

99. MEMANTINE TREATMENT IMPROVES ANTIDOTAL EFFICACY OF ATROPINE, HI-6 AND DIAZEPAM IN RATS POISONED WITH SOMAN

Milos P. Stojiljkovic¹, Matej Maksimovic¹, Vesna Kilibarda¹, Biljana Antonijevic², Zoran A. Milovanovic¹, Milan Jokanovic¹, Bogdan D. Boskovic¹

¹National Poison Control Centre, Military Medical Academy, Crnotravska 17, YU-11002 Belgrade, FR Yugoslavia

²Department of Toxicological Chemistry, Faculty of Pharmacy, Vojvode Stepe 450, YU-11000 Belgrade, FR Yugoslavia

INTRODUCTION

Memantine (1-amino-3,5-dimethyl adamantane) hydrochloride is an adamantane derivative registered as Akatinol[®] by Merz+Co, GmbH & Co., Frankfurt/M, FR Germany. It has been widely used in many European countries for treatment of various neurological and psychiatric disorders, including Parkinson's and Alzheimer's disease (1, 2). However, since 1987 Dr. Ramesh C. Gupta and his associates from Hopkinsville, Kentucky, USA, have published more than a dozen papers on prophylactic use of memantine alone or with atropine, in rats poisoned with various carbamate insecticides carbofuran (3-7) and aldicarb (8-10), organophosphate insecticide methylparathion (11, 12) and nerve agents tabun, sarin, soman, VX and DFP (13-16). According to these research data, memantine efficiently protects acetylcholinesterase (EC 3.1.1.7; AChE) from inhibition and alleviates tremor, convulsions and skeletal muscle lesions induced by sublethal doses of the cholinesterase inhibitors mentioned (14).

Previous experiments performed in our laboratory confirmed the prophylactic efficacy of memantine against toxicity of various carbamates and organophosphates in rats (17, 18) and mice (19-21). The prophylactic efficacy of memantine in rats poisoned with soman was superior over the potency of classical prophylactic antidotes physostigmine and pyridostigmine (22, 23). In these experiments it was clearly shown that pre-treatment of rats with 18, 36 and 72 mg/kg sc of memantine increases atropine/HI-6 protective index (PI) against soman 2-4 times (24).

However, its cardiovascular and especially its behavioural adverse effects limit the prophylactic use of memantine (25-27). Having in mind that the life-threatening intoxication put the adverse effects of the antidote behind its therapeutic potential, the aim of this investigation was to ascertain the antidotal efficacy of these doses of memantine when administered after soman, i.e. along with standard antidotes.

MATERIALS AND METHODS

Animals. The experiments have been performed with male Wistar rats, weighing 180-250 g, with free access to food and tap water.

Chemicals. Memantine hydrochloride (Akatinol[®]) was kindly supplied by Dr. Guenter Quack of Merz + Co. GmbH, Frankfurt am Main, FR Germany. Oxime HI-6 (1-(2-hydroxyimminomethylpyridinium)-1-(4-carboxyamidopyridinium) dimethylether dichloride monohydrate) and soman (1,2,2-trimethylpropylmethylphosphonofluoridate) were synthesised in the Military Technical Institute, Belgrade, FR Yugoslavia. Atropine and diazepam were purchased from commercial sources. Memantine and soman were administered subcutaneously (sc), while atropine, HI-6 and diazepam were injected intramuscularly (im).

Procedures. For the purpose of the protective experiments, groups of eight rats each were poisoned with increasing doses of soman and immediately thereafter treated with one of three doses of memantine (18, 36 or 72 mg/kg), and/or atropine 10 mg/kg, HI-6 50 mg/kg and diazepam 2.5 mg/kg. These antidotes were administered either as single regimens or in combinations. After the 24-hour survival registration and calculation of the median lethal dose (LD₅₀) (28), protective indices were calculated as ratios of LD₅₀ values in treated and untreated animals, respectively (29).

For the purposes of biochemical experiments, rats were poisoned with maximum sub-lethal dose of soman (0.75 LD₅₀), and immediately thereafter treated with HI-6 50 mg alone, memantine (18, 36 or 72 mg/kg), or with the combination of the oxime and one of these three doses of memantine. One hour after soman administration, animals were sacrificed and AChE activity determined in the homogenates of their brains and diaphragms by Ellman's spectrophotometric method (30). The results were expressed as percentages of the enzyme activity in rats treated with saline only. All the comparisons were made with the AChE activity in rats treated only with soman.

