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Neuroprotective effects of currently used antidotes in soman-poisoned rats

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Abstract

The neuroprotective effects of antidotes (atropine, obidoxime, obidoxime/atropine mixture) on rats poisoned with soman at a sublethal dose (54 μg/kg, im, 80% of LD_{50} value) were studied. The soman-induced neurotoxicity was monitored using a functional observational battery (FOB) and an automatic measurement of motor activity. The neurotoxicity of soman was monitored at 24 h and 7 days following soman challenge. The results indicate that obidoxime alone is not able to protect the rats from the lethal effects of soman. Three soman-poisoned rats treated with obidoxime alone died within 24 h. On the other hand, atropine alone or combined with obidoxime allows all soman-poisoned rats to survive within 7 days following soman challenge. Atropine alone and combined with obidoxime seems to be relatively effective antidotal treatment for the elimination of soman-induced neurotoxicity in the case of sublethal poisonings, although the antidotal mixture is significantly less effective than atropine alone because obidoxime can counteract the beneficial effects of atropine. Obidoxime appears to be practically ineffective to diminish soman-induced neurotoxicity. The neuroprotective effects of antidotal mixture consisting of atropine and obidoxime depend on the antimuscarinic effects of atropine only. Thus, the replacement of obidoxime by more effective acetylcholinesterase (AChE) reactivators is necessary to increase the neuroprotective efficacy of antidotal treatment in the case of soman poisonings.

Keywords: Neurotoxicity; Soman; Behavioral screening; Obidoxime; Atropine; Rat

1. 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 [14,20,22]. 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 [14,22]. 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 [4,12].

Soman (pinacolyl methylphosphonofluoridate) is probably one of the most dangerous OP agents since its deleterious effects are especially difficult to counteract [2,4]. Soman seems to cause centrally mediated seizure activity that can rapidly progress to status epilepticus and contribute to profound brain damage [18,23]. Thus, the exposure of experimental animals to soman in convulsions-induced doses may result in irreversible lesions in the central nervous system that can be manifested as behavioral effects in convulsing survivors [13,15]. Unfortunately, the presently used antidotes, such as pralidoxime in combination with atropine, do not appear to ameliorate soman-induced toxic signs including centrally mediated seizure activity and tonic–clonic convulsions [2,11].

The aim of this study was to evaluate the neuroprotective effects of currently used anticholinergic drug atropine and AChE reactivator obidoxime in soman-poisoned rats. The soman-induced neurotoxic signs were determined using a functional observational battery (FOB), a noninvasive and relatively sensitive type of neurological examination in that a wide range of neurobiological functions is assessed,

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Table 1
Functional observational battery

	Scored values only						
Marker	1/*n*	2	3	4	5	6	7
Posture	sitting or standing	rearing	asleep	flattened	lying on side	crouched over	head bobbing
Catch difficulty	normal	passive	defense	flight	aggression		
Ease of handling	very easy	easy	moderately difficult	difficult	very difficult		
Muscular tonus	normal	passive	hypertonia	rigidity			
Lacrimation	none	slight	severe				
Palpebral closure	wide open	slightly drooping	half-way drooping	completely shut			
Endo–exophthalmos	normal	endo	exo				
Piloerection	no	yes					
Skin abnormality	normal	pale	erythema	cyanosis	pigmented	cold	injury
Salivation	none	slight	severe				
Secretion	none	slight	severe	coloured			
Exploratory activity	number of raising and grooming						
Urination	number of pools of urine on the paper for 3 min						
Defecation	number of fecal boluses on the paper for 3 min						
Clonic movements	normal	repetitive movements of mouth and jaws	nonrhythmic quivers	mild tremors	severe tremors	myoclonic jerks	clonic convulsion
Tonic movements	normal	contraction of extensors	opisthotonus	emprosthotonus	explosive jumps	tonic convulsion	
Gait	normal	ataxia	overcompensation of hindlimbs movements	feet point outwards from body	forelimbs are extended	walks on tiptoes	body is flattened against surface
Gait score	normal	slightly impaired	moderately impaired	severely impaired			
Mobility score	normal	slightly impaired	somewhat impaired	totally impaired			
Arousal (level of unprovoked activity)	very low	low	somewhat low	normal	somewhat high	very high	
Tension	normal	present	absent				
Stereotypy	none	head weaving	body weaving	stereotypic grooming	circling		
Approach response	normal	no reaction	slow response	energetic response	very energetic response	exaggerated response	
Touch response	normal	no reaction	slow response	energetic response	very energetic response	exaggerated response	
Click response	normal	no reaction	slow response	energetic response	very energetic response	exaggerated response	
Tail-pinch response	normal	no reaction	slow response	energetic response	very energetic response	exaggerated response	
Pupil size	normal	miosis	mydriasis				
Pupil response	normal reaction	no reaction					
Righting reflex	normal	slightly uncoordinated	lands on side	lands on back			

