Defense Technical Information Center
Compilation Part Notice

ADP013410

TITLE: Antidotal Efficacy and Pharmacokinetics of HI-6 and Trimedoxime in Mice Poisoned with Soman or Paraoxon

DISTRIBUTION: Approved for public release, distribution unlimited

This paper is part of the following report:

TITLE: Chemical and Biological Medical Treatment Symposium - Industry II World Congress on Chemical and Biological Terrorism

To order the complete compilation report, use: ADA411272

The component part is provided here to allow users access to individually authored sections of proceedings, annals, symposia, etc. However, the component should be considered within the context of the overall compilation report and not as a stand-alone technical report.

The following component part numbers comprise the compilation report:
ADP013371 thru ADP013468

40. ANTIDOTAL EFFICACY AND PHARMACOKINETICS OF HI-6 AND TRIMEDOXIME IN MICE POISONED WITH SOMAN OR PARAOXON

[1]Biljana Antonijevic, [1]Matej Maksimovic, [2]Milos P. Stojiljkovic, [2]Vesna Kilibarda, [2]Zoran A. Milovanovic, [1]Mirjana Djukic
[1]Institute of Toxicological Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade,
[2]National Poison Control Centre, Military Medical Academy, Crnotravska 17, 11000 Belgrade; FR Yugoslavia

ABSTRACT

The aim of this work was to examine and correlate the pharmacokinetic, reactivating and protective properties of HI-6 and trimedoxime in mice poisoned with soman or paraoxon.

Male albino mice were poisoned with 1.3 LD-50 iv of soman or paraoxon, at different time intervals after iv administration of the antidotes. Median effective doses and efficacy half times were calculated. In the biochemical set of experiments, brain, diaphragmal and erythrocyte acetylcholinesterase activities were determined. To obtain values of pharmacokinetic parameters, oximes were administered intravenously and analysed in plasma samples by HPLC method. Oxime concentrations versus time curves were estimated using a two-compartment open model.

When HI-6 was applied in soman poisoned animals, acetylcholinesterase activity recovered to 69.30 % and 34.67 % of the control diaphragmal and erythrocyte acetylcholinesterase activity, respectively. In paraoxon treated animals, use of trimedoxime produced significant increase of acetylcholinesterase activity in all examined tissues.

Plasma concentrations of oximes reached maximum values immediately after injection, and then decreased rapidly due to the transport of oximes to the peripheral compartment and elimination. From the calculated pharmacokinetic parameters, it appears that trimedoxime penetrates the tissues better, is slowly transferred back to the circulation and remains longer in the body.

In spite of pharmacokinetic data obtained for trimedoxime, it was practically ineffective against soman poisoning, indicating that antidotal efficacy depends much more on the reactivation potency of the oximes than on their pharmacokinetics. However, better insight into the pharmacokinetic profile of oximes is necessary in order to optimize the antidotal therapy.

INTRODUCTION

Pyridinium oximes are one of the cornerstones of the treatment of poisonings with organophosphate agents. There is no "universal oxime" that could reactivate acetylcholinesterase inhibited by any anticholinesterase. At present, the most efficient oxime against intoxications with nerve agents soman, sarin and VX is HI-6, while trimedoxime and obidoxime are the most efficient ones against tabun poisoning (1, 2, 3, 4, 5, 6). In addition, the mentioned bispyridinium dioximes exert significant reactivating potencies against various organophosphate insecticides (7, 8).

Antidotal efficacy of an oxime depends on its chemical structure, reactivating moiety, concentrations in target tissues and the duration of its maintenance, as well as on the inhibitory potential and toxicokinetic properties of an organophosphate (9, 10, 11, 12).

Therefore, the aim of this work was to investigate and correlate the pharmacokinetic, reactivating and protective properties of HI-6 and trimedoxime in mice poisoned with soman or paraoxon.

METHODS

Chemicals. Soman (98.5 %), paraoxon (99.0 %) and oximes (99.0 %) – pralidoxime (PAM-2), trimedoxime (TMB-4), obidoxime (LuH-6) and HI-6 were obtained from the Military Medical Academy, Belgrade. All the other chemicals of analytical or HPLC grade were purchased from the commercial sources.

Stock solutions of organophosphates were prepared in isopropanol. Oximes were dissolved in distilled water and diluted to the required concentration immediately before use.