RESULTS

Protective experiments. Among the classical antidotes, only HI-6 significantly protected rats from soman, increasing its LD₅₀ by 55%. Atropine + HI-6 was the only dual combination of the classical antidotes, which yielded a significant increase in PI to 2.14. Addition of diazepam only slightly increased that PI to 2.69. Memantine 36 mg/kg alone also assured a significant PI of 1.36. All three used doses of memantine potentiated PIs of all the antidotal regimens investigated. The greatest increases in PI were registered after atropine + HI-6, from 2.14 to 4.70, and atropine + HI-6 + diazepam treatment, from 2.69 to 5.03 (Figure 1).

Biochemical experiments. Soman per se decreased brain AChE activity to 53%. While treatment of rats with HI-6 did not result in higher brain AChE activity in comparison with the untreated but poisoned animals (51% vs. 53%), all three doses of memantine alone significantly protected brain AChE, preserving thus 69-74% of its control activity. Combination of two greater doses of memantine and HI-6 assured the maintenance of enzyme activities close to the control levels (89-97%) (Figure 2).

The activity of AChE in the diaphragms of the rats poisoned with soman and left untreated was decreased to only 30% of its control values. Administration of HI-6 alone doubled it to 60%. All three doses of memantine, administered as monotherapy, assured similar or even higher enzyme activity (56-70%). Combinations of these three doses of memantine with HI-6 resulted in the dose-dependant increase of the diaphragmal AChE activity to 51%, 88% and 107% of the control (Figure 3).

DISCUSSION

HI-6 is, along with HLö-7, the most effective oxime in the treatment of experimental soman intoxication (31-34). These results confirm our previous finding that the administration of the oxime HI-6 monotherapy can significantly prevent death in soman-poisoned rats (18), although no reactivation of brain AChE could be seen. Therefore, its therapeutic effect should be ascribed to the combination of the reactivation of the diaphragmal AChE and the so-called “direct pharmacological effects” of the oxime (35-38).

Addition of atropine, an antagonist of the muscarinic receptors in brain, heart and bronchi, further increased PI via blockade of the effects of the excess of acetylcholine in these vital organs (39), which frequently results in a clear therapeutic synergism (40). Although it is well known that diazepam acts as an anticonvulsive drug counteracting soman-induced seizures (41, 42), it did not in this experiment significantly further increase the PI of atropine + HI-6 combination. One of the reasons could be the erratic absorption of diazepam emulsion from the skeletal muscle (43); the reason why attempts have been made in order to replace diazepam with hydrosoluble benzodiazepine midazolam (44, 45).

The results presented here and preliminary reported earlier (46) clearly show that memantine protects rats from death caused by soman intoxication and potentiates the antidotal effects of atropine, HI-6 and diazepam and their combinations. Also, administration of memantine leads to a significant preservation of AChE activities both in the brains and in the diaphragms of the poisoned animals. Moreover, it strongly potentiates reactivating effects of HI-6 in the diaphragm and even makes it significant in the brain, where HI-6 alone exerted no biochemical effect.

These results confirm Dr. Gupta's and our previous findings that memantine prevents or delays AChE inhibition by soman both *in vitro* (16, 18, 47) and *in vivo* (12, 13, 48). Since memantine lacks the oxime group, it is hard to believe that it can reactivate already inhibited enzyme, like HI-6 does. A more plausible hypothesis concerning the exact biochemical mechanism of memantine interaction with AChE could be allosteric modulation of the active centre of the enzyme, which in turn could lead to its hindrance and unavailability to soman. Another proposed biochemical mechanism could be interaction with soman detoxification by plasma carboxylesterases (EC 3.1.1.1) (49).

The biochemical nature of the antidotal activity of memantine in the peripheral tissues, i.e. respiratory muscles was corroborated by a finding that memantine could alleviate soman-induced deficit in the contractility of the rat phrenic nerve-diaphragm preparation in situ, but failed to induce a similar effect on the neuromuscular deficit induced by decamethonium, a direct nicotinic receptor agonist (50). This experiment ruled out the hypothesis that memantine could exert its peripheral antidotal effect via curariform blockade of the neuromuscular nicotinic receptors (14).

Memantine is also well-known as an anticonvulsive agent, the action of which is ascribed to its neuroprotective effect (51). Memantine is, along with ketamine and dizocilpine (MK-801), one of the drugs that block the action of the excitotoxic amino acid glutamate on the N-methyl-D-aspartate (NMDA) receptors in the central nervous system (14, 15, 52, 53). While soman-induced convulsions are undoubtedly initiated via cholinergic mechanism (54), they are supposedly propagated and the neuronal damage is produced by glutamatergic pathway. This pathway involves not only NMDA, but also AMPA receptors (55). Although this study was not aimed to investigate this mechanism

of antidotal activity of memantine, there is a considerable body of evidence that this mechanism could be of equal value as the biochemical one described above.

