ORIGINAL ARTICLE

NEUROPROTECTIVE EFFECTS OF ANTIDOTES IN SOMAN-POISONED RATS

Jiří Kassa, Marie Koupilová

Purkyně Military Medical Academy, Hradec Králové: Department of Toxicology

Summary: 1. The neuroprotective effects of antidotes (atropine, obidoxime/atropine mixture, HI-6/atropine mixture) on rats poisoned with soman at a sublethal dose (48 μg/kg i.m.; 60% of LD_{50} value) were studied. The neurotoxicity was monitored using a functional observational battery (FOB) and an automatic measurement of motor activity. The neurotoxicity of soman was monitored at 24h and 7d following soman poisoning. 2. The results indicate that atropine alone and the oxime HI-6 in combination with atropine seem to be effective antidotal treatment for the elimination of soman-induced neurotoxicity in the case of sublethal poisonings. 3. On the other hand, the combination of obidoxime with atropine appears to be practically ineffective in diminishing neurotoxic soman-induced symptoms. 4. Dealing with neuroprotective effects of antidotes, the oxime HI-6 in combination with atropine seems to be more suitable antidotal mixture than obidoxime in combination with atropine even in the case of sublethal poisoning with nerve agents.

Key words: *Neurotoxicity; Soman; Behavioral screening; Obidoxime; Atropine; Rat*

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 Konárovice (Czech Republic). They were kept in an air-conditioned room and allowed to access to standard food and tap 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 Zemianské 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 analysed 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 LD_{50}). One minute following soman injection, the rats were treated with atropine (21

mg/kg b.w.) alone or with atropine (21 mg/kg b.w.) in combination with the oxime HI-6 or obidoxime at equimolar doses (100 μmol/kg b.w.). The neurotoxicity of soman was monitored using the FOB at 24 hours and 7 days following soman poisoning.

The FOB consists of 40 measurements of sensory, motor and autonomic nervous functions (4,5,19,22) (Table 1). The first evaluation was made when soman-poisoned rats were in the home cage. The observer evaluated each animal's posture, palpebral closure and gait and the presence or absence of convulsions was noted. Each rat was then removed from the home cage and briefly held in the hand. The presence or absence of spontaneous vocalization, piloerection and other fur and skin abnormalities as well as the irritability were noted too. Then, lacrimation and salivation were registered and scored. Other signs such as exophthalmus, crustiness around the eyes or emaciation were recorded too.

Then rats were placed on a flat surface which served as an open field. A timer was started for three minutes during which the frequency of rearing responses was recorded. At the same time, gait characteristics were noted and ranked, and arousal, tremor, convulsions and abnormal posture were evaluated. At the end of the third minute, the number of fecal boluses and urine pools on the absorbent pad were registered. Then, a reflex testing, that consists of recording each rat's response to the frontal approach of the blunt end of a pen, a touch of the pen to the posterior flank and an auditory clic stimulus, was used. The responsiveness to a pinch on the tail and the ability of pupils to constrict in response to light were then assessed. These measures were followed by a test for the aerial righting reflex, then by the measurements of forelimb and hindlimb grip strength, body weight, rectal temperature and finally hindlimb landing foot splay. The whole battery of tests required approximately 6-8 minutes per a rat.

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 BMDP programme P7D: analysis of variance (ANOVA) and t-test with Bonfferoni'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 outlasted 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 soman-poisoned rats treated with obidoxime/atropine mixture. Ptosis 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 neurotoxic signs were diminished when soman-poisoned animals were treated with atropine alone or atropine in combination with the oxime HI-6 (Table 2).

Tab. 1: Functional Observational Battery (FOB).