Animal experiments. Male albino mice (18-24 g) were obtained from the Military Medical Academy, Belgrade, Yugoslavia. The mice were acclimatised for at least one week prior to use and received food and tap water *ad libitum*. All tested substances were administered intravenously via the tail vein at a volume of 0.1 ml/20 g of body mass.

Experimental animals were poisoned with 1.3 LD-50 *iv* of soman or paraoxon at different time intervals (1-60 min) after intravenous administration of the antidotes. Median effective doses were calculated according to the method of Litchfield and Wilcoxon (13), with 95 % confidence limits. These data were used to calculate ED-50 at null time (ED-50$_0$) and efficacy half time (t$_{1/2}$ eff.).

In the biochemical set of experiments, brain, diaphragmal and erythrocyte acetylcholinesterase activities were determined. Oxime HI-6 (17.36 mg/kg, 48.33 ꓳmol/kg) and trimedoxime (24.43 mg/kg, 68.38 ꓳmol/kg) were injected 5 min before 1.3 LD-50 of soman and paraoxon, respectively. Mice were decapitated and exsanguinated at different time intervals (10, 40 and 60 min) after antidote administration. Diaphragms and brains were removed and homogenised in isotonic saline. The brain and diaphragm enzyme activities were measured by the spectrophotometric method (14, 15) while the erythrocyte acetylcholinesterase activity was determined titrimetrically (16) by using acetylthiocholine iodide as substrate.

To obtain intravenous pharmacokinetics, mice (n = 4-6) were sacrificed at various times (2, 5, 10, 15, 20, 30, 40, 50 and 60 min) after administration of HI-6 (132.54 ꓳmol/kg) or trimedoxime (55.98 ꓳmol/kg). Whole blood of each animal (0.6-1 ml) was collected into the heparinised tubes. Oximes were analysed by ion-pair HPLC method in separated plasma samples (17, 18).

Data analysis. Oxime concentrations versus time curves were estimated by using a two-compartment open model. Statistical significance was determined by means of Student's t-test and Mann-Whitney U-test, and the differences were considered significant when $p < 0.05$.

RESULTS

In mice poisoned with soman, HI-6 afforded the best protection (Table 1). In paraoxon poisoning, calculated ED-50 values at null time increased by the following order: obidoxime < trimedoxime < HI-6 < pralidoxime. Efficacy half times in mice poisoned with paraoxon for pralidoxime, trimedoxime, obidoxime and HI-6 were 6.61, 11.96, 13.36 and 19.17 min, respectively. According to the calculated antidotal potency, the most efficient oximes were trimedoxime and obidoxime.

Following administration of organophosphates alone, some tissue acetylcholinesterase activities (about 4 % in brain and about 20 % in diaphragm) remained functional, while erythrocyte acetylcholinesterase activity was not detectable (Figures 1 and 2).

Administration of HI-6 significantly increased diaphragmal and erythrocyte acetylcholinesterase activities in soman-poisoned mice up to 69.30 % and 34.67 % of the control activities, respectively. Brain acetylcholinesterase activity remained the same or even lower, compared to that in animals treated with soman alone (Figure 1).

Trimedoxime, injected into mice before intoxication with paraoxon, induced a significant reactivation of acetylcholinesterase at all the times and in all the tissues tested, with maximum enzyme activities of 62.76 %, 80.91 % and 56.67 % attained in brain, diaphragm and erythrocytes, respectively (Figure 2).

After fast intravenous application, the concentration of oximes in plasma reached maximum values immediately after injection, and then decreased due to transport of oximes to the peripheral compartment and elimination.

During the distribution phase, the oxime concentrations in plasma decreased rapidly, while the corresponding half times of HI-6 and trimedoxime were similar and amounted about 9 min (Table 2). After the phase of distribution, the oximes were eliminated more slowly. Both oximes penetrated from the central compartment to the tissues at approximately the same rate. However, trimedoxime was transferred back to the circulation about two times more slowly. The rate constants of the oxime elimination from the central compartment (k_{13}) showed that this process proceeded practically at the same level for both oximes. The total clearance (Cl_{tot}) of HI-6 was about 25 % higher than that of trimedoxime. The central compartment distribution volume of HI-6 was about 35 % higher, indicating that the dose of trimedoxime should be increased in order to achieve concentration similar to that of HI-6. In the peripheral compartment, however, the ratio of distribution volumes of the tested oximes was completely the opposite. Consequently, the dose of HI-6 should be increased by about 35 % to reach the concentration range of trimedoxime.

