

EFFECT OF ATROPINE AND THE OXIME HI-6 ON LOW-LEVEL SARIN-INDUCED ALTERATION OF PERFORMANCE OF RATS IN A T-MAZE

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Summary: 1. To study the influence of antidotes on low-level sarin-induced alteration of cognitive functions, male albino Wistar rats were exposed to three various low concentrations of sarin for 60 minutes in the inhalation chamber. One minute following sarin exposure, the rats were i.m. treated with the oxime HI-6 in combination with atropine. Control rats were treated with antidotes as experimental rats but exposed to the pure air instead of sarin. Cognitive functions of the rats were tested using a T-maze where spatial memory and spatial orientation were evaluated. The performance of sarin-exposed and treated rats in the T-maze was tested several times within six weeks (single exposure) or five weeks (repeated exposure) following inhalation exposure to evaluate cognitive impairments. 2. In the case of single exposure to sarin, no statistically significant differences between the performances of the control and the experimental groups in the alteration of spatial memory and spatial orientation were observed. The repeated exposure of treated rats to clinically asymptomatic dose of sarin (LEVEL 2) did not change the effect of low-level sarin exposure on spatial memory of the experimental rats compared to the single exposure to the same dose of sarin. 3. The decrease in the T-maze performance of the control rats was caused by the impairments of rat's mobility due to the features of a solution of antidotes.

Key words: Sarin; Atropine; Oxime HI-6; Spatial memory; Multiple T-maze; Rat

Introduction

Organophosphorus compounds (OP) exhibit their toxicity by irreversible inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) in the peripheral and central nervous system. They include various compounds used as insecticides or extremely toxic AChE inhibitors classified as nerve agents. Nerve agents such as sarin are rapidly acting compounds (21) which reach their maximal cholinesterase inhibition within 5–15 min (16).

The exposure to high doses of OP results in severe brain neuropathology that involves not only neuronal degeneration and necrosis of various brain regions (14,18,19) but also persistent severe alteration of behavior and cognitive functions, especially impairments of learning and memory (4,17,20). One of the most significant injury caused by OP is neuronal degeneration of the hippocampus that is associated with the spatial learning and memory. Therefore, impairments of cognitive functions, especially incapacitation of learning and memory, belongs to the most frequent central signs of acute OP poisoning (15,17).

Our previous study demonstrated the ability of sarin to induce dose-dependent alteration of cognitive functions in

the case of the inhalation exposure of rats to its low concentrations. The adverse effect of low-level sarin inhalation exposure was manifested in the time determining rate of orientation (latency time). Thus, significant, clinically manifested AChE inhibition in the central nervous system leading to the neuronal degeneration of some brain regions including hippocampus, associated with the spatial learning and memory, is not necessary for the clinically manifested cognitive impairments (9).

The aim of this study was to evaluate the influence of the oxime HI-6 in combination with atropine on low-level sarin induced alteration of some cognitive functions using the T-maze as a testing apparatus. Atropine alone is able to antagonize the effect of acetylcholine at muscarinic cholinergic receptor sites without changes of OP - induced inhibition of AChE activity and, thus, diminish neurotoxic effects of OP in the case of sublethal poisoning (4, 10, 15). The oxime HI-6 is not only a relatively efficacious reactivator of OP-inhibited AChE, especially in the peripheral compartment, but it also has secondary antidotal effects that probably arise from its antimuscarinic, ganglion-blocking, postjunctional nondepolarizing action and effects on cardiovascular and respiratory systems (7,22).

Material and methods

Male albino Wistar rats weighing 180–200 g were obtained from VÚFB Konárovice (Czech Republic). They were kept in an air-conditioned room. The animals were maintained on a 23 h food deprivation schedule with food available only in the maze and for 60 min after the daily trial. Tap water was available ad libitum. The rats ($n = 60$) were divided into groups of ten. Handling of the experimental animals was done under supervision of the Ethics Committee of the Medical Faculty of Charles University and the Purkyně Military Medical Academy in Hradec Králové (Czech Republic).

Sarin was obtained from Zemianské Kostolany (Slovak Republic) and was 98.5% pure. The oxime HI-6 of 98% purity was synthesized at the Department of Toxicology of the Purkyně Military Medical Academy in Hradec Králové (Czech Republic). Its purity was analysed using HPLC. All other drugs and chemicals of analytical grade were obtained commercially and used without further purification.

The rats were exposed to various low concentrations of sarin for 60 minutes in the inhalation chamber. Three low concentrations of sarin were chosen:

- clinically and laboratory asymptomatic concentration (0.8 $\mu\text{g/L}$) - LEVEL 1
- clinically asymptomatic concentration with a significant inhibition of erythrocyte AChE by 30% (1.25 $\mu\text{g/L}$) - LEVEL 2. This concentration was used for a single or repeated (three times during one week) exposure
- non-convulsive symptomatic concentration (2.5 $\mu\text{g/L}$) - LEVEL 3

One minute following sarin exposure, the rats were treated i.m. with the oxime HI-6 (13.3 mg/kg) in combination with atropine (25.3 mg/kg) at human relevant doses (2% LD_{50}). The control rats were exposed to the pure air and then, they were treated with antidotes as the experimental rats.