CONCLUSIONS

It is concluded that memantine treatment represents an efficient adjunct to standard antidotes against soman intoxication in rats, which is at least partly a consequence of protection of acetylcholinesterase activity in vital tissues. Memantine's anticonvulsive action, however, cannot be ruled out as a one of the antidotal mechanisms.

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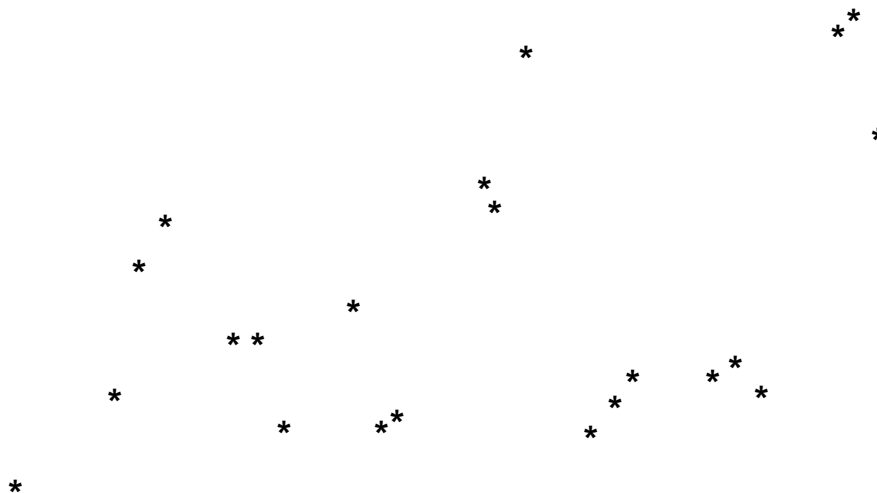
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KEYWORDS

Memantine; soman; atropine; HI-6, diazepam

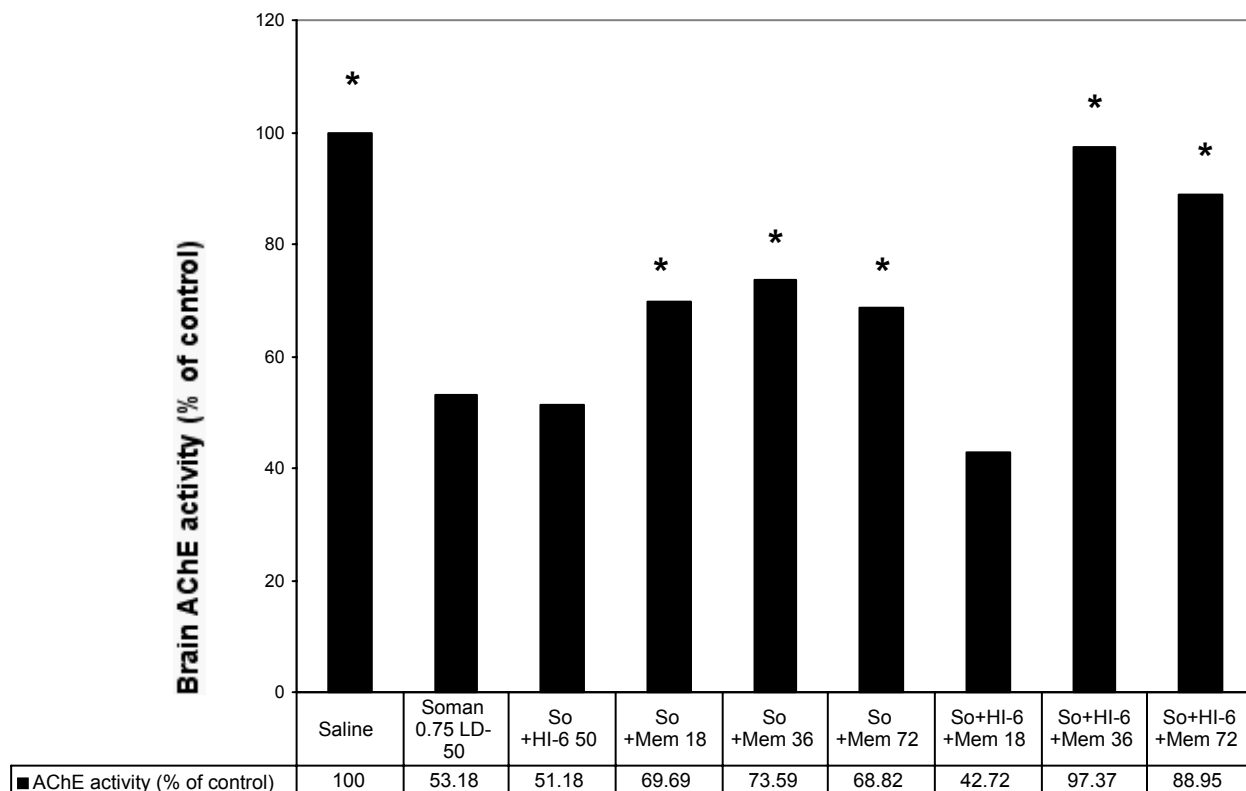
FIGURES AND TABLES

Figure 1 - Protective indices of various antidotal combinations with memantine (18, 36 and 72 mg/kg sc) in rats acutely poisoned with soman sc



