Introduction

The nerve agents are potent organophosphorus (OP) acetylcholinesterase (AChE, EC 3.1.1.7) inhibitors. An exposure to these agents causes a progression of toxic signs, including hypersecretions, fasciculations, tremor, convulsions, coma, respiratory distress and death (16,21,23). These toxic effects are due to hyperactivity of the cholinergic system as a result of AChE inhibition and the subsequent increase in the amount of the neurotransmitter acetylcholine (ACh) at central and peripheral sites (16, 23). The antidotal treatment of OP agent-induced acute poisoning usually consists of anticholinergic drugs to antagonize the effects of ACh excess at cholinergic receptor sites and oximes to reactivate OP agent-inhibited AChE (2,14).

Soman (pinacolyl methylphosphonofluoridate) is probably one of the most dangerous OP agents since its deleterious effects are especially difficult to counteract (1,2). Soman seems to cause centrally mediated seizure activity that can rapidly progress to status epilepticus and contribute to the profound brain damage (6,20). Thus, the exposure of experimental animals to soman in doses induced convulsions may result in irreversible lesions in the central nervous system that can be manifested as behavioral effects in convulsing survivors (11,15,17,18). Unfortunately, the presently used antidotes, such as pralidoxime or obidoxime in combination with atropine, do not appear to ameliorate soman-induced toxic signs including centrally mediated seizure activity and motor convulsions (1,13).

The aim of this study was to compare the neuroprotective effects of various antidotes in soman-poisoned rats. The soman-induced neurotoxic symptoms were determined using a functional observational battery (FOB), a non-invasive and relatively sensitive type of neurological examination in that a wide range of neurobiological functions is assessed, including measurements of sensory, motor and autonomic nervous functions.

Methods

Male albino Wistar rats weighing 180-230 g were purchased from Konarovec (Czech Republic). They were kept in an air-conditioned room and allowed to access standard food and water ad libitum. The rats were divided into groups of eight animals. Handling of the experimental animals was done under the supervision of the Ethics Committee of the Medical Faculty of Charles University and the Military Medical Academy in Hradec Králové (Czech Republic).

Soman was obtained from Zemianske Kostolany (Slovak Republic) and was 98.5% pure. The oximes of 98.0% purity were synthesized at the Department of Toxicology of the Military Medical Academy in Hradec Králové (Czech Republic). Their purities were assessed using HPLC. All other drugs and chemicals of analytical grade were obtained commercially and used without further purification. All substances were administered intramuscularly (i.m.) at a volume of 1 mL/kg body weight (b.w.).

Soman was administered at a sublethal, convulsive dose (48 µg/kg b.w. - 60% of LD50). One minute following soman injection, the rats were treated with atropine (21
Motor activity data were collected shortly after FOB testing, using an apparatus for testing of a spontaneous motor activity of laboratory animals (constructed in Purkyně Military Medical Academy, Hradec Králové, Czech Republic). The animals were placed for a short period (10 minutes) in the measuring cage and their movements (total horizontal activity, stereotypical activity, rearing, jumping, scratching, total vertical activity) were recorded. Statistical analyses were performed on a PC with BMOP programme PTD: analysis of variance (ANOVA) and test with Bonferroni’s corrections.

Results

The results of the experiments related to the measurement of soman-induced neurotoxicity at 24h and 7d following soman poisoning are summarized in Table 2. The observation of neurotoxic signs indicated that some functional disorders of poisoned organisms lasted at least 24 hours not only in untreated soman-poisoned rats but also in soman-poisoned rats treated with obidoxime and atropine. Some registered markers of neuronal damage of soman-poisoned rats are shown in Figures 1-5.

Diminished eating, including body weight loss, pronounced changes in piloerection and significantly decreased exploratory activity (p < 0.05) was observed in untreated soman-poisoned rats as well as in soman-poisoned rats treated with obidoxime/atropine mixture. Posis and the bloody secretion from the nose were observed in the case of untreated soman poisoning and poisoned treated with obidoxime/atropine mixture. On the other hand, practically all above mentioned neurotoxic signs were diminished when soman-poisoned animals were treated with atropine alone or atropine in combination with the oxime HI-6 (Table 2).

