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The Influence of Antidotal Treatment of Low-level Tabun Exposure on Cognitive Functions in Rats Using a Water Maze

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In this study, the influence of antidotal treatment of tabun poisoning on cognitive function, in the case of low-level tabun exposure, was studied. The impairment of cognitive function was evaluated by the measurement of spatial learning and memory in rats poisoned with a sublethal dose of tabun and treated with atropine alone or in combination with newly developed oximes {K027 [1-(4-hydroxyiminomethyl- pyridinium)-3-(4-carbamoylpyridinium) propane dibromide] and K048 [1-(4-hydroxyimino- methylpyridinium)-3-(4-carbamoylpyridinium) butane dibromide]} or currently available oxime (trimedoxime), using the Morris water maze. While atropine alone caused an impairment of studied cognitive functions, the addition of an oxime to atropine contributes to the improvement of cognitive performance of treated tabun-poisoned rats regardless of the type of oxime. The differences in the ameliorative effects of oximes on atropine-induced mnemonic deficits were not significant. Therefore, each low-level nerve agent exposure should be treated by complex antidotal treatment consisting of anticholinergic drug and oxime.

Keywords: Tabun; Water maze; Cognitive function; Atropine; Trimedoxime; K027; K048; Rats

INTRODUCTION

Tabun (*O*-ethyl-*N,N*-dimethyl phosphoramidocyanidate) belongs to highly toxic organophosphorus compounds, called nerve agents, misused as chemical warfare agents for military as well as terrorist purposes. It differs from other highly toxic organophosphates by its chemical structure and by the fact that tabun-inhibited acetylcholinesterase (AChE, EC 3.1.1.7) is very difficult to reactivate. Thus, its deleterious effects are extraordinarily difficult to counteract because of the existence of a lone electron pair located on an amidic group that makes the nucleophilic attack almost impossible (Koplovitz *et al.*, 1995; Jokanovic *et al.*, 1996; Cabal and Bajgar, 1999).

The mechanism of nerve agent-induced toxicity is based on the irreversible inhibition of AChE, resulting in the accumulation of neurotransmitter acetylcholine at peripheral and central cholinergic receptors and subsequent overstimulation of peripheral and central cholinergic nervous system (Bajgar, 2004). The central cholinergic system is important for locomotion, alertness and memory and for the regulation of a number of cyclic and periodic behaviors (Petras, 1994). Nerve agent-induced cholinergic effects are usually manifested immediately following high-level exposure (Marrs, 1993; Taylor, 1996). Nevertheless, there are numerous studies in both humans and animals showing that survivors of high-level nerve agent exposure can experience subtle but significant long-term neurological and neuropsychological outcomes that are detectable months or even years following the recovery from acute poisoning (Brown and Kelly, 1998). In addition, behavioural alterations and impairments of cognitive functions were found following acute exposure to organophosphorus compounds in the absence of any classic signs of cholinergic toxicity (Kassa *et al.*, 2001; Sánchez-Amate *et al.*, 2001). Therefore, all nerve agent poisonings including low-level tabun exposure should be treated by antidotes.

The current standard treatment for poisoning with nerve agents usually consists of the combined administration of anticholinergic drugs (preferably atropine) and oximes (preferably pralidoxime or obidoxime). Anticholinergic drugs block effects of overstimulation

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by accumulated acetylcholine at muscarinic receptor sites while oximes, compounds with nucleophilic activity, repair biochemical lesions by dephosphonylating tabun-inhibited AChE and restoring its activity (Marrs, 1993; Kassa, 2002). In the case of tabun poisoning, anticholinergic drugs such as atropine are able to counteract the effects of tabun at peripheral cholinergic receptors (Marrs, 1993; Bajgar, 2004), commonly used reactivators of tabun-inhibited AChE based on monopyridinium (*e.g.*, pralidoxime) and bispyridinium aldoximes (*e.g.*, obidoxime, trimedoxime, HI-6) are not able to counteract the toxic effects of tabun because of very low reactivating efficacy. Therefore, the replacement of commonly used oximes with a more effective oxime has been a long-standing goal for the treatment of tabun poisoning. New asymmetric bispyridinium oximes, called K027 [1-(4-hydroxyiminomethyl- pyridinium)-3-(4-carbamoylpyridinium) propane dibromide] and K048 [1-(4-hydroxyimino- methylpyridinium)-3-(4-carbamoylpyridinium) butane dibromide] were synthesized at our Department of Toxicology (Kuca *et al.*, 2003a,b) to improve the efficacy of antidotal treatment in eliminating tabun-induced acute toxic effects.

