ACUTE EXPERIMENTAL TABUN-INDUCED INTOXICATION AND ITS THERAPY IN RATS

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SUMMARY

Pharmacological pretreatment and antidotal treatment on tabun-induced neurotoxicity were studied in male albino rats that were poisoned with a lethal dose of tabun (280 μg/kg i.m.; 100% of LD_{50} value) and observed at 24 hours and 7 days following tabun challenge. The neurotoxicity of tabun was evaluated using a Functional observational battery and an automatic measurement of motor activity.

Pharmacological pretreatment as well as antidotal treatment were able to reverse most of tabun-induced neurotoxic signs observed at 24 hours following tabun poisoning. However, there was not significant difference between the efficacy of profylaxis and antidotal treatment to eliminate tabun-induced neurotoxicity. The combination of profylactic pretreatment and antidotal treatment seems to be slightly more effective in the elimination of tabun-induced neurotoxicity in rats at 24 hours following tabun challenge in comparison with the administration of profylactic pretreatment or antidotal treatment alone. At 7 days following tabun poisoning, very few neurotoxic signs in tabun-poisoned rats were observed regardless of administration of pharmacological pretreatment or antidotal treatment.

Thus, our findings confirm that the combination of pharmacological pretreatment and antidotal treatment is not only able to protect the experimental animals from the lethal effects of tabun but also to eliminate most of tabun-induced signs of neurotoxicity in tabun-poisoned rats.

Key words: neurotoxicity, PANPAL, tabun, FOB, atropine, obidoxime, rats

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INTRODUCTION

Development of the inactivation of extremely toxic organophosphorus compounds (nerve agents) has become a subject of major importance in connection with international events of the recent years. Nerve agents effect is related to their potency to irreversibly inhibit acetylcholinesterase (AChE, EC 3.1.1.7), the enzyme responsible for the regulation of neurotransmitter acetylcholine (ACh) concentration at cholinergic synapses (1). The inhibition of AChE induces a major increase in ACh level in the cholinergic nervous system producing muscle fasciculations, respiratory distress and epileptic fits leading to the generalized seizures. In surviving animals, the seizures lead to severe incapacitation and to irreversible brain damage with lesions especially in hippocampus, piriform cortex and other cortical structures (2, 3).

The current antidotal treatment of nerve agent-induced acute poisoning usually consists of anticholinergic drugs to antagonize the effects of ACh excess at cholinergic receptor sites and oximes to reactivate nerve agent-inhibited AChE (4). Unfortunately, some organophosphates were found to be resistant to standard antidotal treatment. One of the most resistant organophosphorus compound is tabun (ethyl-N,N-dimethyl phosphoramidocyanidate). Its deleterious effects are extraordinarily difficult to counteract because of the existence of a free electron pair located on amidic nitrogen that makes the nucleophilic attack of oximes almost impossible (5). According to various studies, obidoxime has higher reactivating efficacy for tabun-inhibited AChE than currently used oximes such as pralidoxime and HI-6 (6).

The relatively unsatisfactory treatment available for acute nerve agent poisoning has prompted studies of pretreatment pos-

Table 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	aggrression	
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	piloerection
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	nonrhythmic quivers	mild tremors	severe tremors	myoclonic jerks	clonic convul-sions	wet dog shakes
Tonic movements			normal	contraction of exten-sors	opisthotonus	emprosthotonus	explosive jumps	tonic convulsions		
Gait			normal	ataxia	overcompensation of hindlimbs movements	feet point outwards from body	forelimbs are extended	walks on tiptoes	hunched body	body is flattened against surface
Ataxia			none	slight	severe					
Gait score				normal	slightly impaired	somewhat impaired	totally impaired			
Mobility score				normal	slightly impaired	somewhat impaired	totally impaired			
Arousal (level of unpro-voked activity)				very low	sporadic	reduced	normal	enhanced	permanent	
Tension			none	partial (ears)	stupor					
Stereotypy			none	head weaving	body weaving	grooming	circling	others		
Bizarre behavior			none	head	body	self-mutilation	abnormal move-ments	others		
Approach response				no reaction	normal	freeze	energetic reaction	exaggerated reaction		

to be continued…

sibilities that allow survival and increase resistance of organisms exposed to nerve agents. Currently used method of protection against nerve agent poisoning is the use of pyridostigmine bromide, a reversible carbamate AChE inhibitor in combination with anticholinergic drugs benactyzine (BNZ) and trihexyphenidyle (THP), designated PANPAL, has been developed in the CR and introduced to the Czech Army (7).