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Table 1: Functional Observational Battery (FOB).

| Home-cage and Handling Consists | Open Field | Other Measures | Reaction on Stimulations | Other Measures |
|--------------------------------|------------|----------------|-------------------------|----------------|
| Posture                        |            |                |                         |                |
| Muscle tone                    |            |                |                         |                |
| Locomotion                     |            |                |                         |                |
| Piloerection                   |            |                |                         |                |
| Skin Abnormalities             |            |                |                         |                |
| Motor Activity                 |            |                |                         |                |
| Activity                       |            |                |                         |                |
| Stereotypy                     |            |                |                         |                |

| Summary of Measures in the Functional Observational Battery |
|------------------------------------------------------------|
| Scored Values | Values in Absolute Units |
|----------------|-------------------------|
| Home-cage and Handling Consists | Open Field | Other Measures | Reaction on Stimulations | Other Measures |
| Posture | Muscle tone | Locomotion | Piloerection | Skin Abnormalities | Motor Activity | Activity | Stereotypy |
| Exploratory Activity | Pupil Response to Light | Approach Response | Landing Foot Splay (cm) | Touch Response | Hindlimb Grip Strength (kg) | Defecation | Fall from Vertical Position | Locomotion | Body Weight (g) | Piloerection | Mobility | Activity | Stereotypy |

| Motor Activity | Activity | Stereotypy |
|----------------|---------|-----------|
| Stereotypy | Activity | Stereotypy |

The convulsions were not observed in any experimental group of animals at 24h nor at 7d following soman poisoning although they were manifested intensively shortly following soman administration. The significant alteration of gait (p < 0.001) occurred in untreated soman-poisoned animals and animals treated with obidoxime/atropine mixture at 24h following soman poisoning (Table 2, Figure 1). These animals had awkward hindlimbs and their mobility was markedly diminished or totally eliminated (p < 0.001) (Table 2). Their posture was hump-backed or they were lying on their abdomen without stretched limbs (Table 2). Their reaction to sensory stimuli, including tail-pinch response, was markedly affected (p < 0.05) (Table 2) and their rectal temperature was reduced at 24h following soman poisoning (p < 0.01) (Table 2). Their forelimb and hindlimb grip strength as well as the distance between hindpaws after a jump were significantly diminished (p < 0.001) (Table 2, Figure 3) and their spontaneous horizontal as well as vertical activity was markedly decreased (p < 0.001) (Table 2). The shortening of the latency period of rearing responses was not balanced by the increased number of the responses, which remained almost stable during the whole observation period.

Tab. 2: The values of soman-induced neurotoxic markers measured by FOB (No 1 - % of control values, No 2-32 - scored values, No 33-40 - values in absolute units). Statistical significance: * p < 0.05, ** p < 0.01, *** p < 0.001. X - arithmetical mean of values.

| Marker | Controls | Soman | Soman+Atropine | Soman+Atropine + HI-6 | Soman+Atropine + Obidoxime |
|--------|---------|------|----------------|----------------------|--------------------------|
| Food   | 100%    | 100% | 100%           | 100%                 | 100%                     |
| Posture| 3.00    | 2.12 | 3.30           | 3.14                 | 3.25                     |
| Crisis | 3.00    | 3.15 | 1.08           | 1.00                 | 1.00                     |
| Gait   | 3.00    | 1.50 | 1.00           | 1.00                 | 1.00                     |
| Tailpinch | 3.00 | 2.12 | 1.50           | 1.00                 | 1.00                     |
| Rectal temperature | 3.00 | 3.15 | 1.08           | 1.00                 | 1.00                     |
| Body weight | 3.00 | 3.15 | 1.08           | 1.00                 | 1.00                     |
| Activity | 3.00 | 3.15 | 1.08           | 1.00                 | 1.00                     |
| Stereotypy | 3.00 | 3.15 | 1.08           | 1.00                 | 1.00                     |
Motor activity data were collected shortly after FOB testing, using an apparatus for testing of a spontaneous motor activity of laboratory animals (constructed in Purdue Military Medical Academy, Hradec Kralove, Czech Republic). The animals were placed for a short period (10 minutes) in the measuring cage and their movements (total horizontal activity, stereotypic activity, rearing, jumping, scratching, total vertical activity) were recorded.

Statistical analyses were performed on a PC with BMDP programme P7D: analysis of variance (ANOVA) and t-test with Bonferroni’s corrections.

The convulsions were not observed in any experimental group of animals at 24h nor at 7d following soman poisoning although they were manifested indirectly shortly following soman administration. The significant alteration of gait (p < 0.001) occurred in untreated soman-poisoned animals and animals treated with obidoxime/atropine mixture at 24h following soman poisoning (Table 2, Figure 1). These animals had awkward hindlimbs and their mobility was markedly diminished or totally eliminated (p < 0.001). Their posture was hump-backed or they were lying on their abdomen without stretched limbs (Table 2). Their reaction to sensory stimuli, including tail-pinch response, was markedly affected (p < 0.05) (Table 2, Figure 2) and their rectal temperature was reduced at 24h following soman poisoning (p < 0.01) (Table 2). Their forelimb and hindlimb grip strength as well as the distance between hindpaws after a jump were significantly diminished (p < 0.001) (Table 2, Figure 3) and their spontaneous horizontal as well pronounced changes in piloerection and significantly decreased exploratory activity (p < 0.05) was observed in untreated soman-poisoned rats as well as in soman-poisoned rats treated with obidoxime/atropine mixture. Posisis and the bloody secretion from the nose were observed in the case of untreated soman poisoning and poisoning treated with obidoxime/atropine mixture. On the other hand, practically all above mentioned neurotic signs were diminished when soman-poisoned animals were treated with atropine alone or atropine in combination with obidoxime (HI-6) (Table 2).
When soman-poisoned rats were treated with anticholinergic drug atropine, a relatively large decrease in neurotoxic symptoms induced by soman at a sublethal dose was observed. Atropine alone is able to antagonize the effects of Ach at muscarinic cholinergic receptor sites without changes of soman-induced inhibition of AChE activity (16,23) and thus diminish neurotoxic effects of soman in the case of sublethal poisoning (3,10). Nevertheless, atropine alone fails to prevent seizures and motor convulsions as well as mortality following exposure to soman at lethal and supra-lethal doses (8,9,18).