There are several behavioral tests used for the evaluation of effects of various compounds on memory functions, *i.e.*, memory formation, consolidation and retrieval (Bures *et al.*, 1979). Especially, a water maze is often used for its advantages and broad utilization. The water maze (WM; Morris, 1984) is a widely used measurement of visuospatial learning that has been demonstrated to have high validity in identifying cognitive effects of various brain lesions and the effects of drugs used to treat cognitive deficits (Myhrer, 2003). Special motivation such as food and water deprivation is not required for the WM performance. The effect of odour cues is eliminated in the WM. In addition, rats are forced to swim in the WM. They cannot choose whether or not to move, so failure to respond is not a confound (Shukitt-Hale *et al.*, 2004). The place learning version with submerged platform can be used for working memory test (Myhrer, 2003). The WM can be used to measure spatial learning and memory in the case of the evaluation of cognitive impairment in rats because of these forementioned advantages.

The rats perform cognitive tasks that require spatial learning and memory - the ability to acquire a cognitive representation of location in space and the ability to effectively navigate the environment in the WM (Shukitt-Hale *et al.*, 2004). Memory alterations appear to occur mostly in secondary memory systems and are reflected in the storage of newly acquired information (Bartus *et al.*, 1989; Joseph, 1992). It is thought that hippocampus mediates allocentric spatial navigation (*i.e.*, place learning) and prefrontal cortex is critical to acquiring the rules that govern performance in particular tasks (*i.e.*, procedural knowledge), while the dorsomedial striatum mediates egocentric spatial orientation (*i.e.*, response and cue learning) (McDonald and White, 1994; Oliviera *et al.*, 1997).

The aim of our study was to evaluate the potency of low-level tabun poisoning and the antidotal treatment of low-level tabun poisoning (atropine alone or in combination with an oxime) to influence spatial working orientation and memory in tabun-poisoned rats.

METHODS

Animals

Animals used in our experiments were male albino Wistar rats weighing 200-220 g, purchased from Biotest Konarovice (Czech Republic). They were kept in an air-conditioned room (22 ± 2°C and 50 ± 10% relative humidity, with lights from 7.00 to 19.00 h) and were allowed to access standard food and tap water *ad libitum*. The rats were divided into groups of eight. This study with experimental animals was done under the supervision of the Ethics Committee of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic).

Chemicals and Drugs

Tabun of 89.25% purity was obtained from the Military Technical Institute in Brno (Czech Republic). Its purity was assayed by acidimetric titration. Oximes were synthesized at the Department of Toxicology of the Faculty of Military Health Sciences and were 98% pure. Their purity was analysed using HPLC. All other chemicals and drugs of analytical grade were obtained commercially and used without further purification.

Apparatus

The water maze consists of a black circular pool (180 cm diameter x 80 cm high) filled to a depth of 25 cm with water of room temperature (Raveh *et al.*, 2002). The pool was imaginarily divided into four identical compartments numbered 1-4 clockwise. The black antireflective circular escape platform (15 cm diameter) was placed into compartment no. 1 or 4 (see Procedure of experiments) 20 cm off the pool wall. The platform was immersed 2 cm below the water surface, so it was not visible to rat viewing, owing to a water mirror effect. A yellow rectangle (30 cm x 40 cm) was fixed on the pool wall, immediately close to the platform, as the spatial conditional cue (Robinson *et al.*, 2004). Its place was variable in accordance to platform position.

Another dark rectangle was randomly fixed on the pool wall in different compartments (without platform) as the negative conditional cue. Around the pool, there were several stable extramaze cues in the room that the rat could use to navigate the maze (Morris, 1984). However, the impact of extramaze cues was not significant due to high maze walls.

