ORIGINAL ARTICLE

ANTICHOLINERGIC DRUGS – FUNCTIONAL ANTIDOTES FOR THE TREATMENT OF TABUN INTOXICATION

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Summary: 1. To study the influence of antidotes on tabun-induced neurotoxicity, the rats were injected intramuscularly with organophosphate tabun (LD_{50}). The efficacy of choice antidotal treatment consisting of acetylcholinesterase reactivator obidoxime and one of four anticholinergic drugs (atropine, benactyzine, biperiden, scopolamine) was compared. 2. Testing of tabun-induced neurotoxicity progress was carried out using the method Functional observational battery. The experimental animals as well as controls were observed at 24 hours and 7 days following tabun or saline administration. 3. The results were compared to the condition of animals without anticholinergic drug (oxime alone) and control rats that received physiological solution instead of tabun and treatment. Antidotal treatment involving centrally acting anticholinergic drugs (benactyzine, biperiden, scopolamine) showed significantly higher neuroprotective efficacy compared to antidotal treatment containing atropine.

Key words: *Nerve agents; Tabun; Atropine; Benactyzine; Biperiden; Scopolamine; Functional observational battery*

Introduction

The inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) by organophosphorus compounds (OPs) causes acute toxicity or death of the intoxicated animals because of endogenic increase in acetylcholine (ACh) level in the cholinergic nervous system, that leads to muscle fasciculations, respiratory distress and epileptic fits. Consequential generalized seizures lead to severe incapacitation and to brain damage with lesions especially in hippocampus, piriform cortex (12,16) and other cortical structures (8). The lesion is a highly selective distal axonopathy affecting the distal axons of sensory, motor, and autonomic neurons, and the longer central tracts. The lower motor neuron axonopathy is reversible, but not that of the upper motor neuron (17). Within the central nervous system, excitotoxicity has been described as a consequence of poisoning. Pathogenesis probably involves a combination of hypoxia and cholinergically mediated excitotoxicity with secondary recruitment of glutamatergic excitotoxicity (14). One group of these compounds, called nerve agents, pose an increasing threat in the world due to their possible use in the battlefield or terrorist acts. Therefore, inactivation of nerve agents has become a subject of major importance. Antidotes containing oxime compounds to reactivate the inhibited enzyme AChE are highly valued for the treatment of OP poisonings (7). Apart from oximes, the current antidotal treatment of nerve agent-induced acute poisoning includes anticholinergic drugs to antagonize the effects of ACh excess at cholinergic receptor sites (15).

Methods

Male albino Wistar rats weighing 180–220 g were purchased from Konárovice (Czech Republic) and used in our experiment. The animals were kept in an air-conditioned room and allowed to access to standard food and tap water ad libitum. They were divided into groups of eight animals. Handling of the experimental animals was done under the supervision of the Special Committee of the Ministry of Defence according to § 23 paragraph 1 a); the low of the Czech Republic no. 246/1992.

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

The anticholinergic drugs (atropine, benactyzine, biperiden, scopolamine) in combination with obidoxime were used as antidotal treatment and were carried out by i.m. injection 1 min following tabun administration at a lethal dose (130 μg/kg b.w. – LD_{50}). The doses of obidoxime (4.2 mg/kg b.w.) and anticholinergic drugs atropine (25.2 mg/kg

Tab. 1: Functional Observational Battery (FOB).