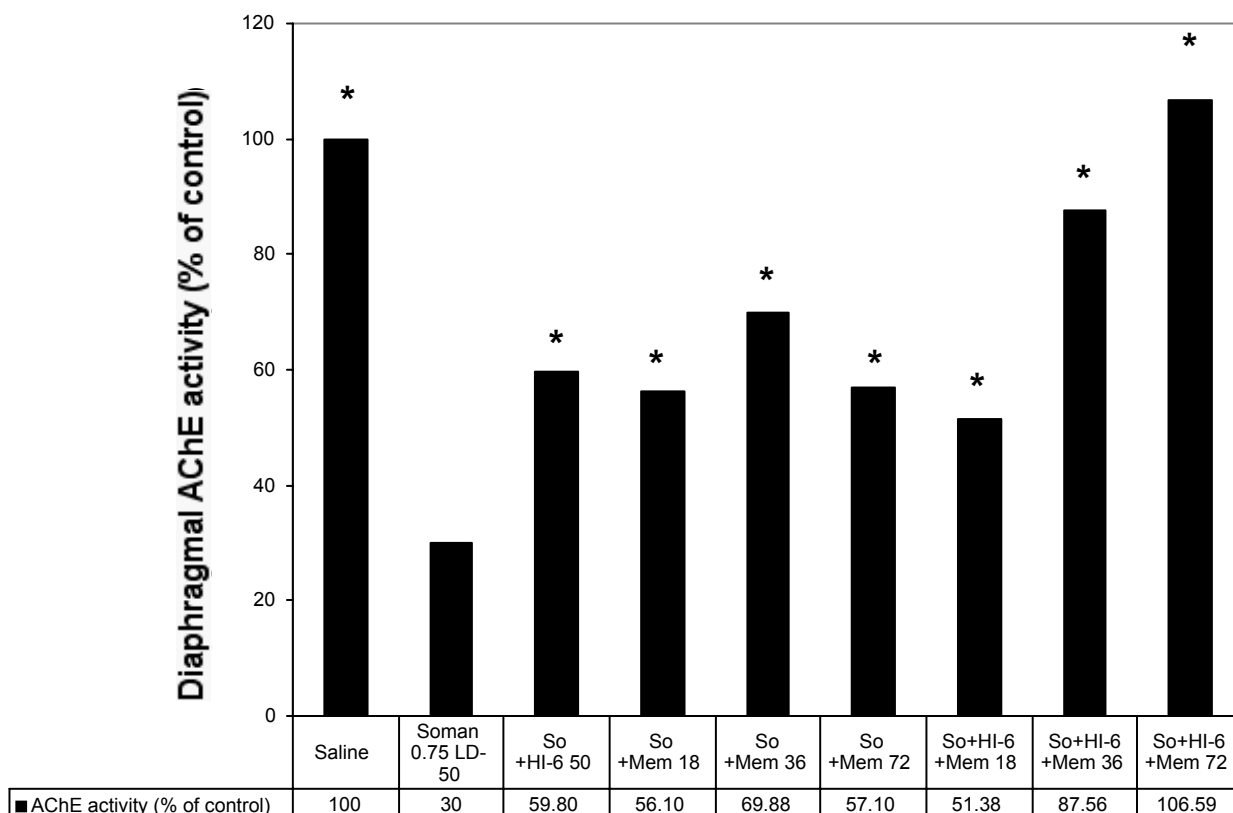
* p< 0.05, in comparison with animals treated without memantine

Figure 2 - Influence of HI-6 (50 mg/kg i. m.) and memantine treatment (18, 36 and 72 mg/kg sc) on brain acetylcholinesterase (AChE) activity in rats acutely poisoned sc with 0.75 LD-50 of soman.



* p < 0.05, in comparison with animals treated with soman only.

Figure 3 - Influence of HI-6 (50 mg/kg im) and memantine treatment (18, 36 and 72 mg/kg sc) on diaphragmal acetylcholinesterase (AChE) activity in rats acutely poisoned sc with 0.75 LD-50 of soman.



* p < 0.05, in comparison with animals treated with soman only.