DISCUSSION

There are numerous articles on the antidotal effects of HI-6 against soman poisoning published so far (1, 3, 5, 10, 19, 20). Based on our results, the protective effect of HI-6 in mice seems to be determined by the extent of acetylcholinesterase reactivation in respiratory muscles. These results are in agreement with those published earlier (11, 19, 21, 22, 23, 24, 25).

Lack of or minimal reactivation of brain acetylcholinesterase by HI-6 in experimental animals intoxicated with soman has been explained by the insufficient access of the oxime through the blood-brain barrier (11, 26). In our experiments, brain acetylcholinesterase activity determined 60 min after HI-6 application, was significantly lower than in animals treated with soman alone (Fig. 1). After an initial enhancement, a decrease of the brain and diaphragmal acetylcholinesterase activity was obtained. This phenomenon could be ascribed to the leakage of soman from its tissue depots (9, 27, 28).

Obidoxime and trimedoxime were superior to HI-6 and pralidoxime in antagonising the toxic effects of paraoxon in mice (Table 1). Trimedoxime caused a significant increase in the activity of both the central and peripheral acetylcholinesterase (Fig. 2). It is well known that trimedoxime and obidoxime have great reactivating potentials, which explains their antidotal efficacy against a number of organophosphate insecticides (7, 8). Worek and co-workers (8, 29) showed that obidoxime was better reactivator of human erythrocyte acetylcholinesterase inhibited with paraoxon than the equimolar concentrations of pralidoxime, HI-6 and HLo-7. In addition, ten times smaller concentration of obidoxime (10 μmol/l) was needed for restoring the contractility of the mouse isolated phrenic nerve-diaphragm in vitro preparation treated with paraoxon, in comparison with pralidoxime (100 μmol/l) (29).

For many years it has been accepted that the minimal plasma concentration of an oxime needed for effective reactivation of the inhibited cholinesterase is 4 mg/l (30). However, it was shown that this statement had been uncritically accepted, regardless of the oxime and inhibitor used (8, 29, 31, 32). Although all the pyridinium oximes are rapidly

241

eliminated from the circulation, they differ in some pharmacokinetic properties (33, 34, 35). According to the results obtained (Table 2), calculated distribution volumes (V_1, V_2 and V_d), rate constants of transfer from the peripheral to the central compartment (k_{21}) and total clearances (Cl_{tot}) suggest that trimedoxime penetrates better into the tissues, is slowly transferred back into the circulation and has longer persistence in the organism than HI-6. Nevertheless, trimedoxime was practically ineffective against soman poisoning, indicating that the antidotal efficacy of an oxime depends much more on the inhibitor-specific reactivation potency than on its pharmacokinetics. However, pharmacokinetic properties of pyridinium oximes are important for proper individualisation of the antidotal therapy.