Cognitive functions of the rats were tested using the T-maze where spatial memory and spatial orientation of the animals were evaluated (11,12). The rats were trained to pass the maze consisting of five segments in less than 10 seconds without entering wrong arm of the maze (a length of correct track from a start space to a goal compartment was 185 cm). A reward (several food pellets) was placed in the goal compartment. The animals were trained once a day for 35 days. The exposure started after period of four days in which animals had not made a mistake in any case. The rats were tested 1 hour, 2 hours, 24 hours and 7 days following the sarin inhalation exposure and then, once a week till the end of the fifth week following the exposure. Time that animals needed to pass the maze was recorded and compared to the value obtained from the same rats immediately before sarin exposure and from control group exposed to pure air instead of sarin.

Statistical analysis was performed on a PC with a programme Statistica '98 Edition. Analysis of variance (ANOVA) and Scheffé method of contrasts were used for the deter-

mination of significant differences between control and experimental groups (1). The differences were considered significant when $p < 0.05$.

Results

The results of our study showed that T-maze performance of rats exposed to all three low sarin concentrations (LEVEL 1, LEVEL 2 or LEVEL 3) and treated one minute after exposure with the oxime HI-6 and atropine was impaired in comparison with the values obtained before the exposure. A decrease in the T-maze performance was observed for a short time - usually one hour and two hours after single sarin exposure (Fig. 1). The time reaction of sarin-exposed rats was similar to the value registered before the exposure when it was recorded one week following the exposure or later (Fig. 1). Unfortunately, a significant decrease in the T-maze performance in the control group till 24h following the exposure to the pure air was also demonstrated. Therefore, no significant differences among time reaction values in the control and the experimental rats regardless of sarin concentration or time of the T-maze performance were demonstrated (Fig. 1).

The repeated LEVEL 2 exposure of rats did not change the effect of low-level sarin exposure on spatial memory in comparison with the single exposure to the same dose of sarin (Fig. 2). In this case, no significant differences between the control and the experimental rats were observed again because T-maze performance in the control group was also decreased. Nevertheless, the control rats showed shorter impairment of T-maze performance than the experimental rats (Fig. 2).

Discussion

The adverse effects of OP on cognitive functions, such as learning and memory, may persist for a relatively long time following their exposure. The results from several studies have demonstrated the presence of OP-induced learning impairments several days after the behavioral signs of OP toxicity have subsided (2,3,6,17). It was also demonstrated that sarin is able to induce dose-dependent alteration of cognitive functions in the case of the inhalation exposure of rats to its low concentrations (9).

The present study demonstrates that T-maze performance of sarin-exposed rats was decreased even in the case of the antidotal treatment of rats with atropine and the oxime HI-6. Moreover, the control rats treated with antidotes showed longer reaction time than non-treated control animals (9). Therefore our results did not confirm some previous studies in which the antidotal mixture consisting of atropine and the oxime HI-6 was effective to decrease the neurotoxicity of OP (8,13). In our present study, the antidotal treatment itself caused a decrease in T-maze performance of the control rats, especially immediately following single as well as repeated exposure to the pure air.