Experimental Procedure

Animals acclimatized to the task on the two training days by being placed into the water for 60 s with no opportunity to escape (*i.e.*, no platform). On zero experimental day, animals were administered with tabun (90 μg/kg intramuscularly, i.m. - 50% LD_{50}) causing moderate muscarinic signs (salivation, chewing), but no nicotinic signs. The clinical signs of poisoning disappeared within 24 hours. One minute later, antidotal treatment was injected i.m.: atropine (21 mg/kg) alone; atropine (21 mg/kg) + trimedoxime (44.6 mg/kg); atropine (21 mg/kg) + K027 (44.6 mg/kg) or atropine (21 mg/kg) + K048 (47.3 mg/kg) to the experimental groups, respectively. Oximes were administered at equimolar doses (100 μmol/kg). Experimental animals were compared to controls administered with saline instead of tabun and antidotes at the same volume (1 ml/kg.). Control animals were exposed to the same experimental conditions in the WM as experimental groups.

WM testing was performed for fifteen days, one trial/day. At the beginning of each trial, the rat was gently immersed in water at the start position facing the wall of the pool. The start position was not variable for all experimental trials and took place in compartment no. 3. Then each animal had to search for the submeged platform in which the location was changed randomly from compartment no. 1 to compartment no. 4. Each rat was allowed 120 seconds to escape onto the platform. If the rat failed to escape within this time, it was guided to the platform. Once the rat reached the platform, it remained there for 20 seconds to learn visual cues to the platform location. Performance on each trial was videotaped and analysed with image tracking software (TSE VideoMot2, Bad Homburg, Germany) that provided dependent measures such as latency to find the platform (s), length of trajectory (cm) and speed (cm/s).

Data Analysis

Statistica´98 Edition® was used for the statistical analysis of behavioral measurements. Data for behavioral tests were compared among groups by one-way analysis of variance (ANOVA). Specific comparisons were performed using Scheffé test for multiple comparisons (Afifi and Azen, 1979; Raveh *et al.*, 2002; Abou-Donia *et al.*, 2003). The differences were considered significant when $P<0.05$.

RESULTS

The influence of low-level tabun exposure and antidotes on the process of spatial working memory formation performance was evaluated in rats. The results show that recorded values of latencies and lengths of trajectories in the WM corresponded to each other. Data describing the ability of rats to pass the WM within fifteen experimental trial days by the evaluation of latencies and lengths of trajectories are shown in Figures 1-2. Each column corresponds to one experimental group. The first column poses animals of the control group that received saline only. Rats from the control group were already able to resolve correct WM-track on

Table I The influence of antidotal treatment on tabun-induced impairment of rat`s performace in water maze.

MARKER	Controls	Tabun	Tabun + atropine	Tabun + atropine + trimedoxime	Tabun + atropine + K027	Tabun + atropine + K027
Latency (s)	14.6 ± 5.2	19.9 ± 5.8	28.8 ± 7.6**	16.9 ± 4.2^{++}	15.3 ± 4.7^{+++}	15.9 ± 4.2^{+++}
Length (cm)	402.5 ± 120.5	524.8 ± 134.6	663.1 ± 150.4***^{o}	460.5 ± 109.7^{+}	451.2 ± 116.4^{+}	371.8 ± 98.6^{+++}
Speed (cm/s)	33.5 ± 1.7	32.4 ± 1.6	32.0 ± 1,8	29.6 ± 1.6	35.6 ± 1.8	33.5 ± 1.7

Statistical significance *vs* control group, $^{**}P<0.01$; $^{***}P<0.001$. Statistical significance *vs* experimental group treated with atropine alone: $^{+}P<0.05$; $^{++}P<0.01$; $^{+++}P<0.001$. Statistical significance *vs* tabun-poisoned experimental group: $^{o}P<0.05$.

the fifth day. Their latencies were about 14.5 seconds and lengths of trajectories were 400 cm on average (Table I). Animals poisoned with tabun showed worse results compared to the control group. Nevertheless, the differences between control and the tabun-poisoned group were not significant. Their latencies were about 20 seconds and lengths of trajectories were 525 cm on average. In addition, our results suggested that learning by the experimental group intoxicated with tabun and treated with atropine alone is impaired not only in comparison with the control group but also in comparison with non-treated tabun-poisoned rats, because the animals needed almost two times longer latency (29 seconds) and length of trajectory (663 cm) on average