Summary of Measures in the Functional Observational Battery				
Scored Values			Values in Absolute Units	
Home-cage and Handling Measures	Open Field	Other Measures	Reaction on Stimulations	Other Measures
Posture	Exploratory Activity	Pupil Response to Light	Approach Response	Landing Foot Splay (cm)
Catch Difficulty	Urination	Righting Reflex	Touch Response	Forelimb Grip Strength (kg)
Ease of Handling	Defecation	Fall from Vertical Position	Click Response	Hindlimb Grip Strength (kg)
Muscular tonus	Clonic Convulsions	Damage of Respiration	Tail-pinch Response	Forelimb and Hindlimg Grip Strength (kg)
Lacrimation	Tremor			Body Weight (g)
Palpebral Closure	Tonic Convulsions			Rectal Temperature (°C)
Endo-Exophthalmus	Gait			Horizontal Activity
Piloerection	Mobility			Vertical Activity
Skin Abnormalities	Tensions			
Salivation	Vocalizations			
Secretion	Stereotypy			
	Bizarre behavior			
	Arousal			

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 ($p < 0.001$) occured 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 stimulus, 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

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.

number of marker	marker	controls		soman		soman+atropine		soman+atropine + HI-6		soman+atropine +obidoxime	
		24 hours	7 days	24 hours	7 days	24 hours	7 days	24 hours	7 days	24 hours	7 days
		x		*x*		*x*		*x*		*x*	
1	food	100%	100%	10%	100%	100%	100%	79%	100%	86%	100%
2	posture	3,00	2,12	3,50	3,14	3,25	2,75	3,12	2,25	4,00	2,60
3	catch difficulty	1,00	1,00	1,50	1,08	1,00	1,00	1,00	1,00	2,00***	1,00
4	ease of handling	1,00	1,00	1,50	1,00	1,00	1,00	1,00	1,00	1,83***	1,00
5	muscular tonus	1,00	1,00	1,62	1,00	1,00	1,00	1,00	1,00	2,00***	1,00
6	lacrimation	1,00	1,00	1,25	1,00	1,00	1,00	1,00	1,00	2,00***	1,00
7	palpebral closure	1,00	1,00	2,25***	1,28	1,00	1,00	1,00	1,00	1,00	1,00
8	endo-exophthalmus	1,00	1,00	1,75*	1,43	1,00	1,00	1,00	1,00	1,00	1,00
9	piloerection	1,00	1,00	6,25***	3,00	1,00	1,00	1,00	1,00	8,00***	2,40
10	skin abnormalities	1,00	1,00	1,25	1,00	1,00	1,00	1,00	1,00	1,00	1,00
11	salivation	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	3,00***	1,00
12	secretion	1,00	1,00	2,62**	1,42	1,75	1,00	1,00	1,00	4,00***	1,00
13	exploratory activity	8,12	5,25	1,37*	2,42	7,12	6,12	11,87	5,37	0,66**	7,20
14	urination	1,75	1,62	0,12*	1,57	2,87	3,50	3,25	6,12	8,66***	1,80
15	defecation	2,37	0,50	0,50	2,42	1,75	1,87	0,00	1,75	2,83	3,80
16	clonic convulsions	1,00	1,00	1,50	1,00	1,00	1,00	1,25	1,00	1,50	1,00
17	tonic convulsions	1,00	1,00	2,37	1,00	1,50	1,00	1,00	1,00	1,16	1,00
18	gait	1,00	1,00	5,12***	2,14	1,25	1,00	1,25	1,00	4,33***	1,00
19	gait score	1,00	1,00	2,87***	1,57	1,00	1,00	1,00	1,00	3,66***	1,00
20	mobility score	1,00	1,00	2,87***	2,42	1,00	1,37	1,00	1,00	3,33***	1,00
21	activity	1,00	1,00	2,75***	2,57	1,00	1,37	1,62	2,62	2,00*	1,20
22	tension	1,00	1,00	2,00***	1,00	1,00	1,00	1,00	1,00	1,00	1,00
23	stereotype	1,00	1,00	1,25***	2,14***	1,00	1,00	1,00	1,00	1,00	1,00
24	approach response	1,50	1,25	2,00	1,14	1,25	1,12	1,00	1,12	2,66*	1,40
25	touch response	1,62	1,75	2,12	2,00	1,87	1,75	2,00	2,00	2,00	2,00
26	click response	1,62	1,00	2,75	1,28	2,50	2,87*	1,75	2,12	3,16	1,60
27	tail-pinch response	1,00	1,25	2,25*	1,14	1,00	1,12	1,37	1,00	2,33*	1,00
28	pupil size	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	3,00**	1,40
29	pupil response	1,00	1,00	1,00	2,42*	1,00	1,25	1,00	1,00	3,00**	1,00
30	righting reflex	1,00	1,00	1,62	1,00	1,00	1,00	1,00	1,00	2,33	1,00
31	fall from vertical position	1,00	1,00	1,37	1,00	1,00	1,00	1,00	1,00	2,00	1,00
32	damage of respiration	1,00	1,00	2,00	1,00	1,25	1,00	1,00	1,00	3,66***	1,00
33	landing foot splay (cm)	88,25	95,75	55,93*	74,14*	83,06	81,75	88,06	88,37	65,00*	76,20*
34	forelimb grip strenght (kg)	6,83	6,26	2,86***	4,92	6,16	5,61	7,20	6,51	3,63*	7,86
35	hindlimb grip strength (kg)	3,29	3,71	1,11**	3,18	2,18	3,05	2,28	3,62	0,78***	2,80
36	grip stren.of for and hindlimb	19,26	18,56	5,90***	15,65	14,23	17,71	13,58	19,92	6,61***	16,96
37	body weight (g)	190,37	205,87	165,5*	181,57	200,37	206,37	196,00	219,75	160,66*	186,60
38	rectal temperature (°C)	38,08	37,80	36,22**	35,94	37,65	37,41	37,62	37,78	34,08***	37,62
39	activity horizont (No/10 min)	522,75	384,00	176,50***	380,14	469,50	479,12	334,62	341,25	62,33***	409,40
40	activity vertical (No/10 min)	178,87	91,12	58,50*	110,28	146,87	181,87	96,12	112,12	2,33***	105,60