REFERENCES

1. Bokonjic, D. et al. (1993) Arch. Toxicol. Kinet. Xenobiot. Metab. 1, 223-232.
2. Clement. J.G. et al. (1987) Arch. Toxicol. 61, 70-75.
3. Dawson, R.M. (1994) J. Appl. Toxicol. 14, 317-331.
4. Hamilton, M. and Lundy, P.M. (1989) Arch. Toxicol. 63. 144-149.
5. Rousseaux, C.G. and Dua, A.K. (1989) Can. J. Physiol. Pharmacol. 67, 1183-1189.
6. Worek, F. et al. (1998) Arch. Toxicol. 72, 237-243.
7. Jokanovic. M. and Maksimovic, M. (1995) Arch. Toxicol. 70, 119-123.
8. Worek, F. et al. (1996) Arch. Toxicol. 70, 497-503.
9. Benschop, H.P. et al. (1987) Toxicol. Appl. Pharmacol. 90. 490-500.
10. Boskovic, B. (1981) Fund. Appl. Toxicol. 1, 203-213.
11. Cassel. G. et al. (1997) Eur. J. Pharmacol. 332. 43-52.
12. Maksimovic, M. et al. (1980) Acta. Pharm. Jugosl. 30. 151-160.
13. Litchfield, J.T. and Wilcoxon. F. (1949) J. Pharmacol. Exp. Ther. 96, 99-113.
14. Ellman, G.L. et al. (1961) Biochem. Pharmacol. 7. 88-95.
15. Wilhelm, K. (1968) Arh. Hig. Rada. Toksikol. 19. 199-207.
16. Augustinsson. K.B. (1971) In: Glick D, editor. Analysis of biogenic amines and their related enzymes. London: John Wiley & Sons. pp. 217-273.
17. Benschop, H.P. et al. (1981) J. Chromatogr. 225. 107-114.
18. Clement, J.G. et al. (1990) Biopharm. Drug. Dispos. 11, 227-232.
19. Clement. J.G. (1981) Fund. Appl. Toxicol. 1. 193-202.
20. Wolthuis. O.L. et al. (1994) Neurosci. Biobehav. Rev. 18, 469-486.
21. Clement, J.G. (1982) Biochem. Pharmacol. 31. 1283-1287.
22. Kassa. J. (1995) Toxicology. 101, 167-174.
23. Kassa. J. and Cabal. J. (1999) Toxicology. 132. 111-118.
24. Lundy. P.M. and Shih, T.M. (1983) J. Neurochem. 40, 1321-1328.
25. Shih, T.M. (1993) Arch. Toxicol. 67, 637-646.
26. Ligtenstein, D.A. and Kossen, S.P. (1983) Toxicol. Appl. Pharmacol. 71, 177-183.
27. Benschop, H.P. and De Jong. L.P.A. (1991) Neurosci. Biobehav. Rev. 15, 73-77.
28. Goransson-Nyberg. A. et al. (1998) Arch. Toxicol. 72. 459-467.
29. Worek, F. et al. (1997) Hum. Exp. Toxicol. 16. 466-472.
30. Sundwall, A. (1961) Biochem. Pharmacol. 8, 413-417.
31. Thiermann. H. et al. (1997) Hum. Exp. Toxicol. 16, 473-480.
32. Willems. J.L. et al. (1993) Arch. Toxicol. 67, 79-84.
33. Jovanovic. D. (1989) Arch. Toxicol. 63, 416-418.
34. Jovanovic, D. et al. (1985) Iugoslav. Physiol. Pharmacol. Acta. 21, 149-150.
35. Schoene, K. et al. (1988) Arch. Toxicol. 61. 387-391.

KEY WORDS

Organophosphates, HI-6, trimedoxime, efficacy, pharmacokinetics

FIGURES AND TABLES

Table 1: Efficacy data on pyridinium oximes in mice poisoned with 1.3 LD-50 *iv* of soman or paraoxon

	PAM-2	TMB-4	LuH-6	HI-6
ED-50$_0$ (Lmol/kg)				
Soman	329,57	4,65	25,78	7,96
Paraoxon	47,62	6,27	3,78	12,83
$t_{1/2}$ eff. (min)				
Soman	5,88	1,31	10,09	16,24
Paraoxon	6,61	11,96	13,36	19,17

Figure 1: Influence of HI-6 on brain, diaphragmal and erythrocyte acetylcholinesterase activity in mice poisoned with soman

Figure 1.

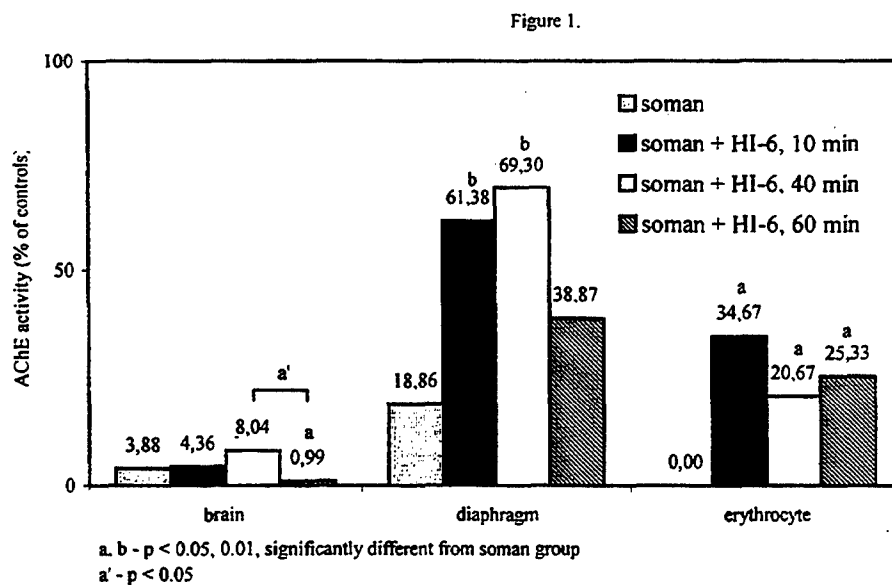a, b - p < 0.05, 0.01, significantly different from soman group
a' - p < 0.05

