to a variety of stimuli (e.g. histamine, methoxamine in the rabbit aorta; Kalsner & Nickerson 1969) and (iii) acts as an agonist at α-adrenoceptors and also potentiates responses to acetylcholine by a postjunctional mechanism in the rat anococcygeus muscle (Doggrell & Waldron 1982).

Cocaine has been used to inhibit neuronal uptake in contractility studies with the rat vas deferens (e.g. Demichel et al 1981; Leedham & Pennefather 1982; Doxey et al 1984). We have examined whether cocaine has additional actions on this tissue. Thus we report the effects of cocaine on the contractile responses of the epididymal half of rat vas to methacholine and noradrenaline (NA). The effects of cocaine on the uptake and the spontaneous overflow of ^3H, following preloading of the tissue with [^3H]noradrenaline, were also investigated.

Methods and materials

Mature male Wistar rats (300–450 g) were stunned and exsanguinated. Vasa deferentia were removed, dissected free of surrounding tissue and the epididymal half retained. All experiments were performed in the presence of a modified Krebs solution [composition (mM): NaCl 116; KCl 5·5; $CaCl_2$ 2·5; $MgCl_2$ 1·2; NaH_2PO_4 1·2; $NaHCO_3$ 22·0; D-glucose 11·2; Na_2EDTA 0·04] equilibrated with 5% CO_2 in oxygen at 37 °C. In each series of experiments the individual values obtained were compared by Student's paired *t*-test and were considered significant when $P < 0.05$. Mean values ± s.e.m. were then determined.

For the contractility studies, each epididymal tissue was mounted under 0·5 g tension in a 5 ml organ bath containing Krebs solution, and allowed to equilibrate for 1 h. Concentration-response curves to methacholine and then noradrenaline were obtained non-cumulatively. Exposure to agonist was continued for 30 s or until the response was maximal. The tissues were then washed by over-flow and allowed to recover, for a minimum of 7 min, before further addition of agonist. Contractile responses were recorded isometrically with force displacement transducers (Grass model FT03.C) and displayed on a polygraph (Grass model 79B). When two response curves were obtained 30 min was allowed to elapse between them.

When the effect of cocaine or prazosin on the responses to agonists was studied, the drug was added to one of a pair of mounted vasa, while the other remained untreated. For experiments in the presence of cocaine, the drug was in the Krebs solution in the bath. After all experiments the tissues were blotted and weighed.

Where maximum responses (mg), with or without drug, were not significantly different, responses were calculated as a percentage of the maximum response of the individual response curve (i.e. normalized) and the slope (difference in percentage maximum of the response/logarithm molar concentration of agonist) for each concentration response curve was computed by regression analysis (over the range 20–80% of the

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