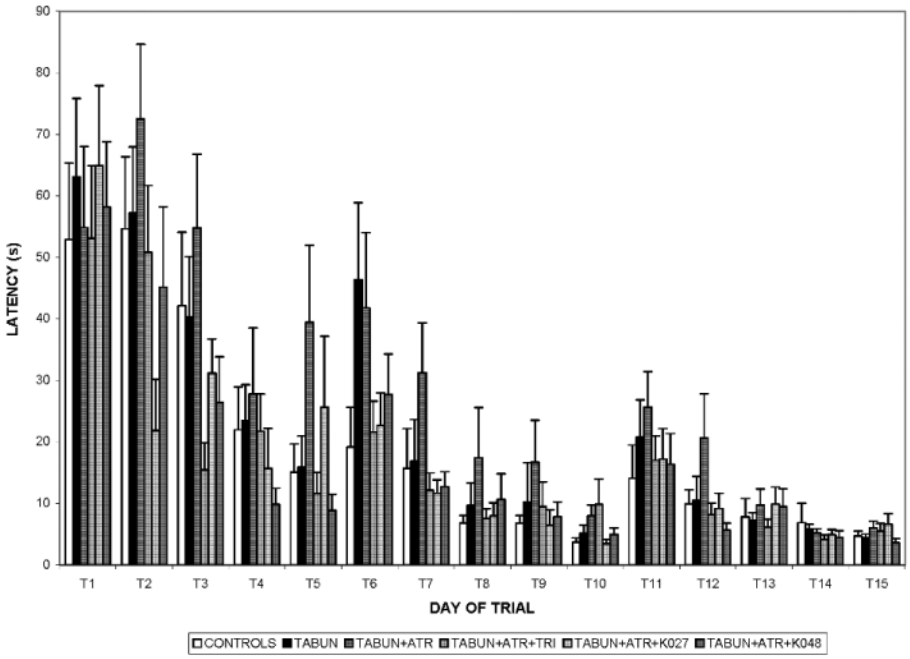

FIGURE 1 The latencies to find the platform in the water maze, performed by rats poisoned with tabun and treated with atropine alone or atropine in combination with the oxime.

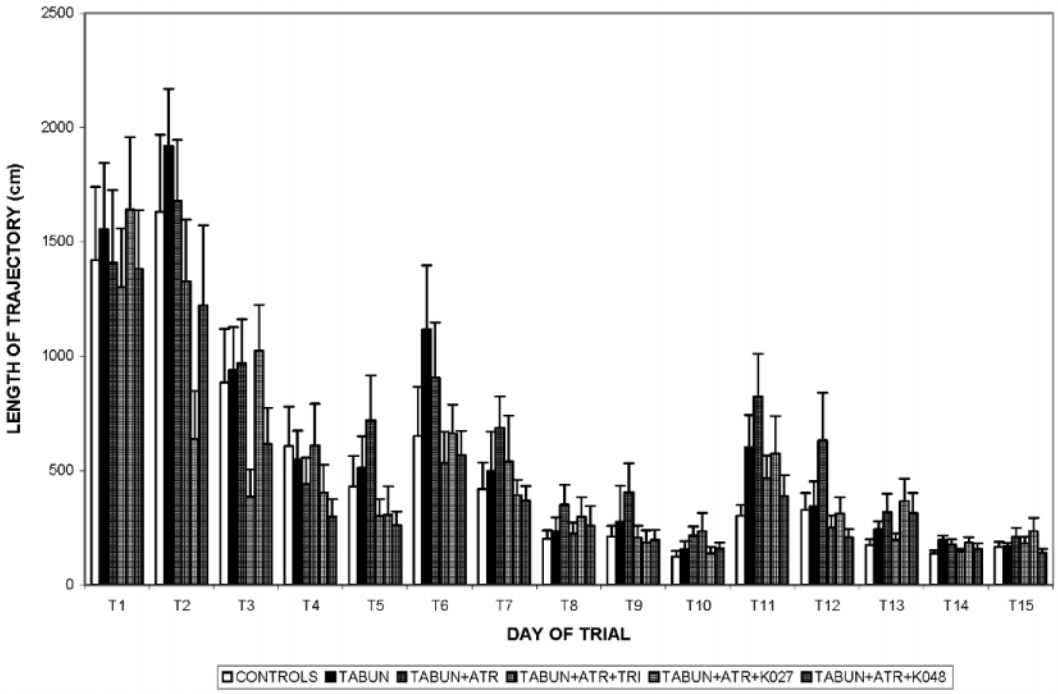

FIGURE 2 The lengths of trajectories in the water maze, performed by rats poisoned with tabun and treated with atropine alone or atropine in combination with the oxime.