MARKER	Scored values only									
	-2	-1	0	1	2	3	4	5	6	7
POSTURE				*sitting or standing*	*rearing*	*asleep*	flattened	lying on side	crouched over	head bobbing
CATCH DIFFICULTY				passive	*normal*	elevated activity	flight	escape	aggrres-sion	
EASE OF HANDLING				passive	*normal*	moderately difficult	difficult			
MUSCULAR TONUS	atonia	hypotonia	*normal*	hypertonia	rigidity	fasciculations				
LACRIMATION			*none*	slight	severe	crusta	coloured crusta			
PALPEBRAL CLOSURE				open	slightly drooping	half-way drooping	completely shut	ptosis		
ENDO-EXOPHTHALMUS		endo	*normal*	exo						
FUR ABNORMALITIES			*normal*	coloured	tousled	color.+ tousl.	blaze	injury	other changes	pilo-erection
SKIN ABNORMALITIES			*normal*	pale	erythema	cyanosis	pigmented	cold	injury	
SALIVATION			*none*	sllight	severe					
NOSE SECRETION			*none*	slight	severe	coloured				
CLONIC MOVEMENTS			*normal*	repetitive movements of mouth and jaws	non-rhythmic quivers	mild tremors	severe tremors	myoclonic jerks	clonic convulsions	wet dog shakes
TONIC MOVEMENTS			*normal*	contraction of extensors	opistho-tonus	emprostho-tonus	explosive jumps	tonic convul-sions		
GAIT			*normal*	ataxia	overcom-pensation of hindlimbs move-ments	feet point outwards from body	forelimbs are extended	walks on tiptoes	hunched body	body is flattened against surface
GAIT SCORE				*normal*	slightly impaired	somewhat impaired	totally impaired			
MOBILITY SCORE				*normal*	slightly impaired	somewhat impaired	totally impaired			
AROUSAL (level of unprovoked activity)				very low	sporadic	reduced	*normal*	enhanced	permanent	
TENSION			*none*	partial (ears)	stupor					
TENSION			*none*	partial (ears)	stupor					
STEREOTYPY			*none*	head weaving	body weaving	grooming	circling	others		
BIZARRE BEHAVIOR			*none*	head	body	self-mutilation	abnormal movements	others		
APPROACH RESPONSE				no reaction	*normal*	freeze	energetic reaction	exaggerated reaction		
TOUCH RESPONSE				no reaction	*normal*	freeze	energetic reaction	exaggerated reaction		
CLICK RESPONSE				no reaction	*normal*	freeze	energetic reaction	exaggerated reaction		
TAIL - PINCH RESPONSE				no reaction reaction	*normal*	ffreeze	energetic reaction	exaggerated reaction		
PUPIL SIZE		miosis	*normal*	mydriasis						
PUPIL SIZE	miosis consider-able	miosis slight	*normal*	mydriasis slight	mydriasis consider-able					
PUPIL RESPONSE			no reaction	*normal reaction*						
RIGHTING REFLEX				*normal*	slightly uncoordin.	lands on side	lands on back	rise from back spon-taneously	rise from back with stimulus	no reaction

b.w.), benactyzine (3.7 mg/kg b.w.), biperiden (4.1 mg/kg b.w.) and scopolamine (15.2 mg/kg b.w.) correspond to human-relevant doses (2 % of their LD_{50}) (1,5). The neurotoxicity of tabun was monitored using the Functional observational battery (FOB) at 24 hours and 7 days following tabun poisoning. The evaluated markers of tabun-induced neurotoxicity in experimental animals were compared to the parameters obtained from control rats, administered with saline instead of tabun and antidotes at the same volume (0.1 ml/100 g b. w.).

The FOB consists of 40 measures of sensory, motor and autonomic functions (Tab. 1) (3,9,10,11). First measurements were made while the animal was in the home cage. The observer evaluated each animal posture, palpebral closure, and presence or absence of convulsions. If convulsions were present, they were further categorized. Following observations in the home cage, the animal was removed and briefly held in the hand. Ease of removal and handling, skin and fur abnormalities, lacrimation, salivation and nose secretion were recorded.

Then, the rat was placed on a flat surface, which served as the open field covered with a clean absorbent pad. A timer was started for three minutes during which time the frequency of rearing responses was noted. At the same time, gait characteristics were noted and ranked, and arousal, tremor, convulsions and abnormal postures were evaluated. At the end of the time period (3 min), the number of faecal boluses and urine pools on the absorbent pad were recorded. Reflex testing followed next and consisted of recording each rat's responses to the frontal approach of a blunt object such as a pencil, a touch of an object to the posterior flank, and an auditory click stimulus. Reactivity to a pinch on the tail and the ability of the pupil to constrict in response to light were also assessed. These measures were followed by a test for the righting reflex, then by measures of forelimb and hindlimb grip strength, body weight, rectal temperature and finally hindlimb foot splay. The entire battery of tests required approximately from six to eight minutes per one 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 Purkyne Military Medical Academy, Hradec Králové, Czech Republic). The animals were placed for a short time (10 minutes) in the measuring cage and their movements (total, horizontal and vertical activity) were recorded.

Data recorded with the FOB and motor activity assessment include categorical, ordinal and continuous values. Statistical analyses were performed on a PC with a special interactive programme NTX (3). The categorical and ordinal values were formulated as contingency tables and judged consecutively by Chi-squared test of homogeneity, Concordance-Discordance test and Kruskal-Wallis test respectively. The continual data were assessed by successive statistical tests: CI for Delta, Bartlett test for Equality of Variance, Williams test and Test for Distribution Function. The differences were considered significant when $p<0.05$.

