

94. EFFICACY OF OXIMES AND ADAMANTANES AGAINST SOMAN POISONING IN MICE

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INTRODUCTION

The "classical" nerve agents, such as tabun, sarin, soman, and VX are extremely toxic cholinesterase inhibiting organophosphates. The majority of currently available oximes are quite effective in reactivating cholinesterase inhibited by sarin, VX, and depending on the oxime, tabun. Of many oximes, HI-6 is most effective in restoring soman-inhibited acetylcholinesterase activity. However, despite this, the treatment of soman has not been completely solved (1,2). In contrast, besides the fact that HI-6 is clearly the oxime that is the most effective in restoring soman-inhibited acetylcholinesterase activity, the treatment of soman intoxication still represents a problem that has not been completely solved (1,2). Soman-inhibited acetylcholinesterase is very difficult to reactivate following poisonings with multiple lethal doses of soman. In addition, the soman-acetylcholinesterase complex "ages" very rapidly making it resistant to reactivation and does not undergo spontaneous reactivation. Also, convulsive activity in soman intoxication creates a problem that has been linked to irreversible brain damage. Some experiments have shown that memantine (Mem), 1-amino-3, 5-dimethyl adamantane, prophylaxis potentiates the therapeutic activity of standard antidotes used in organophosphate intoxications (3-7). Therefore, the possible antidotal interactions of anticonvulsive drug, memantine as well as its active and less toxic metabolite, 1-amino-3-hydroxymethyl-5-methyl adamantane (Mrz 2/373) and oximes were tested in mice poisoned with soman.

MATERIALS AND METHODS

Chemicals. Soman and oximes - pralidoxime (PAM-2), trimedoxime (TMB-4), obidoxime (LüH-6) and HI-6 - were obtained from the Military Medical Academy, Belgrade, Yugoslavia. Soman was 98.5% pure and oximes, analyzed using HPLC technique were greater than 99% pure. Memantine and Mrz 2/373 were kindly supplied as a gift by Dr. Guenter Quack, from Merz+Co, GmbH & Co., Frankfurt/M, FR Germany. All the other chemicals of analytical grade were obtained from Merck AG (Darmstadt, FR Germany). Soman stock solution was prepared in isopropanol. Oximes were dissolved in distilled water and diluted to the required concentration immediately prior to use. (Was the soman purity determined from HPLC?)

Animal experiments. Male albino mice (18-24 g) were obtained from the Military Medical Academy, Belgrade, Yugoslavia. The mice were acclimatized 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 body mass. Experimental animals were pretreated *iv* with oximes, memantine, Mrz 2/373 as well as with combinations of oxime and memantine or Mrz 2/373, at different time intervals (1-60 min) before 1.3 LD-50 (63.70 µg/kg; 0.35 µmol/kg) soman *iv*. The dose of memantine and Mrz 2/373 was fixed at 10 mg/kg. Median effective doses were calculated according to the method of Litchfield and Wilcoxon (8), with 95 % confidence limits. These data were used to calculate ED-50 at null time (ED-50₀) and efficacy half time ($t_{1/2}$ eff.), i.e., half time of the ED-50 decrease.

In a separate experiment, the brain, diaphragmal and erythrocyte acetylcholinesterase (AChE) and plasma carboxylesterase (CarbE) activities were determined. The dose of HI-6 equivalent to the dose of ED-50₅, i.e. the dose of HI-6, which protects 50% of the poisoned animals when administered 5 min before 1.3 LD-50 of soman (3.1 mg/kg; 7.96 µmol/kg) and/or adamantanes (10 mg/kg) were administered 5 min before the animals received soman 0.75 LD-50 (36.75 µg/kg; 0.20 µmol/kg). Mice were decapitated and exsanguinated 5 min following soman poisoning. Diaphragm and brain were removed and homogenised in isotonic saline. The enzyme activity in brain and diaphragm was measured by the spectrophotometric method (9) and erythrocyte acetylcholinesterase was determined titrimetrically (10) using acetylthiocholine iodide as substrate. Plasma was assayed for CarbE by the method of Boskovic et al. (11) using tributyrin as substrate.

Data analysis. Statistical significance was determined by means of Student's t-test and Mann-Whitney U-test, and differences were considered significant when $p < 0.05$.

RESULTS

Calculated ED-50 values at null time of memantine, pralidoxime, trimedoxime, obidoxime and HI-6 were 39.98, 329.57, 4.65, 25.78 and 7.98 μ mol/kg, respectively (Table 1). These doses are expected to keep 50 % of the animals alive, if oxime and soman are administered simultaneously. Mrz 2/373 did not counteract the toxic effects of soman at any time of pretreatment. Along the tested time intervals, HI-6 afforded the best protection for experimental animals. Efficacy half times increased according to the following order: trimedoxime < pralidoxime < obidoxime < HI-6 < memantine. Co-administration of memantine significantly decreased the ED-50 of pralidoxime (7.67 times), obidoxime (9.34 times) and HI-6 (4.45 times) (Table 2). When trimedoxime was applied with memantine, no changes of its antidotal effect could be seen. However, in mice pretreated with trimedoxime, its efficacy half time was increased by 4.82 fold.