Figure 2: Influence of trimedoxime on brain, diaphragmal and erythrocyte acetylcholinesterase activity in mice poisoned with paraoxon
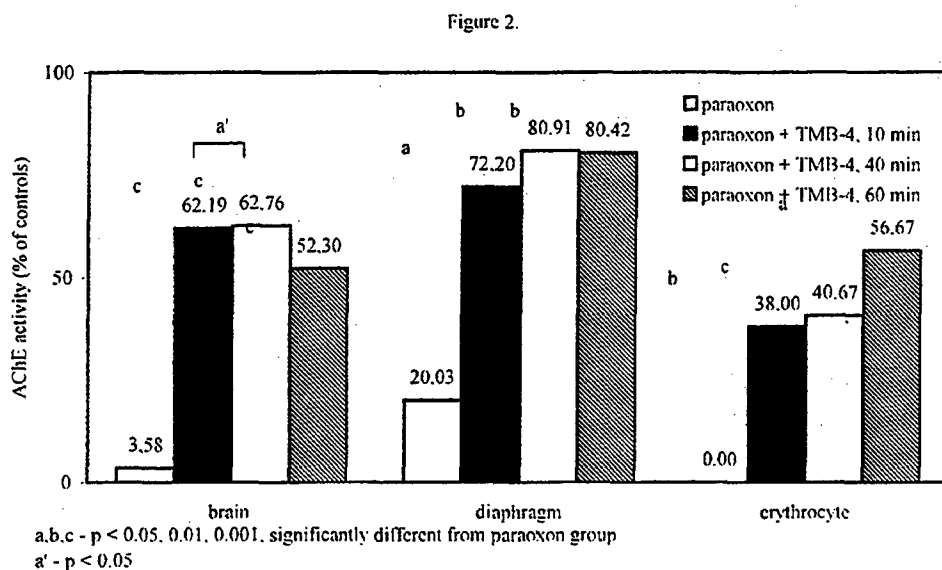
Figure 2.

a,b,c - $p < 0.05$, 0.01, 0.001, significantly different from paraoxon group
a' - $p < 0.05$

Table 2: Pharmacokinetic parameters of HI-6 and trimedoxime administered intravenously to mice

Parameter[a]	HI-6	TMB-4
Dose, mol/kg*	139,19	55,98
A, mol/l*	428,88	240,73
a, min^{-1}	0,0788	0,0773
$t_{1/2}$, min	8,79	8.96
B, mol/l*	34,56	8,71
b, min^{-1}*	0,0116	0,0064
$t_{1/2}$, min*	59,73	108,08
k_{12}, min^{-1}	0,0188	0,0191
$t_{1/2 k12}$, min	36,9	36,3
k_{21}, min^{-1}*	0,0166	0,0089
$t_{1/2 k21}$, min*	41,7	77,9
k_{13}, min^{-1}	0,0550	0,0557
$t_{1/2 k13}$, min	12,6	12,4
AUC, mol/l min*	8421,9	4475,2
Cl_{tot}, l/min kg*	0,0165	0,0125
V_d, l/kg*	1,42	1,95
V_1, l/kg*	0,30	0,22
V_2, l/kg*	0,34	0,47

*$p < 0.001$a

244

Pharmacokinetic abbreviation used:

A - intercept of the distribution phase;

B - intercept of the elimination phase;

α - distribution rate constant;

b - elimination rate constant;

t1/2a - distribution half time; t1/2b - elimination half time;

k12 - rate constant of transfer from central to peripheral compartment;

k21 - rate constant of transfer from peripheral to central compartment;

t1/2k12 - half time of transfer from central to peripheral compartment;

t1/2k21 - half time of transfer from peripheral to central compartment;

k13 - rate constant of elimination from central compartment;

t1/2k13 - half time of elimination from central compartment;

AUC - area under the curve (0 to infinity);

Cltot - total body clearance;

Vd - apparent volume of distribution;

V1 - volume of the central compartment;

V2 - volume of the peripheral compartment.