including measurements of sensory, motor, and autonomic nervous functions.

2. Methods

2.1. Subjects

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 ($n=8$). 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).

2.2. Chemicals

Soman was obtained from Zemianské Kostolany (Slovak Republic) and was 98.5% pure. The oxime of 98.0% purity was synthesized at the Department of Toxicology of the Military Medical Academy in Hradec Králové. Their purities were analyzed using HPLC. All other drugs and chemicals of analytical grade were obtained commercially and used without further purification. All substances were administered intramuscularly at a volume of 1 ml/kg bw.

Table 2
The values of soman-induced neurotoxic markers ($x \pm s$) measured at 24 h following soman challenge by FOB (No. 1–29 — scored values, No. 30–38 — values in absolute units)

		Controls ($n=8$)		Soman ($n=6$)		Soman + atropine ($n=8$)		Soman + obidoxime ($n=5$)		Soman + atropine + obidoxime ($n=8$)	
No.	Marker	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
1	posture	2.00	0.00	2.33	0.81	2.00	0.00	3.16	1.02	2.25	0.70
2	catch difficulty	1.00	0.00	1.50	0.65	1.00	0.00	2.00	0.95	1.00	0.00
3	ease of handling	1.00	0.00	1.50	0.60	1.00	0.00	1.83	0.85	1.00	0.00
4	muscular tonus	1.00	0.00	1.62	0.75	1.00	0.00	2.00	1.05	1.00	0.00
5	lacrimation	1.00	0.00	1.25	0.60	1.00	0.00	2.00	1.00	1.00	0.00
6	palpebral closure	1.00	0.00	2.25 *	0.60	1.00	0.00	1.00	0.00	1.00	0.00
7	endo–exophthalmos	1.00	0.00	1.75	0.90	1.00	0.00	1.00	0.00	1.00	0.00
8	piloerection	1.00	0.00	2.00 *	0.10	1.00	0.00	2.00 *	0.15	1.00	0.00
9	skin abnormalities	1.00	0.00	1.25	0.45	1.00	0.00	1.00	0.00	1.00	0.00
10	salivation	1.00	0.00	1.00	0.00	1.00	0.00	2.00	0.60	1.00	0.00
11	secretion	1.00	0.00	2.62 *	0.54	1.00	0.00	4.00 *,x	0.58	1.75 *	0.38
12	exploratory activity	7.87	3.83	1.37 *	0.97	7.12	2.42	0.66 *	0.40	2.73 *	1.30
13	urination	1.85	1.30	3.33	2.80	2.87	1.52	8.66 *	4.80	4.25	3.52
14	defecation	1.18	0.62	0.81	0.33	0.37	0.24	0.74	0.37	2.13	1.50
15	clonic movements	1.00	0.00	1.50	0.60	1.00	0.00	1.50	0.54	1.25	0.40
16	tonic movements	1.00	0.00	2.37	1.03	1.16	0.40	1.50	0.66	1.25	0.75
17	gait	1.00	0.00	5.12 *	1.87	1.25	0.44	4.83 *	2.07	4.33 *	1.98
18	gait score	1.00	0.00	2.87 *	1.02	1.00	0.00	3.86 *	1.23	3.66 *	1.09
19	mobility score	1.00	0.00	2.87 *	0.87	1.00	0.00	3.33 *	0.53	3.30 *	1.16
20	arousal	4.00	0.00	2.75 *	0.43	4.00	0.00	2.16 *	0.53	3.00 *	0.26
21	tension	1.00	0.00	2.00 *	0.32	1.00	0.00	1.00	0.00	1.00	0.00
22	stereotypy	1.00	0.00	1.25	0.55	1.00	0.00	1.00	0.00	1.00	0.00
23	approach response	1.50	0.35	2.00	0.40	1.25	0.37	2.66 *	0.50	1.60	0.55
24	touch response	1.62	0.46	2.12	0.51	1.38	0.48	2.00	0.51	1.87	0.65
25	click response	1.75	1.38	2.75	1.43	2.50	1.39	3.16	1.90	2.87	1.78
26	tail-pinch response	1.37	0.51	2.25	0.87	1.14	0.37	2.33	1.06	1.73	0.96
27	pupil size	1.00	0.00	1.00	0.00	1.00	0.00	1.33	0.51	1.00	0.00
28	pupil response	1.00	0.00	1.00	0.00	1.00	0.00	1.33	0.51	1.00	0.00
29	righting reflex	1.00	0.00	1.62	0.59	1.00	0.00	2.33 *,x	0.54	1.26	0.43
30	landing foot splay (mm)	89.06	11.56	55.93 *	7.36	83.06	15.14	65.00 *	4.73	78.41	15.04
31	forelimb grip strength (kg)	6.83	1.25	2.86 *	1.27	6.16	1.99	3.63 *	1.06	3.66 *	0.63
32	hindlimb grip strength (kg)	3.72	1.01	1.11 *	1.08	2.18	1.12	0.78 *	0.51	2.05 *	0.69
34	grip strength of all limbs (kg)	19.26	2.52	5.90 *	1.90	14.23	3.19	6.61 *	1.90	7.16 *	1.89
35	body weight (g)	226.25	10.32	218.75	9.91	216.75	15.98	206.66	17.51	213.75	11.87
36	body temperature (°C)	37.35	0.53	37.25	0.25	37.00	0.41	37.16	0.45	37.10	0.55
37	vertical activity (No./10 min)	178.87	42.49	58.50 *	28.76	146.87	40.57	2.33 *,x	1.46	4.79 *,x	1.48
38	horizontal activity (No./10 min)	522.75	137.52	176.50 *	59.87	469.50	59.55	62.33 *,x	16.05	96.23 *,x	25.45