Results

The results obtained from testing at 24 hours or 7days following tabun poisoning are shown in Table 2 and 3. One non-treated tabun-poisoned rat and one tabun-poisoned rat treated with obidoxime alone died within 24 hours following tabun administration. All fully treated tabun-poisoned rats survived till the end of experiment (7 days following the intoxication). The evaluation of tabun-induced neurotoxic signs at 24 hours following intoxication proved significant alteration of 23 observed parameters (Tab. 2). All tested possibilities of antidotal treatment of tabun-poisoned rats brought marked improvement in many of studied parameters. The results confirm that an addition of atropine to obidoxime does not cause to an increase in neuroprotective effect in comparison with obidoxime alone. On the other hand, if obidoxime is combined with the centrally acting anticholinergic drugs (benactyzine, biperiden, scopolamine), the tabun-poisoned rats were higher protected from acute neurotoxicity compared to obidoxime alone. The sitting posture of animal, low rearing and significant differences in landing foot splay, grip strength, body weight and body temperature were recorded at 7 days following tabun administration. Nevertheless, it is not possible to differenciate the efficacy of all combinations of antidotes of tabun-poisoned rats because of very few tabun-induced signs of neurotoxicity (Tab. 3).

Discussion

Tabun-induced toxic effects are extraordinarily difficult to counteract due to very low reactivating efficacy of currently used oximes (2,4,13). The recent development of pharmacological protection from tabun-induced neurotoxicity focuses to revelation suitable prophylaxis and/or to current antidotal treatment. The anticholinergic drugs seem to be appropriate to enhance effectiveness of our protective possibilities. The previous published experiments (5,6) established potency of the anticholinergics as prophylaxis. Nowadays, the commonly used anticholinergic drug for antidotal treatment is peripherally acting atropine. In our study, we examined the efficacy of centrally acting anticholinergics in a role of antidote. Our results suggest that all studied centrally acting anticholinergic drugs (benactyzine, biperiden, scopolamine) in the combination with oxime are able to increase the protection of animals against tabun-induced neurotoxicity. Their neuroprotective effect was more marked compared to atropine, because no improvement of improvement of therapeutic effect of antidotal treatment consisting of atropine and obidoxime was observed compared to obidoxime alone. Among centrally acting anticholinergics, biperiden showed the highest effect. In conclusion, the administration of centrally acting anticholinergic drugs in combination with the obidoxime appears to be the hopeful antidotal treatment of poisoning with nerve agents such as tabun.