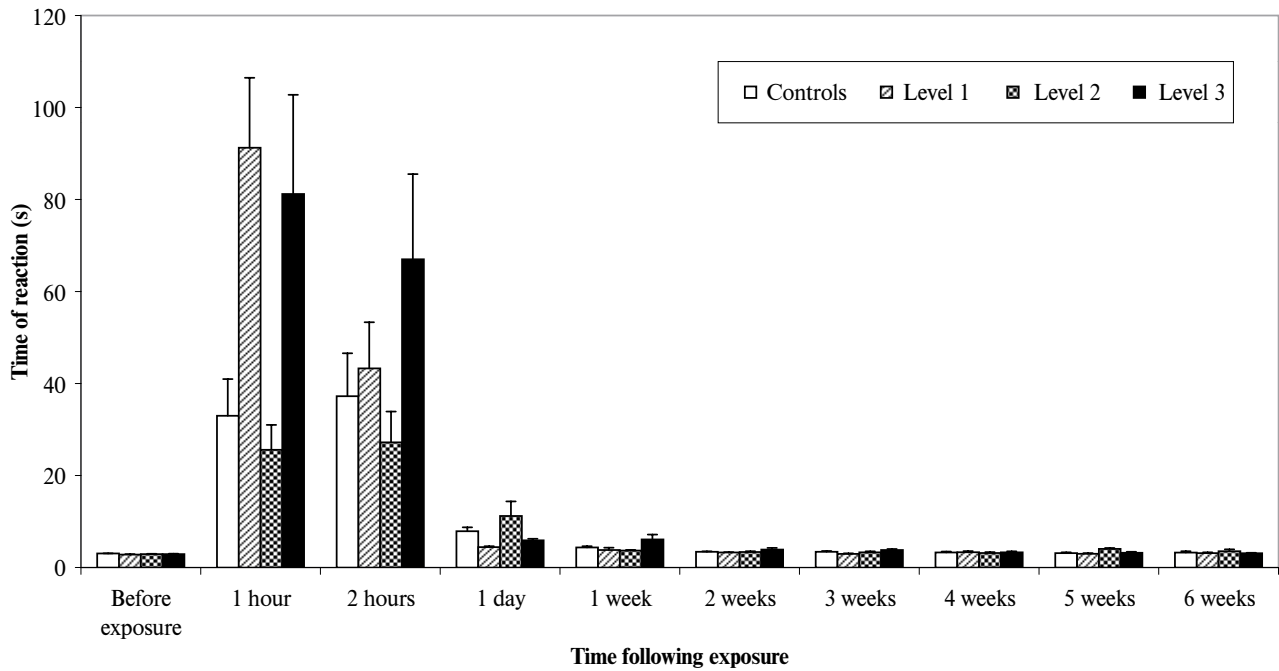


Fig. 1: Protective effect of antidotal treatment on T-maze performance in rats singly exposed to sarin.

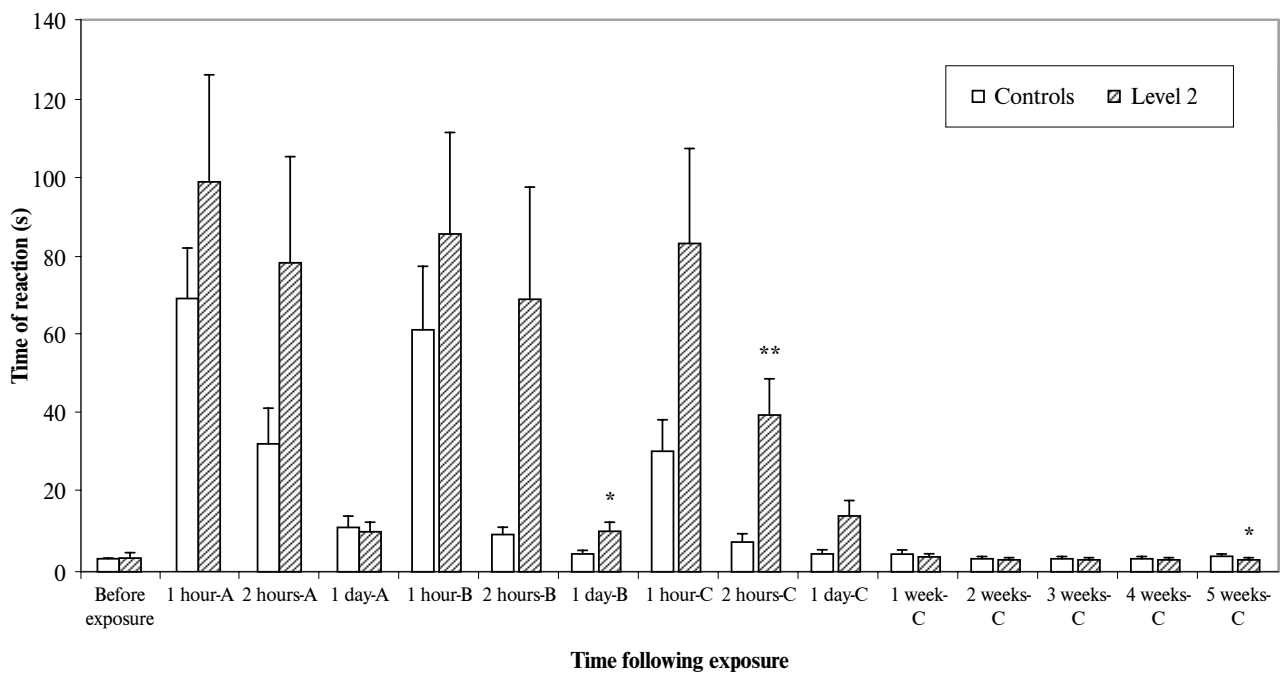


Fig. 2: Protective effect of antidotal treatment on T-maze performance in rats repeatedly exposed to sarin (A - the first exposure, B - the second exposure, C - the third exposure). Statistical significance: * $p < 0.05$, ** $p < 0.001$.

These data can be explained by features of antidotal mixture. The solution of atropine and the oxime HI-6 is slightly hypotonic and acid (its pH is 3-4). These properties obviously influence a mobility power of the rat's limb where antidotes were administered. Our results indicate marked deterioration of the reaction time to pass the maze although the animals did not enter wrong arms. These results do not suggest the impairments of memory but only the harm of rat's mobility. Therefore, our experiments can not relevantly evaluate the ability of atropine and HI-6 to eliminate OP-induced impairments of memory because the T-maze performance is the method where the mobility of animals remarkably participates in the total performance of rats. Besides this, we have to admit the fact, that atropine itself is able to moderately affect memory of rats (5).

Acknowledgement

The authors thank to Mrs. E. Reslová and Mrs. E. Vodáková for their technical assistance and to Mgr. V. Bláha for the statistical evaluation.

This study was supported by the grant of Ministry of Defence, No 03021100006.

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Submitted April 2002.

Accepted May 2002.

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