* Statistical significance: $P<.05$ (comparison with the control values).
x Statistical significance: $P<.05$ (comparison with the values from nontreated soman-poisoned rats).

2.3. Procedure

Soman was administered at a sublethal, convulsive dose (54 μg/kg bw, 80% of LD_{50}). One minute following soman challenge, the rats were treated with atropine (21 mg/kg bw) or obidoxime (35.9 mg/kg) alone or with the combination of atropine and obidoxime at the same doses. The neurotoxicity of soman was monitored using the FOB at 24 h and 7 days following soman poisoning. The evaluated markers of soman-induced neurotoxicity in experimental animals were compared with the parameters obtained from control rats, that saline was administered instead of soman and antidotes at the same volume.

The FOB consists of 38 measurements of sensory, motor, and autonomic nervous functions. Some of them are scored and the others are measured in absolute units [5,6,17,21] (Table 1). The first evaluation was obtained when soman-poisoned rats were in the home cage. The observer evaluated each animal's posture, palpebral closure, and involuntary motor movements. Then, each rat was removed from the home cage and briefly held in the hand. The exploratory activity, piloerection, and other fur and skin abnormalities were noted too. Lacrimation, salivation, and nose secretion were also registered and scored.

Then, the rats were placed on a flat surface that served as an open field. A timer was started for 3 min 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

Table 3
The values of soman-induced neurotoxic markers ($x \pm s$) measured at 7 days following soman challenge by FOB (No. 1–29 — scored values, No. 30–38 — values in absolute units)