Fig. 1: The neuroprotective effect of antidotes on soman-induced alteration of the gait at 24h and 7d following soman challenge. C - control values, treatment 1 - atropine alone, treatment 2 - atropine + the oxime HI-6, treatment 3 - atropine + obidoxime.

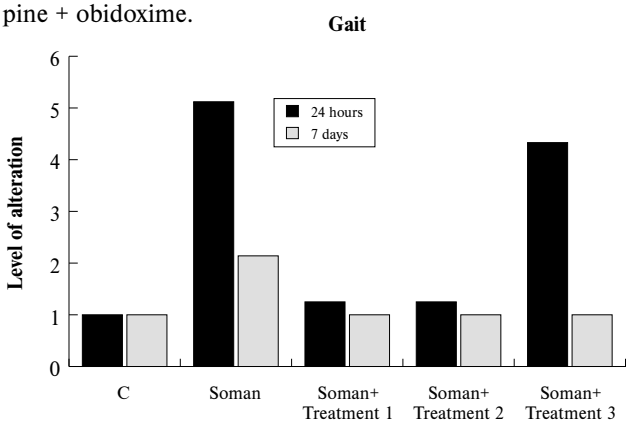


Fig. 2: The neuroprotective effect of antidotes on soman-induced latency of the tail-pinch response at 24h and 7d following soman challenge. For symbols - see Fig. 1.