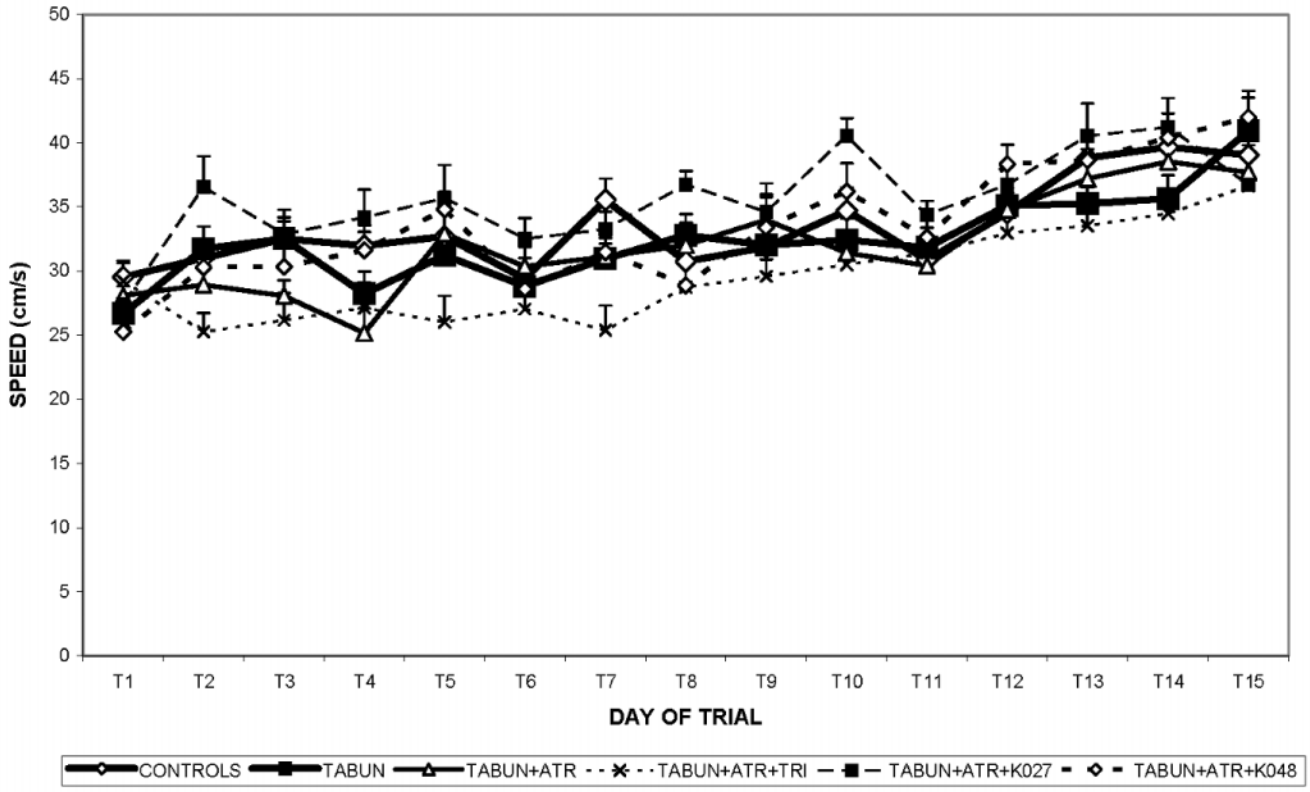

FIGURE 3 The speed of rats poisoned with tabun and treated with atropine alone or atropine in combination with the oxime during the performance in water maze.

to achieve the goal, in comparison with control animals. The adverse effects of atropine were demonstrated till the twelfth day of our experiment (FIGs. 1-2). On the other hand, the treatment with antidotal combination (atropine + oxime) caused improvement of tabun-induced impairment of the learning process, because average latencies and lengths of trajectories to find the hidden platform were comparable to control values and shorter than average values obtained from non-treated tabun-poisoned animals or tabun-poisoned animals treated with atropine alone. The antidotal combination makes the performance of rats comparable to the control group till the end of the experiment (FIGs. 1-2). The performance of rats treated with antidotal combination was similar to each other regardless of the type of oximes. No statistically significant differences among experimental groups treated with the antidotal mixture (atropine+oxime) were found (Table I).

