performance liquid chromatography (HPLC). Neurosci. Lett. In press
Chan, K., Lai, B., Chu, S. M. (1990) A pilot study on the dependent consumption of cough and cold remedies in Hong Kong youths. Ibid. 40 (Suppl.): S18
Dambisya, Y. M., Chan, K., Wong, C. L. (1991a) Ephedrine and phenylpropanolamine potentiate the lethal toxicity of morphine and codeine in naive mice. Asia Pacific J. Pharmacol. 6: 255–258
Dambisya, Y. M., Chan, K., Wong, C. L. (1991b) Role of adrenoceptors in the potentiation of opioid antinociception by ephedrine and phenylpropanolamine in mice. Psychopharmacology 105: 563–567
Dambisya, Y. M., Wong, C. L., Chan, K. (1990) Effects of ephedrine and phenylpropanolamine on the antinociceptive effects of morphine and codeine in mice. Arch. Int. Pharmacodyn. 308: 5–12
Dambisya, Y. M., Wong, C. L., Chan, K. (1991c) Effects of sympathomimetic agents on opiate analgesia, tolerance and dependence in mice. Meth. Find. Exp. Clin. Pharmacol. 13: 239–248
D'Armour, F. E., Smith, D. L. (1941) A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72: 74–79
Findlay, J., Jones, E. C., Butz, R., Welch, R. (1978) Plasma codeine and morphine concentrations after therapeutic oral doses of codeine-containing analgesics. Clin. Pharmacol. Ther. 24: 60–68
Lake, C. R., Rosenberg, D. R., Gallant, S., Zaloga, G., Chernow, B. (1990) Phenylpropanolamine increases plasma caffeine levels. Ibid. 47: 675–685
Leza, J. C., Lizasoain, I., Lorezo, P. (1990) H_1- and H_2-histamine receptor blockers and opiate analgesia in mice. Meth. Find. Exp. Clin. Pharmacol. 12: 671–678
Monasky, M. S., Zinsmeister, A. R., Stevens, C. W., Yaksh, T. L. (1990) Interaction of intrathecal morphine and ST-91 on antinociception in the rat: dose-response analysis, antagonism and clearance. J. Pharmacol. Exp. Ther. 254: 383–392
Rogers, J. F., Findlay, J. W., Hull, J. H., Butz, R. F., Jones, E. C., Burstrack, J. A., Welch, R. M. (1982) Codeine disposition in smokers and nonsmokers. Clin. Pharmacol. Ther. 32: 218–227
Shah, J. C., Mason, W. D. (1990) Plasma codeine and morphine concentrations after a single oral dose of codeine phosphate. J. Clin. Pharmacol. 30: 764–766
Sjöqvist, F. (1991) Interethnic differences in polymorphic drug oxidation—clinical implications. Proceedings of the Sixth South East Asian/Western Pacific Regional Meeting of Pharmacologists, Hong Kong. Abstract S.6-2
Sjöqvist, F., Von Bahr, C. (1973) Interindividual differences in drug oxidation: clinical importance. Drug Metab. Dispos. 1: 469–482
Svensson, J. O., Rane, A., Sawe, J., Sjöqvist, F. (1982) Determination of morphine, morphine-3-glucuronide and (tentatively) morphine-6-glucuronide in plasma and urine using ion-pair high performance liquid chromatography. J. Chromatogr. 230: 427–432
Weinberger, M., Bronsky, E., Bensch, G. W., Bock, G. N., Yecies, J. J. (1975) Interaction of ephedrine and theophylline. Clin. Pharmacol. Ther. 17: 585–592
Yue, Q. Y., Svensson, J. O., Alm, C., Sjöqvist, F., Sawe, J. (1989a) Interindividual and interethnic differences in the demethylation and glucuronidation of codeine. Br. J. Clin. Pharmacol. 28: 629–637
Yue, Q. Y., Svensson, J. O., Alm, C., Sjöqvist, F., Sawe, J. (1989b) Codeine *O*-demethylation co-segregates with polymorphic debrisoquine hydroxylation. Ibid. 28: 639–645

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HI-6 pharmacokinetics in rabbits after intravenous and intramuscular administration[1]

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Abstract—The pharmacokinetics of HI-6 ((4-carboxamidopyridinium (1) methyl)-(2′-hydroxyiminomethyl-pyridinium (1′) methyl) ether dichloride) have been studied in rabbits receiving an intramuscular (50 $\mu g\ kg^{-1}$) or intravenous (12·5 $\mu g\ kg^{-1}$) dose. The plasma concentration-time profile for the intramuscular dose (n = 8) fits a one-compartment open model with first-order absorption and elimination. The absorption half-life was 2 min and maximum concentration (51 $\mu g\ mL^{-1}$) was reached in 9 min. The pharmacokinetics for the intravenous dose (n = 8) was described by a two-compartment open model with first-order distribution and elimination. The apparent volume of distribution was 0·1 $L\ kg^{-1}$. Half-lives of distribution and elimination were 5 and 38 min, respectively. The results indicate HI-6 is rapidly absorbed, distributed and eliminated in rabbits receiving an intramuscular dose.