The aim of this study was to evaluate the neuroprotective effects of pharmacological pretreatment PANPAL with or without antidotal treatment consisting of obidoxime and atropine in tabun-poisoned rats.

METHODS

Animals used in our experiments were male albino Wistar rats weighing 180-220g purchased from Konárovice (CR). 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 ten animals (n=10). Handling of the experimental animals was done under the supervision of the Ethics Committee of the Medical Faculty of Charles University and Purkyne Military Medical Academy in Hradec Králové (CR).

Tabun of 89.25% purity was obtained from Military Technical Institute in Brno (CR). The oxime was synthesised at the Department of Toxicology of the Military Medical Academy and was 98% pure. All other chemicals and drugs of analytical grade were obtained commercially and used without further purification. Pyridostigmine (5.82 mg/kg of body weight) in combination with BNZ (70 mg/kg of body weight) and THP (16 mg/kg of body weight) was administered perorally (p.o.) as solution in distilled water (0.2 ml/100g of body weight) 120 min before intramuscular (i.m.) tabun challenge at a lethal dose (280 μg/kg b.w. – LD_{50}). Antidotal treatment (obidoxime in combination with atropine) was carried out by i.m. injection 1 min following tabun administration. The doses of obidoxime (3.2 mg/kg of body weight) and anticholinergic drug atropine (25.2 mg/kg of body weight) correspond to human-relevant doses (2% of their LD_{50}) (8, 9). The neurotoxicity of tabun was monitored using the method 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 (Table 1) (10). 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, CR).

Statistical analyses were performed on a PC with a special interactive programme NTX (11). The differences were considered significant when $p<0.05$.

RESULTS

The list of the evaluation results of tabun-induced neurotoxicity is recorded in Table 2. The evaluation of tabun-induced neurotoxic signs at 24 hours following intoxication proved significant alteration of 29 observed parameters (Table 2). All three combinations of protection of tabun-poisoned rats brought marked improvement

Table 1. *Functional Observational Battery (FOB) – continued*

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	normal	freeze	energetic reaction	exaggerated reaction			
Pupil size	miosis considerable	miosis slight	mydriasis slight	mydriasis considerable						
Pupil response			normal reaction							
Righting reflex			normal	slightly uncoordin.	lands on side	lands on back	rise from back spontaneously	rise from back with stimulus	no reaction	

Table 2. *The values of tabun-induced neurotoxic markers (x ± s) measured at 24 hours following tabun challenge by FOB*