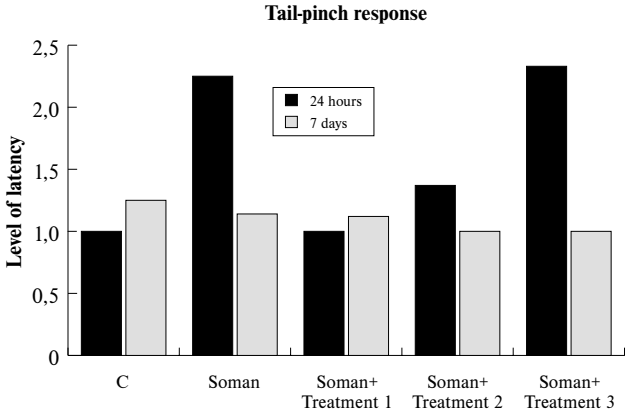


Fig. 3: The neuroprotective effect of antidotes on soman-induced decrease in forelimb and hindlimb grip strength at 24h and 7d following soman challenge. For symbols - see Fig. 1.

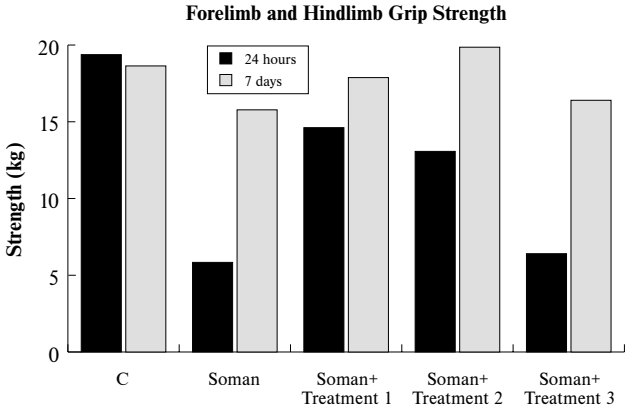


Fig. 4: The neuroprotective effect of antidotes on soman-induced decrease in horizontal motor activity at 24h and 7d following soman challenge. For symbols - see Fig. 1.

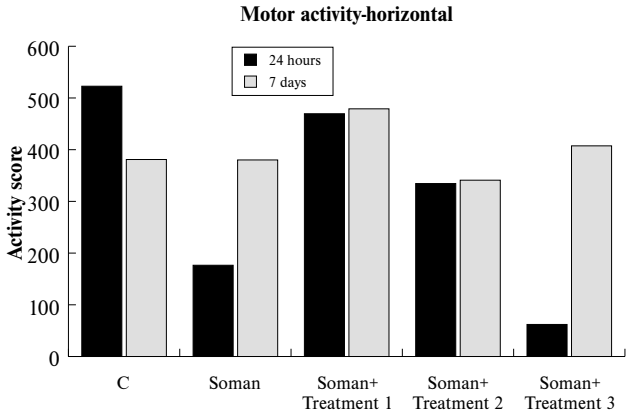
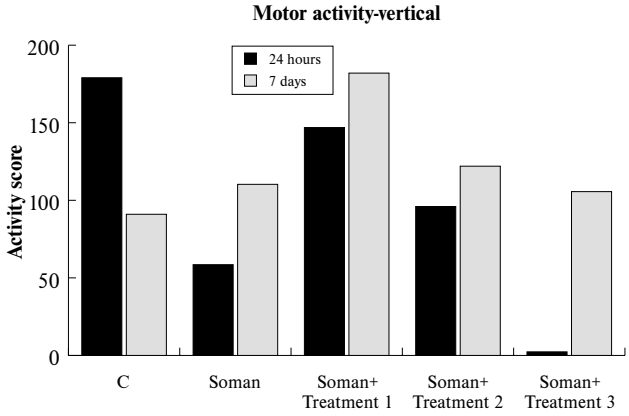


Fig. 5: The neuroprotective effect of antidotes on soman-induced decrease in vertical motor activity at 24h and 7d following soman challenge. For symbols - see Fig. 1.



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 at lethal and supralethal 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 reactivator of soman-inhibited AChE, especially in the peripheral compartment (7), 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 (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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Doc. MUDr. Jiří Kassa, CSc.,
P.O. Box 35/T, VLA JEP,
500 01 Hradec Králové,
Czech Republic.
e-mail: kassa@pmfhk.cz