Following administration of soman alone, mice still had 44.53 % of their diaphragmal, 28.07 % of their brain and 4.67 % of erythrocyte acetylcholinesterase functional. When relatively low dose of HI-6 (3.1 mg/kg) was applied, acetylcholinesterase activity increased in all tissues examined (Figure 1). The results obtained in diaphragms showed that administration of either HI-6 or memantine provided significant recovery of diaphragmal acetylcholinesterase activity. In mice pretreated with the combination of HI-6 and memantine, diaphragmal acetylcholinesterase activity was even higher than in mice, which received HI-6 only. Among tested tissues, the highest recovery of inhibited acetylcholinesterase was obtained in the diaphragm. Co-administration of adamantanes (memantine, Mrz 2/373) produced a statistically significant increase in erythrocyte acetylcholinesterase activity. In all the prophylactic regimens used, plasma carboxylesterase activity was higher than after soman alone, but yet not significantly different.

DISCUSSION

The results presented in numerous previous studies demonstrate that HI-6 is the least toxic and most efficacious oxime examined as an antidote of soman poisoning in mice. The lack of or low antidotal action of the other oximes is due to their relatively low reactivating potency combined with relatively high toxicity. Until now, it was undoubtedly shown that HI-6 provided best protection and reactivating effect in soman poisoning (12, 13). A comparison of the various congeners of HI-6 illustrated that an oxime group and an amide group were essential for therapeutic activity. The oxime group in position two of the first pyridinium ring was required for reactivation of soman-inhibited acetylcholinesterase and the amide group in position four of the second pyridinium ring was required to reduce the inherent toxicity of the bispyridinium oxime structure (14, 15).

In our experiment, administration of HI-6, five minutes before soman, provided a significant increase of brain and especially diaphragmal acetylcholinesterase activity. For many years, it has been assumed that if an oxime could reactivate the organophosphate-inhibited acetylcholinesterase in the CNS, a beneficial effect should result. However, various studies have reported results, which do not confirm this hypothesis. Clement (16) found that mice, which died following soman poisoning, had higher brain cholinesterase activity than those which survived soman poisoning by the prophylactic administration of HI-6. This and other similar results question the significance of reactivation of soman-inhibited acetylcholinesterase in the CNS and suggest that inhibition of acetylcholinesterase in the CNS is not the primary lesion (?) in the lethality produced by soman. Having in mind the fact that the greater extent of acetylcholinesterase reactivation was seen in diaphragm than in brain, it could be assumed that reactivation of diaphragmal acetylcholinesterase was more important in the beneficial therapeutic action than reactivation of central acetylcholinesterase in mice poisoned with soman.

Although co-administration of memantine produced a decrease of ED-50 doses for all the oximes used, with the exception of trimedoxime, special attention was paid to its combination with HI-6, as the most relevant one in the case of soman poisoning. It was already shown that memantine in combination with atropine can prevent or antagonize the acute as well as subacute anticholinesterase poisoning due to carbamate or organophosphate (3-7). Also, it was suggested that memantine could counteract the effects of anticholinesterases by protection of acetylcholinesterase from inhibition, rapid reactivation of inhibited acetylcholinesterase, protection of carboxylesterase, and by speeding-up the bioelimination of the poison. Stojiljkovic (17) found that memantine and its metabolite (Mrz 2/373) along with atropine, HI-6 and diazepam provided better prophylaxis than physostigmine or pyridostigmine in soman-intoxicated rats. Co-administration of HI-6 and Mrz 2/373 produced some recovery of the enzyme activity in all the groups and tissues tested, but since a significant difference from soman group was achieved only in erythrocyte acetylcholinesterase activity, it was concluded that obtained results were not fully in accordance with those previously cited. Some of these discrepancies could be explained by differences in experimental design. The other explanation for variance of Mrz 2/373 efficiency observed in mice and rats could be ascribed to interspecies differences in importance of carboxylesterases for detoxification of soman (18). The major prophylactic influence of memantine and its combination with HI-6 was observed in acetylcholinesterase activity in

peripheral tissues. In accordance with this finding, it was recently published that in the treatment of acute carbofuran poisoning, memantine provided complete protection and reversal of the induced biochemical changes in diaphragm muscle by preventing depletion of high-energy phosphates and maintaining normal cell membrane characteristics, including permeability and integrity (19).

Soman exposure can lead to irreversible lesions of the central nervous system. Numerous studies have demonstrated that the excitatory amino acid glutamate plays a prominent role in the maintenance of soman induced seizures and in the subsequent neuropathology via an overstimulation of glutamatergic N-methyl-D-aspartate (NMDA) receptors. Memantine is known as a substance with NMDA antagonistic properties (20-22). Therefore, memantine NMDA receptor blocking anticonvulsant properties should be also included in its prophylactic potency against soman.