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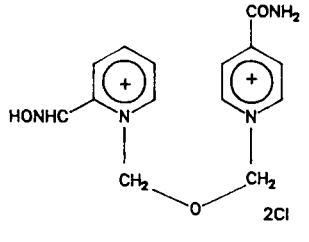
[1]The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985). The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

The oxime, 2-pralidoxime chloride is the standard antidote against organophosphate insecticides due to its ability to reactivate inhibited acetylcholinesterase (Harris et al 1969). However, 2-pralidoxime chloride is ineffective in the treatment of soman poisoning (Loomis & Salafsky 1963). Hagedorn et al (1976, 1978) synthesized a number of bispyridinium oximes in an effort to obtain an effective antidote for soman poisoning. Of the many tested, HI-6 (I) proved to be extremely effective in reactivating acetylcholinesterase, before ageing inhibited by soman (DeJong & Wolring 1980) and protecting against soman lethality (Boskovic 1981, 1985).

Our organization has used rabbits as an animal model in testing HI-6 efficacy for organophosphate poisoning. However,



I. (4-Carboxamidopyridinium (1)methyl)-(2′hydroxyiminomethyl-pyridinium (1′) methyl) ether dichloride.

HI-6 pharmacokinetic data in rabbits are non-existent. Only a few studies have investigated HI-6 pharmacokinetics in any species. These species include rat (Maksimovic 1979; Simons & Briggs 1985), dog (Simons & Briggs 1983), and man (Kusic et al 1985). The pharmacokinetics in a variety of species provide a good means to correlate interspecies efficacy. Therefore the purpose of this study was to define HI-6 absorption, distribution and elimination in the rabbit.

Materials and methods

Eight male New Zealand White rabbits (2–4 kg) were randomly divided into two groups. The first group received HI-6 (50 μmol kg^{-1}) intramuscularly (i.m.) followed two weeks later by HI-6 (12·5 μmol kg^{-1}) intravenously (i.v.). The second group received the i.v. dose first followed two weeks later by the i.m. dose. Both groups were studied on the same weeks.

For each animal, a 22 gauge i.v. catheter was placed in the central artery of an ear. The catheter was sealed with an intermittent injection cap and kept patent by periodically flushing with a 5% dextrose water (D_5W)-heparin (10 μg mL^{-1}) solution. HI-6 was either administered as an intramuscular bolus in the right quadriceps or an i.v. bolus in the marginal vein of the ear opposite the one with the i.v. catheter. Before blood sample collection, 0·5 mL of fluid was withdrawn from the arterial catheter to clear it of dead volume. Blood samples were taken immediately before and 2·5, 5, 10, 20, 25, 30, 40, 50, 60, 80, 100, 120, 150, 180 and 240 min after dosing. Blood was collected in heparinized collection tubes that were subsequently placed on ice until all samples that day were collected. Samples were then spun at 1500 *g* for 15 min and the plasma removed. Plasma samples were frozen at −70°C for batch analysis at the end of each week.

Plasma concentrations of HI-6 were determined by slightly modifying an automated dialysis-spectrophotometric technique (Groff & Ellin 1969). Briefly, the method consists of an automated sampler that dilutes plasma into a sample stream which then enters a dialyser. HI-6 passes through the dialyser to the recipient stream that is pumped to the spectrophotometer. The sample and recipient streams are 0·05 M Tris (hydroxymethyl) aminomethane buffer in aqueous solution and the wavelength for maximum absorption is 355 nm. Concentration of HI-6 were determined from daily standard curves covering a range of 0–50 μg mL^{-1}.

Data analysis. The concentration-time profile for each animal was fitted to standard pharmacokinetic models using the PCNONLIN computer program (Statistical Consultants Inc. 1986). Initial estimates of coefficients and exponentials required by PCNONLIN were obtained from the exponential curve-fitting program JANA (Statistical Consultants Inc., Lexington, KY, USA). Data for model fitting were iteratively reweighted by the square reciprocal of the predicted concentration. Selection of the most appropriate model was based upon the simplest curve (fewest exponentials) possessing a small sum of squared residual, large correlation coefficient of observed vs predicted concentrations, even distribution patterns of residuals as a function of calculated concentrations, and small standard deviations of pharmacokinetic parameter estimates.