The speed data of swimming animals were recorded, too. An average speed of all experimental animals was about 30-35 cm per second. Significant differences in speed values between control and experimental groups were not demonstrated (FIG. 3).

DISCUSSION

It is known that the cholinergic system plays an important role during encoding and retrieval of spatial information (Rogers and Kesner, 2003). Activity of the cholinergic system in hippocampus has been shown to decrease during training, such as a necessary requirement for consolidation (Hasselmo, 1999). It has been demonstrated that cholinergic agonists as well as AChE inhibitors increase acetylcholine level beyond the testing-time into time when acetylcholine level needs to be reduced, thereby interfering with the consolidation process (Rogers and Kesner, 2003); and, in addition, they can cause brain damage with lesions especially in hippocampus, piriform cortex (Petras, 1983; Tonduli *et al.*, 2001; Segura-Aguilar and Kostrzewa, 2004) and other cortical structures (McLeod *et al.*, 1984). In this study, low-level tabun poisoning was evaluated. The small dose of tabun brings an impairment of studied cognitive functions but tabun-induced changes in rats` performance were not significant compared to control animals. Tabun-induced impairment of cognitive functions is caused by subsequent desensitisation and internalization of cholinergic receptors as a reaction of tabun-exposed organisms to hyperstimulation of cholinergic receptors, especially in parts of the brain with a high density of cholinergic synapses such as hippocampus (McDonald *et al.*, 1988; Stone *et al.*, 2000).

The current antidotal treatment of nerve agent-induced

acute poisoning consisting of anticholinergic drugs and oximes should be beneficial to eliminate nerve agent-induced toxic effects (Taylor, 1996). Atropine is used to eliminate nerve agent-induced hyperstimulation of muscarinic cholinergic receptors, especially in the peripheral compartment. On the other hand, atropine as well as other anticholinergic drugs (scopolamine, biperiden) can also produce cognitive dysfunction by blocking the action of acetylcholine at muscarinic receptors (Patocka, 1998). Generally, atropine is combined with an oxime to treat acute poisoning with organophosphorus compounds including nerve agents. Oximes are considered to be beneficial to recover memory function because they restore the physiological function of cholinergic nervous system by the reactivation of AChE and, thus, make cholinergic receptors normally functioning (Kassa, 2002; Bajgar, 2004).

The results of our study indicate that the administration of tabun causes non-significant impaired visuospatial working learning performance in the water maze, that is believed to reflect learning of trial-dependent information and ability of the subject to retain this trial-dependent information in memory (Frick *et al.*, 1996; Luine *et al.*, 1998). The mentioned memory deficits were intensified by the therapy containing atropine alone because of its central antimuscarinic effects. Thus, when tabun-poisoned rats were treated with atropine alone, the discriminatory learning was impaired compared to non-treated tabun-poisoned rats. This fact corresponds with previously published data describing the potency of atropine to affect the ability of rodents to search for the goal (platform) (Day and Schallert, 1996; Boccia *et al.*, 2003). Nevertheless, atropine cannot be excluded because it is an integral component of antidotal treatment in the case of organophosphate poisoning (Shih and McDonough, 2000; Raveh *et al.*, 2003).

On the other hand, low-level tabun-induced moderate impairment of working memory was improved, if tabun-poisoned rats were treated with antidotal combination consisting of atropine and an oxime regardless of the oxime used. So, the difference between the potency of newly developed oximes and trimedoxime to eliminate moderate cognitive dysfunction in the case of low-level tabun poisoning in rats was not significant. As recorded values of speed of animals were not significantly different, we can exclude a motoric dysfunction as a cause of impaired performance of tabun-poisoned rats treated with atropine alone.

In conclusion, our findings clearly demonstrate that atropine should not be used alone to treat low-level nerve agents exposure because it can intensify nerve agent-induced moderate impairment of cognitive functions by its central antimuscarinic effects. Therefore, each low-level nerve agent exposure should be treated by antidotal treatment consisting of anticholinergic drug and oxime, although the laboratory and clinical signs of poisoning are not marked.

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