When soman-poisoned rats were treated with atropine in combination with the oxime HI-6, a significant neuroprotective effect was demonstrated. This antidotal mixture seems to be effective in decreasing in the neurotoxicity of soman because of the beneficial effects of both antidotes (12). The oxime HI-6 is not only a relatively efficacious re-activator of soman-inhibited AChE, especially in the peripheral compartment (7), but it also has secondary antidotal effects that probably arise from its antismuscarinic, gangli-on-blocking, postjunctional nondepolarizing action and effects on cardiovascular and respiratory systems (24).

On the other hand, another oxime obidoxime in combination with atropine is practically ineffective in the treatment of soman poisoning (8). In addition, our results confirm that obidoxime even diminishes the neuroprotective effect of atropine because our findings demonstrate the absence of any neuroprotective effect of this antidotal mixture in rats poisoned with the sublethal dose of soman.

In conclusion, atropine in combination with the oxime HI-6 is worth using in the antidotal treatment of soman poisoning for the elimination of soman-induced neurotoxicity.

Atropine alone is also sufficient for the elimination of neurotoxic symptoms in rats poisoned with soman at sublethal doses but in the case of lethal soman poisoning the effect of atropine is not enough to allow poisoned experimental animals to survive at least 24h following soman challenge (9,25). The antidotal mixture that consists of obidoxime and atropine is not able to eliminate soman-induced neurotoxicity. Therefore, this antidotal mixture is not suitable for the treatment of soman acute poisoning even in the case of sublethal poisoning with nerve agents.

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References

1. Bajgar J. Present views on toxidynamics of soman poisoning. Acta Med. 1998;59:105-6.
2. Dawson RM. Review of oximes available for treatment of nerve agent poisoning. J Appl Toxicol. 1994;14:317-31.

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In conclusion, atropine in combination with the oxime HI-6 is practically ineffective in the treatment of soman poisoning (8). In addition, our results confirm that oxime even diminishes the neuroprotective effect of atropine because our findings demonstrate the absence of any neuroprotective effect of this anticholinesterase in rats poisoned with the sublethal dose of soman.

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As vertical motor activity was markedly reduced at 24h following soman injection (Figures 4,5). On the other hand, the above mentioned soman-induced neurotoxic symptoms were significantly diminished at 24h as well as 7d following soman poisoning when soman - poisoned rats were treated with atropine alone or with atropine in combination with the oxime HI-6 (Table 2, Figures 1-5).

Discussion

When soman-poisoned rats were treated with anticholinergic drug atropine, a relatively large decrease in neurotoxic symptoms induced by soman at a sublethal dose was observed. Atropine alone is able to antagonize the effects of ACh at muscarinic cholinergic receptor sites without changes of soman-induced inhibition of AChE activity (16,23) and thus diminish neurotoxic effects of soman in the case of sublethal poisoning (3,10). Nevertheless, atropine alone fails to prevent seizures and motor convulsions as well as mortality following exposure to soman and supralethal doses (8,9,18).

When soman - poisoned rats were treated with atropine in combination with the oximes HI-6, a significant neuroprotective effect was demonstrated. This anticholinesterase mixture seems to be effective in decreasing in the neurotoxicity of soman because of the beneficial effects of both anticholinesterases (12). The oxime HI-6 is not only a relatively efficacious reactivator of soman-inhibited AChE, especially in the peripheral compartment (7), but it also has secondary anticholinergic properties that probably arise from its antispasmodic, ganglioblocking, postjunctional nondepolarizing action and effects on cardiovascular and respiratory systems (24).

On the other hand, another oxime obidoxime in combination with atropine is practically ineffective in the treatment of soman poisoning (9). In addition, our results confirm that oxime even diminishes the neuroprotective effect of atropine because our findings demonstrate the absence of any neuroprotective effect of this anticholinesterase in rats poisoned with the sublethal dose of soman.

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