To determine systemic clearance, the product of dose and bioavailability was divided by area under the concentration-time curve. Intramuscular bioavailability was calculated as ($Dose_{i.v.}$/$Dose_{i.m.}$) ($AUC_{i.m.}$/$AUC_{i.v.}$). Volume of distribution for the i.m. route was determined by multiplying the reported volume of distribution for the PCNONLIN program by the i.m. bioavailability. All other parameters were obtained from the PCNONLIN program. Parameters for each route of administration were averaged and the averages were used to draw the best fit line by calculating expected concentrations with small increments in time from an electronic spreadsheet (Lotus 123, Lotus Development Corporation, Cambridge, MA, USA). Data were then transferred to a computer graphics program (Sigmaplot, Jandel Scientific, Sausalito, CA, USA) for plotting. All errors represent 95% confidence limits.

Results and discussion

The concentration-time profile of HI-6 administered i.v. to rabbits fit a two-compartment open model with first-order distribution and elimination (Fig. 1):

$$C(t) = Ae^{-k_{10}t} + Be^{-k_{12}t}$$

where C = plasma concentration (ng mL^{-1}), t = time (min), k_{12} = rate constant of distribution (min^{-1}), and k_{10} = rate constant of elimination (min^{-1}), and where A and B are coefficient constants for the elimination and distribution phase, respectively. This model was also employed to describe the pharmacokinetics of HI-6 intravenously administered to dogs (Simons & Briggs 1983) and rats (Simons & Briggs 1985). The $t_{\frac{1}{2}}$ of the distribution phase for rabbits (5·0 ± 2·6 min) was short and in agreement with the 4·1 min value for rats (Simons & Briggs 1985) and 6·3 min value for dogs (Simons & Briggs 1983). The $t_{\frac{1}{2}}$ of the elimination phase for rabbits (38 ± 11 min) was also in agreement with the $t_{\frac{1}{2}}$ for dogs (48 ± 18 min (Simons & Briggs 1983)) and rats (65 ± 21 (Simons & Briggs 1985)).

HI-6 pharmacokinetics for an i.m. dose was described using a one-compartment open model with first-order absorption and elimination:

$$C(t) = \frac{FD}{Vd}\,\frac{k_{01}}{k_{01}-k_{10}}\,(e^{-k_{10}t} - e^{-k_{01}t})$$

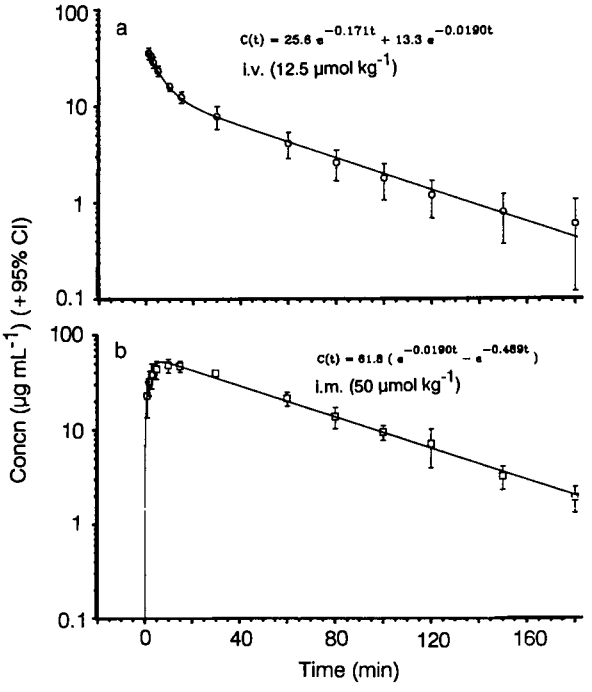


FIG. 1. The concentration-time profiles of HI-6 administered to rabbits. The best fit line was determined for an (a) intravenous and (b) intramuscular dose by averaging pharmacokinetic estimates individually obtained for each animal within a group (n = 8). Mean concentrations with respect to time were not used to estimate pharmacokinetic parameters but are displayed here with 95% confidence limits to represent raw data.

Table 1. Pharmacokinetic estimates of HI-6 administered to rabbits.

	HI-6 dose (μg kg^{-1})	
Parameter[a]	50 (i.m.)	12·5 (i.v.)
Vd(L kg^{-1})	0·32 ± 0·06	0·12 ± 0·01
AUC (ng min mL^{-1})	3147 ± 592	885 ± 186
Clearance (mL min^{-1} kg^{-1})	5·4 ± 1·1	5·5 ± 1·1
$t_{\frac{1}{2}}$ absorption (min)	2·3 ± 1·9	b
$t_{\frac{1}{2}}$ distribution (min)	c	5·0 ± 2·6
$t_{\frac{1}{2}}$ elimination (min)	35·7 ± 3·3	37·9 ± 11·0
T_{max} (min)	9·0 ± 5·0	b
C_{max} (ng mL^{-1})	51·3 ± 9·4	38·9 ± 4·2
Bioavailability (%)	89 ± 20	100