Application of memantine, along with the oximes used in this study, significantly lowered the oxime doses necessary to protect experimental animals from soman toxicity. Among the mechanisms explained, our results suggest that its efficacy could be ascribed to the combination of the protection of acetylcholinesterase activity in some vital tissues, such as diaphragm, and direct antagonistic effect of memantine on NMDA receptors in the brain of mice poisoned with soman.

SUMMARY

The aim of this study was to investigate the efficacy of the four pyridinium oximes - pralidoxime (PAM-2), trimedoxime (TMB-4), obidoxime (LüH-6) and HI-6 - alone or in the combination with memantine and its principal metabolite 1-amino-3-hydroxymethyl-5-methyl adamantane (Mrz 2/373) against soman in mice.

Male Albino mice were pretreated *iv* with oximes and adamantanes at various times before 1.3 LD-50 of soman *iv* in order to obtain their ED-50_t. In a separate experiment, the brain, diaphragmal and erythrocyte acetylcholinesterase and plasma carboxylesterase activities were determined after sacrificing mice 5 min after soman 0.75 LD-50. Oxime HI-6 (ED-50 value at 5 min before soman) and adamantanes were administered 5 min before soman. In the combination regimens and in the biochemical experiments the dose of memantine and Mrz 2/373 was fixed at 10 mg/kg *iv*.

Along tested time intervals, HI-6 afforded the best protection of experimental animals, and calculated ED-50₀ value of HI-6 was 7.96 μ mol/kg. Memantine significantly (up to 9 times) decreased ED-50₀ values of all the oximes used, with the exception of TMB-4. Among tested tissues, the highest recovery of inhibited acetylcholinesterase was obtained in the diaphragm. Co-administration of adamantanes (memantine, Mrz 2/373) produced a statistically significant increase in erythrocyte acetylcholinesterase activity. It could be concluded that memantine antidotal efficacy could be ascribed to the protection of acetylcholinesterase activity.

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KEYWORDS

Soman, memantine, adamantanes, oximes, HI-6, mouse, acetylcholinesterase, carboxylesterase.

FIGURES AND TABLES

Figure 1. Acetylcholinesterase and carboxylesterase activities in mice following soman poisoning: effect of HI-6 and/or adamantanes

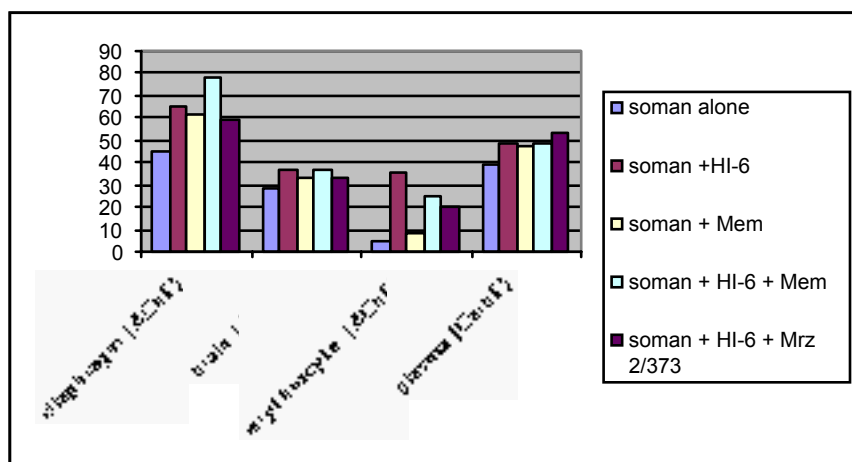


Table 1. Efficacy data: oximes and memantine in mice poisoned with soman

	Mem	PAM-2	TMB-4	LuH-6	HI-6
time (min)	ED-50 _t (mmol/kg)				
1		370.76			
2		417.10	13.35		
5	45.47		64.82	35.63	8.63
12.5	52.05			64.70	
20	64.84			100.28	21.44
40					48.30
60					93.45
	ED-50 ₀ (mmol/kg)				
	39.98	329.57	4.65	25.78	7.96
	t _{1/2} eff.(min)				
	28.88	5.88	1.31	10.09	16.24

Table 2. Efficacy data: combinations of oximes and adamantanes in mice poisoned with soman

	PAM-2 + Mem	TMB-4 + Mem	LuH-6 + Mem	HI-6 + Mem	HI-6 + Mrz 2/373
time (min)	ED-50 _t (mmol/kg)				
1	74.27				
2	129.07				
5		6.16	4.70	2.56	23.47
12.5		28.55			
20		59.62	21.07	26.22	91.36
27.5		74.82			
40			174.88	150.99	138.86
	ED-50 ₀ (mmol/kg)				
	42.95	5.00	2.76	1.79	23.08
	t _{1/2} eff.(min)				
	1.26	6.31	6.71	6.02	14.15