Tab. 2

24 hours		Controls		Tabun + Obidoxime + Biperiden		Tabun + Obidoxime + Benactyzine		Tabun + Obidoxime + Atropine		Tabun + Obidoxime + Scopolamine		Tabun + Obidoxime		Tabun	
No	Marker	x/m	±s	x/m	±s	x/m	±s	x/m	±s	x/m	±s	x/m	±s	x/m	±s
1	posture	1,63		1,50		1,57		2,75*		1,00		2,38		3,25*	
2	catch difficulty	2,00		1,63		1,14***		1,50***		1,43*		1,63*		1,63***	
3	ease of handling	2,00		1,63		1,14***		1,25***		1,43*		1,57**		1,75**	
4	muscular tonus	0,00		-0,38		-0,86***		-0,88***		-0,43		-0,63		-1,13***	
5	lacrimation	0,00		0,00		0,00		0,00		0,00		1,00		1,50*	
6	palpebral closure	1,00		1,00		1,00		1,00		1,00		1,50		1,50	
7	endo-exophthalmus	0,00		0,14		0,14		0,25		0,33		0,25		1,00***	
8	fur abnormalities	0,00		0,00		0,00		0,38		0,00		0,88		0,88	
9	skin abnormalities	0,00		0,00		0,00		0,25		0,43		0,00		0,00	
10	salivation	0,00		0,13		0,00		0,14		0,00		0,63		0,75***	
11	nose secretion	0,00		0,00		0,00		1,88**		0,00		0,75		1,75**	
12	rearing	18,00	5,86	15,38	29,68	7,86***	6,04	7,43**	5,26	3,00***	3,37	13,57	10,37	1,88***	1,36
13	urination	0,25		0,63		0,57		0,00		2,00		0,00		0,38	
14	defecation	0,00		0,13		0,38		0,00		0,63		0,25		0,00	
15	tremor	0,00		1,88		1,29		1,88		1,14		1,38		2,50	
16	clonic movements	0,00		0,25		0,14		0,00		0,00		0,38		0,38	
16	tonic movements	0,00		0,38		0,00		0,38		0,13		1,00		2,50	
17	gait	0,00		0,75*		0,86**		1,13***		0,29		1,38		1,75***	
18	gait score	1,00		1,88***		2,00***		2,00***		1,43*		1,88*		2,63***	
20	ataxia	0,00		0,25		0,75*		0,86***		0,29		0,50		1,13***	
19	mobility score	1,00		1,25		1,25		1,63**		1,13		1,63		2,38***	
20	arousal	4,00		3,75		3,50		3,63		3,63		3,75		2,75	
21	tension	0,00		0,25		0,00		0,00		0,00		0,25		0,25	
22	stereotypy	0,00		0,00		0,50		0,00		0,00		0,50		0,00	
23	bizzare behavior	0,00		0,00		0,00		0,00		0,00		0,00		0,00	
24	approach response	2,25		2,00		1,88		2,00		1,88		2,25		2,38	
25	touch response	2,00		2,00		1,80		2,00		2,10		1,80		1,80	
26	click response	2,00		2,00		2,13		2,00		2,25		1,88		2,25	
27	tail-pinch response	2,00		1,50*		2,00		1,75		1,75		1,50*		1,25***	
28	pupil size	0,00		-0,50		-0,13		-0,25		-0,38		-0,50		0,00	
29	pupil response	1,00		1,00		1,00		1,00		0,88		0,69		0,50	
30	righting reflex	1,00		1,00		1,00		1,13		1,13		2,13		2,00	
31	landing foot splay (mm)	94,13	20,14	103,31	26,75	86,71	13,05	69,75*	12,66	96,79	21,19	58,13*	32,67	58,44**	30,34
32	forelimb grip strength (kg)	3,53	1,44	2,94	0,97	2,67	0,43	2,74	1,09	2,47	0,36	2,08*	1,17	2,08*	1,11
33	hindlimb grip strength (kg)	0,96	0,43	0,97	0,43	0,92	0,12	0,63	0,43	0,81	0,14	1,18	1,44	0,48*	0,35
34	grip strength of all limbs (kg)	7,64	1,46	8,22	3,58	7,24	0,97	5,76*	1,11	6,76	0,83	5,28*	2,63	5,22*	2,36
35	food receiving (%)	100,00	0,00	100,00	0,00	100,00	0,00	100,00	0,00	100,00	0,00	50,00	0,00	50,00	0,00
36	body weight (g)	237,25	13,35	220,86	20,65	212,14**	12,29	219,13*	11,81	202,43**	16,43	209,14*	17,98	181,88	73,64
37	body temperature (oC)	37,16	0,39	37,14	0,30	37,27	0,29	36,60*	0,34	37,13	0,23	37,33	0,44	36,44*	0,57
38	vertical activity (No/10 min.)	95,86	24,86	60,50***	35,34	43,86***	31,40	0,88***	0,83	22,86***	44,93	29,13***	24,87	8,17***	13,38
39	horizontal activity (No/10 min.)	332,43	80,59	168,57***	88,68	178,14***	108,40	43,75***	23,37	120,71***	120,94	176,50**	146,86	66,17***	52,07
40	total motor activity (No/10 min.)	428,29	165,69	229,07***	101,07	222,00	135,96	44,63***	22,65	143,57***	163,76	205,63	170,62	74,34***	63,96
		n=8		n=8		n=8		n=8		n=8		n=7		n=7	