	24 hours	**Controls**		**Tabun**		**Panpal+Tabun**		**Tabun + A + Obidoxime**		**Panpal+Tabun + A + Obidoxime**	
No	Marker	x/M	±s	x/M	±s	x/M	±s	x/M	±s	x/M	±s
1	posture	1.00		3.00		1.00		1.00		3.00	
2	catch difficulty	2.00		1.00***		2.00		1.00***		2.00	
3	ease of handling	2.00		1.00***		2.00		2.00		2.00	
4	muscular tonus	0.00		-1.00***		-1.00***		0.00		1.00*	
5	lacrimation	0.00		4.00*		0.00		0.00		0.00	
6	palpebral closure	1.00		1.00*		1.00		1.00		1.00	
7	endo-exophthalmus	0.00		0.00		0.00		0.00		0.00	
8	fur abnormalities	0.00		0.00*		0.00		0.00		0.00	
9	skin abnormalities	0.00		0.00*		0.00		0.00		0.00	
10	salivation	0.00		1.00***		0.00		0.00		0.00	
11	nose secretion	0.00		3.00***		0.00		0.00		0.00	
12	rearing	11.60	6.57	2.43***	2.51	3.30***	0.00	6.00*	3.59	1.90***	2.08
13	urination	0.00		0.00		0.00		0.00		0.00	
14	defecation	1.00		0.00***		1.00		0.00***		1.00	
15	clonic movements	0.00		0.00*		0.00		0.00		0.00	
16	tonic movements	0.00		0.00*		0.00		0.00		0.00	
17	gait	0.00		6.00***		1.00***		0.00		1.00*	
18	ataxia	0.00		2.00***		1.00***		0.00		0.00	
19	gait score	1.00		3.00***		2.00***		1.00		2.00*	
20	mobility score	1.00		2.00*		1.00		1.00		1.00	
21	arousal	4.00		4.00		2.00*		4.00		4.00	
22	tension	0.00		0.00		0.00		0.00		0.00	
23	stereotypy	0.00		0.00		0.00		0.00		0.00	
24	bizzare behavior	0.00		0.00		0.00		0.00		0.00	
25	approach response	2.00		1.00***		2.00		2.00		2.00	
26	touch response	1.00		1.00		1.00		1.00		1.00	
27	click response	2.00		2.00		2.00		2.00		2.00	
28	tail-pinch response	2.00		2.00		1.00		2.00		2.00	
29	pupil size	0.00		0.00*		0.00		0.00		0.00	
30	pupil response	1.00		0.50*		1.00		1.00		1.00	
31	righting reflex	1.00		1.00*		1.00		1.00		1.00	
32	landing foot splay (mm)	94.80	15.75	43.00***	30.25	84.35	16.55	82.1*	10.06	87.30	17.70
33	forelimb grip strength (kg)	2.26	0.74	1.43***	1.20	2.68	0.47	2.05	0.51	2.34	0.63
34	hindlimb grip strength (kg)	1.04	0.20	0.74***	0.65	0.89	0.22	0.76***	0.12	0.29	0.89
35	grip strength of all limbs (kg)	5.63	0.72	2.64***	1.79	5.22	0.55	4.63***	0.63	4.73**	0.61
36	food receiving (%)	100.00	0.00	50.00***	0.00	95.00*	5.27	100.00	0.00	100.00	0.00
37	body weight (g)	221.90	16.43	193.57**	14.36	221.80	7.41	216.40	10.45	223.10	12.68
38	body temperature (oC)	37.61	0.42	37.22*	0.20	37.87	0.24	37.20*	0.36	37.84	0.41
39	vertical activity	78.00	44.56	3.00***	6.03	40.90*	27.82	23.30***	24.21	12.70***	13.35
40	horizontal activity	320.70	90.60	38.57***	50.22	200.60*	104.41	156.9***	81.38	115.20***	78.17
41	total motor activity (No/10 min.)	398.70	130.20	41.57***	55.79	241.50*	129.77	180.20***	102.40	127.90***	89.22
		n=10		n=7		n=10		n=10		n=10	

*No 1-30 – scored values, No 31-40 – values in absolute units. Statistical significance: * p < 0.05; ** p < 0.01; *** p < 0.005 (comparison with the control values).*

in the most of altered parameters. Rats pretreated by PANPAL and treated by atropine in combination with obidoxime showed the best protection from tabun-induced neurotoxicity compared to other groups at 24 hours following tabun challenge. The combination of PANPAL pretreatment and antidotal treatment was able to eliminate the most of tabun-induced signs of neurotoxicity (Table 2).

The change in mobility score, a decrease in the distance between hindpaws after a jump and a decrease in forelimb grip strength in tabun-poisoned rats were only observed at 7 days following tabun administration. Almost all signs of tabun-induced neurotoxicity were eliminated by PANPAL pretreatment as well as by antidotal treatment with atropine in combination with obidoxime.

DISCUSSION

PANPAL seems to be sufficiently effective pretreatment of tabun-exposed laboratory animals. The similar beneficial effect of PANPAL was also observed in the case of experimental soman poisoning. The beneficial effect of PANPAL is probably caused not only by pyridostigmine-induced protection of peripheral AChE from irreversible inhibition by nerve agents but also by anticholinergic drug-induced decrease in the cholinergic and stress causing effects of nerve agents (12). Therefore, the ability of PANPAL to increase the neuroprotective effects of antidotal treatment of acute tabun poisoning may be expected.

According to our results, PANPAL was found to be suitable pharmacological pretreatment of tabun exposure and beneficial to be combined with antidotal treatment to counteract tabun-induced toxic effect. Our findings tally with the previous study where PANPAL was presented to be effective in enhancing the efficacy of antidotal treatment to protect experimental animals poisoned with lethal doses of tabun (9). Thus, PANPAL seems to be effective to reduce signs of nerve agent-induced neurotoxicity not only in the case of soman poisoning but also in the case of tabun poisoning. Therefore, it should be considered as means for the currently used pretreatment of the nerve agent poisoning, especially in the case of the threat of exposure to soman or tabun.

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