		Controls ($n=8$)		Soman ($n=6$)		Soman + atropine ($n=8$)		Soman + obidoxime ($n=5$)		Soman + atropine + obidoxime ($n=8$)	
No.	Marker	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
1	posture	2.12	0.54	3.14	0.76	2.75	0.65	2.96	0.87	2.60	0.70
2	catch difficulty	1.00	0.00	1.08	0.32	1.00	0.00	1.00	0.00	1.00	0.00
3	ease of handling	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
4	muscular tonus	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
5	lacrimation	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
6	palpebral closure	1.00	0.00	1.28	0.43	1.00	0.00	1.00	0.00	1.00	0.00
7	endo–exophthalmos	1.00	0.00	1.43	0.45	1.00	0.00	1.00	0.00	1.00	0.00
8	piloerection	1.00	0.00	1.30	0.35	1.00	0.00	1.24	0.15	1.00	0.00
9	skin abnormalities	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
10	salivation	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
11	secretion	1.00	0.00	1.42	0.32	1.00	0.00	1.00	0.00	1.00	0.00
12	exploratory activity	5.25	2.97	2.42	0.97	6.12	2.23	6.66	2.40	6.75	2.30
13	urination	1.62	1.36	1.57	1.80	0 *	0.00	1.80	0.76	0 *	0.00
14	defecation	0.50	0.23	1.42	0.73	0.12	0.08	0.83	0.63	1.12	0.65
15	clonic movements	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
16	tonic movements	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
17	gait	1.00	0.00	2.14	0.73	1.00	0.00	1.00	0.00	1.00	0.00
18	gait score	1.00	0.00	1.57	0.82	1.00	0.00	1.00	0.00	1.00	0.00
19	mobility score	1.00	0.00	2.16	0.98	2.37 *	0.51	1.22	0.50	2.06	0.50
20	arousal	4.00	0.00	4.80	1.34	5.12	1.65	3.16	0.75	3.52	0.62
21	tension	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
22	stereotypy	1.00	0.00	1.14	0.35	1.00	0.00	1.00	0.00	1.00	0.00
23	approach response	1.25	0.46	1.33	0.51	1.12	0.35	1.50	0.54	1.50	0.53
24	touch response	1.87	0.35	1.50	0.54	1.50	0.53	2.00	0.62	1.75	0.46
25	click response	1.00	0.00	1.28	0.34	2.87 *	0.93	1.60	0.76	2.12	1.18
26	tail-pinch response	1.25	0.32	1.14	0.37	1.12	0.41	1.00	0.00	1.00	0.00
27	pupil size	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
28	pupil response	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
29	righting reflex	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
30	landing foot splay (mm)	95.75	5.75	74.14 *	7.20	87.15	8.76	76.20 *	7.43	87.40	10.54
31	forelimb grip strength (kg)	6.26	1.74	4.92	2.03	5.61	1.76	6.30	1.89	7.86	1.65
32	hindlimb grip strength (kg)	3.29	0.91	3.18	0.41	3.05	0.64	2.63	1.07	2.80	0.63
34	grip strength of all limbs (kg)	18.56	2.78	15.65	1.33	17.71	3.66	12.15 *	2.13	16.96	1.25
35	body weight (g)	236.25	15.05	237.50	14.40	224.12	15.03	213.33	28.54	219.75	9.83
36	body temperature (°C)	37.77	0.37	37.91	0.42	37.97	0.34	37.16	0.75	37.52	0.34
37	vertical activity (No./10 min)	75.75	24.90	30.33 *	9.06	121.12 *,x	17.55	38.16 *	7.72	109.25x	26.25
38	horizontal activity (No./10 min)	276.12	27.79	175.83 *	20.26	382.62 *,x	37.41	201.50 *	23.31	349.87 *,x	31.30

* Statistical significance: $P<.05$ (comparison with the control values).
x Statistical significance: $P<.05$ (comparison with the values from nontreated soman-poisoned rats).

fecal boluses and urine pools on the absorbent pad were registered. A reflex testing consisting 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 click stimulus was also 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 and 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 min/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 min) in the measuring cage and their movements (total horizontal and vertical activity) were recorded.

2.4. Data analysis

Statistical analyses were performed on a PC with BMDP programme P7D: analysis of variance (ANOVA) and *t* test with Bonferroni's corrections [1]. The differences were considered significant when $P < .05$.

3. Results

Six nontreated soman-poisoned rats only survived till the end of experiment (7 days following the intoxication) because two nontreated soman-poisoned rats died within 2 h following soman challenge. While all soman-poisoned rats, treated with atropine alone or the combination of atropine and obidoxime, survived till the end of experiment, five soman-poisoned rats treated with obidoxime alone only survived till the end of experiment because three soman-poisoned rats treated with obidoxime alone died within 2 h following soman administration.

The results of the experiments related to the measurement of soman-induced neurotoxicity at 24 h and 7 days following soman poisoning are summarized in Tables 2 and 3. The observation of neurotoxic signs indicated that some functional disorders of poisoned organisms outlasted at least 24 h not only in untreated soman-poisoned rats but also in soman-poisoned rats treated with obidoxime alone or with obidoxime in combination with atropine.