Tab. 3

7 days		Controls		Tabun + Obidoxime + Biperiden		Tabun + Obidoxime + Benactyzine		Tabun + Obidoxime + Atropine		Tabun + Obidoxime + Scopolamine		Tabun + Obidoxime		Tabun	
No	Marker	x/m	±s	x/m	±s	x/m	±s	x/m	±s	x/m	±s	x/m	±s	x/m	±s
1	posture	1,88		1,38*		1,25***		1,63		1,25***		2,38*		2,38*	
2	catch difficulty	2,00		1,71		2,00		2,57		1,86		2,00		1,86	
3	ease of handling	2,00		1,75		2,00		1,75		1,88		2,00		1,88	
4	muscular tonus	0,00		0,00		0,00		0,25		-0,13		-0,38		-0,25	
5	lacrimation	0,00		0,00		0,00		0,00		0,00		0,50		0,50	
6	palpebral closure	1,00		1,00		1,00		1,00		1,00		1,50		1,50	
7	endo-exophthalmus	0,00		0,00		0,00		0,00		0,13		0,13		0,13	
8	fur abnormalities	0,00		0,00		0,00		0,00		0,00		1,13		0,88	
9	skin abnormalities	0,00		0,00		0,00		0,00		0,00		0,38		0,00	
10	salivation	0,00		0,00		0,00		0,00		0,00		0,25		0,29	
11	nose secretion	0,00		0,00		0,00		0,00		0,00		0,38		0,38	
12	rearing	11,25	5,28	7,38	7,03	6,86	5,24	1,88***	2,70	4,38*	4,10	3,83**	5,19	7,13	6,85
13	urination	0,38		0,00		1,71		0,00		0,00		0,14		0,14	
14	defecation	0,38		0,88		0,29		0,00		0,00		0,00		1,14	
15	tremor	0,00		0,00		0,00		0,38		0,00		0,63		0,71	
16	clonic movements	0,00		0,00		0,00		0,00		0,13		0,25		0,25	
16	tonic movements	0,00		0,25		0,00		0,13		0,13		0,63		0,86	
17	gait	0,00		0,75		0,25		0,75		0,38		1,38		1,14	
18	gait score	0,00		1,50		1,38		1,75		1,13		1,63		1,86	
20	ataxia	0,00		0,25		0,25		0,63		0,00		0,50		0,43	
19	mobility score	1,00		1,25		1,25		1,13		1,13		1,38		1,43	
20	arousal	3,88		3,00		3,38		3,13		3,50		2,75		3,71	
21	tension	0,00		0,13		0,13		0,13		0,13		0,25		0,71	
22	stereotypy	0,00		0,00		0,00		0,00		0,00		0,00		0,00	
23	bizzare behavior	0,00		0,00		0,00		0,00		0,00		0,00		0,00	
24	approach response	1,75		2,00		2,00		2,00		1,88		1,88		1,86	
25	touch response	1,50		2,00		2,00		2,00		1,88		1,88		1,86	
26	click response	2,13		2,00		1,63		2,25		1,88		2,00		2,29	
27	tail-pinch response	2,00		2,00		1,88		2,00		2,25		1,63		1,71	
28	pupil size	0,00		0,00		-25,00		-0,38		-0,13		-0,25		-0,29	
29	pupil response	1,00		1,00		1,00		0,88		1,00		0,88		0,86	
30	righting reflex	1,00		1,00		1,13		1,25		1,00		1,75		1,86	
31	landing foot splay (mm)	108,63	13,52	93,63	22,56	88,69	22,94	87,56	25,70	81,94*	16,94	74,86*	35,52	73,00*	37,29
32	forelimb grip strength (kg)	3,56	0,49	3,13	0,63	3,05*	0,37	3,06	0,71	2,67*	0,58	2,73*	0,41	2,42*	0,61
33	hindlimb grip strength (kg)	0,76	0,32	1,16*	0,18	1,54*	0,90	1,13*	0,32	1,06	0,22	1,08	0,36	0,80	0,29
34	grip strength of all limbs (kg)	8,78	0,60	8,46	1,77	8,98	1,32	7,52*	1,17	8,66	1,11	7,67	1,21	7,75	1,33
35	food receiving (%)	100,00	0,00	100,00	0,00	100,00	0,00	100,00	0,00	100,00	0,00	100,00	1,00	100,00	0,00
36	body weight (g)	262,50	14,78	245,50*	16,23	242,50*	10,54	253,63	13,14	239,25*	18,70	238,67	32,30	246,43*	8,56
37	body temperature (oC)	37,21	0,44	37,65	0,58	37,83*	0,62	36,6**	0,34	37,81*	0,55	37,68	0,44	37,80	0,29
38	vertical activity (No/10 min.)	33,63	26,25	60,00	50,52	48,86	34,552	20,71	22,79	53,50	79,24	13,33	12,06	10,29	14,66
39	horizontal activity (No/10 min.)	168,50	101,55	229,00	87,80	217,29	123,39	158,71	123,77	204,50	157,52	125,17	114,58	74,14*	49,49
40	total motor activity (No/10 min.)	202,13	125,25	289,00	132,80	266,15	170,63	179,42	148,67	258,00	222,47	138,50	123,54	84,43	64,79
		n=8		n=8		n=8		n=8		n=8		n=7		n=7	

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