[a] Pharmacokinetic parameters were individually determined for each animal grouped within a given route of administration (n = 8). Average estimates with 95% confidence limits are reported. [b]Intravenous absorption is assumed to be instantaneous and therefore maximum concentration is reached immediately. [c]The distribution phase could not be detected from the intramuscular dose.

where C = plasma concentration (ng mL^{-1}), t = time (min), F = fraction of drug absorbed, D = dose (ng kg^{-1}), Vd = apparent volume of distribution (mL kg^{-1}), k_{01} = rate constant of absorption (min^{-1}) and k_{10} = rate constant of elimination (min^{-1}). The same model was used to describe the concentration time profile of HI-6 intramuscularly given to man (Kusic et al 1985), dog (Simons & Briggs 1983) and rat (Simons & Briggs 1985). The absorption $t_{\frac{1}{2}}$ of 2 min in rabbits was similar to that in rats (4·6 ± 2·2 min (Simons & Briggs 1985)) but shorter than in man (7–8 min (Kusic et al 1985)) and dogs (8·0 ± 3·1 min (Simons & Briggs 1983)). Bioavailability from an i.m. administration was essentially 100% ($P < 0·05$). A summary of the average pharmacokinetic estimates for i.m. and i.v. administration is listed in Table 1.

The results for the present study in rabbits indicate that HI-6 is very rapidly and completely absorbed after an i.m. administration. Maximum plasma concentrations are reached within 9 min. HI-6 distribution is nearly as quick, and therefore only detectable for the i.v. route of administration. The apparent volume of distribution is fairly small (0·1 L kg^{-1}) indicating little if any tissue binding. Systemic clearance (5·5 mL min^{-1} kg^{-1}) and elimination half-life (38 min) are also quick, suggesting a need for multiple dosing if a sustained plasma level is required.

This study indicates that HI-6 should be rapidly available from an i.m. administration to reactivate organophosphate-inhibited acetylcholinesterase in the rabbit. However, if reactivation of acetylcholinesterase is still required several hours after organophosphate poisoning, multiple dosing or a sustained release of HI-6 may be necessary.

References

Boskovic, B. (1981) Treatment of soman poisoning and its perspectives. Fund. Appl. Toxicol. 1: 203–213

Boskovic, B. (1985) Therapeutic efficacy of aprophen, atropine, and HI-6 in mice poisoned by highly toxic organophosphorus compounds. Naucno-Tehnicki Pregled 35: 19–24

DeJong, L. P., Wolring, G. Z. (1980) Reactivation of acetylcholinesterase inhibited by 1,2,2′-erimethylpropyl methylphosphonofluididate (soman) with HI-6 and related oximes. Biochem. Pharmacol. 29: 2379–2387

Groff, W. A., Ellin, R. I. (1969) A new and rapid method for the determination of pyridinium aldoximes in blood and urine. Clin. Chem. 15: 72–83

Hagedorn, I., Grundel, W. H., Hoose, J., Jenter, C. (1976) Darstellung und Quaternierung von Pyridinaldoxim-alkylathern. Arznei. Forsch. 26: 1273–1275

Hagedorn, I., Stark, E., Schoene, K., Schenkel, H. (1978) Reaktivierung phosphorylierter Acetylcholin-Esterase. Ibid. 28: 2055–2057

Harris, L. W., Fleisher, J. H., Innerebner, T. A., Cliff, W. J., Sim, V. M. (1969) The effects of atropine-oxime therapy on cholinesterase activity and the survival of animals poisoned with *O*, *O*-diethyl-*O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate. Toxicol. Appl. Pharmacol. 15: 216–224

Kusic, R., Boskovic, B., Voivodic, V., Joanovic, D. (1985) HI-6 in man: blood levels, urinary excretion, and tolerance after intramuscular administration of the oxime to healthy volunteers. Fund. Appl. Toxicol. 5: S89–S97

Loomis, T. A., Salafsky, B. (1963) Antidotal action of pyridinium oximes in anticholinesterase poisoning; comparative effects on soman, sarin, and neostigmine on neuromuscular function. Toxicol. Appl. Pharmacol. 5: 685–701

Maksimovic, M. (1979) Oximes HI-6 and 2-PAM-Cl: comparative pharmacokinetic studies after intramuscular and oral administration to the rat. Arh. Hig. Rada Toksikol. 30: 227–239

Simons, K. J., Briggs, C. J. (1985) Disposition of HI-6 oxime in rats after intravenous and intramuscular administration. J. Pharm. Pharmacol. 37: 367–369

Simons, K. J., Briggs, C. J. (1983) The pharmacokinetics of HI-6 in beagle dogs. Biopharm. Drug Dispos. 4: 375–388