While atropine alone is able to eliminate soman-induced signs of neurotoxicity observed at 24 h following soman challenge (palpebral closure, piloerection, a significant increase in the secretion from the nose, a significant decrease in exploratory activity, the alteration of gait including the decrease in mobility and unprovoked activity, forelimb and hindlimb grip strength, the distance between hindpaws after a jump, and spontaneous horizontal as well as vertical motor activity — $P < .05$), neither obidoxime alone nor obidoxime in combination with atropine is able to eliminate or at least decrease the intensity of above mentioned soman-induced signs of neurotoxicity. On the contrary, some signs of neurotoxicity are even more intensive compared to nontreated soman-poisoned rats (Table 2).

The significant decrease in landing foot splay and spontaneous horizontal as well as vertical motor activity in soman-poisoned rats were only observed at 7 days following soman administration ($P < .05$). While atropine alone or in combination with obidoxime is able to eliminate these signs of soman-induced neurotoxicity, soman-poisoned rats treated with obidoxime alone showed the same neurotoxic signs as nontreated soman-poisoned rats (Table 3). On the other hand, compared to the control animals, some signs of atropinization (the decrease in urination, the increase in mobility and horizontal as well as vertical motor activity — $P < .05$) were observed in soman-poisoned rats treated with atropine alone or with atropine in combination with obidoxime (Table 3).

4. Discussion

In the case of the treatment of soman-poisoned rats with atropine alone, the elimination of soman-induced neurotoxic effects at 24 h as well as 7 days following soman challenge was demonstrated. Thus, atropine alone is able to antagonize soman-induced neurotoxic effects in the case of sublethal poisoning due to its peripheral as well as central antimuscarinic effects [14,15,22]. Nevertheless, atropine alone fails to prevent soman-induced seizures and subsequent neurotoxic effects including the brain damage following an exposure to soman at lethal and supralethal doses because it is considered to be a muscarinic blocker with a relatively low central antimuscarinic activity in comparison with other anticholinergic drugs such as benactyzine, biperiden, and scopolamine [8,15,16].

Therefore, the anticholinergic drug such as atropine should be combined with AChE reactivator for the antidotal treatment of soman poisonings to improve its efficacy. One of the currently used, commercially available oximes for the treatment of poisonings with highly toxic organophosphates is obidoxime [4]. Unfortunately, obidoxime alone seems to be practically ineffective to prevent soman-induced neurotoxicity according to our findings. In addition, it is not able to protect poisoned animals from the lethal effects of soman at the dose studied. Our results even show that obidoxime can cause the increase in the intensity of some soman-induced neurotoxic signs. The absence of therapeutic efficacy of obidoxime against soman is possible to explain by very low affinity for intact as well as soman-phosphonylated AChE and by very low potency of obidoxime in reactivating soman-inhibited AChE in vitro as well as in vivo. It is practically without chance to reactivate soman-inhibited AChE when it is injected following soman challenge

[9,10,19]. Moreover, it penetrates the blood–brain barrier in not enough concentrations to counteract soman-induced central cholinergic effects (i.e., central respiratory depression) [3]. In addition, obidoxime was found to be not only ineffective in antagonizing soman-induced neurotoxicity but also to make some neurotoxic signs worse. This fact is supported by earlier in vitro demonstration of the ability of obidoxime in human relevant doses to make AChE inhibition by soman worse [9].

The combination of atropine with obidoxime is able to protect the animals from the lethal effects of soman at the dose studied, nevertheless, this antidotal mixture is significantly less effective compared to atropine alone. Therefore, the obidoxime when combined with atropine counteracts the beneficial effect of atropine because of increasing soman-induced AChE inhibition, perhaps, by the production of phosphonyloxime complexes with AChE inhibiting activity [9].

In conclusion, obidoxime is not suitable oxime for the treatment of soman poisonings because it is not sufficiently effective to reactivate soman-inhibited AChE and eliminate soman-induced toxic effects and, even, it can counteract the beneficial effects of atropine. Therefore, it is necessary to replace this presently used, commercially available oxime by more effective AChE reactivators. According to the recent data, H oximes, especially HI-6, seem to be more effective to counteract soman-induced toxic effects [4,7,9,19,24].

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