A Comparison of the Neuroprotective and Reactivating Efficacy of a Novel Bispyridinium Oxime K870 with Commonly Used Pralidoxime and the Oxime HI-6 in Tabun-Poisoned Rats

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ABSTRACT

Aim: The comparison of neuroprotective and central reactivating effects of the oxime K870 in combination with atropine with the efficacy of standard antidotal treatment in tabun-poisoned rats.

Methods: The neuroprotective effects of antidotal treatment were determined in rats poisoned with tabun at a sublethal dose using a functional observational battery 2 h and 24 h after tabun administration, the tabun-induced brain damage was investigated by the histopathological evaluation and central reactivating effects of oximes was evaluated by the determination of acetylcholinesterase activity in the brain using a standard spectrophotometric method.

Results: The central reactivating efficacy of a newly developed oxime K870 roughly corresponds to the central reactivating efficacy of pralidoxime while the ability of the oxime HI-6 to reactivate tabun-inhibited acetylcholinesterase in the brain was negligible. The ability of the oxime K870 to decrease tabun-induced acute neurotoxicity was slightly higher than that of pralidoxime and similar to the oxime HI-6. These results roughly correspond to the histopathological evaluation of tabun-induced brain damage.

Conclusion: The newly synthesized oxime K870 is not a suitable replacement for commonly used oximes in the antidotal treatment of acute tabun poisonings because its neuroprotective efficacy is only slightly higher or similar compared to studied currently used oximes.

KEYWORDS

tabun; acetylcholinesterase; neurotoxicity; functional observational battery; histopathology; oximes; rats

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INTRODUCTION

Nerve agents including tabun are highly toxic organophosphorus compounds that were developed and stockpiled for use as chemical warfare agents (1). Their main toxic mechanism is based on their covalent binding to the active site of acetylcholinesterase (AChE, EC 3.1.1.7) resulting in AChE irreversible inhibition and development of cholinergic crisis. As some nerve agents including tabun easily penetrate through the blood-brain barrier (BBB), they can cause centrally mediated seizure activity that can rapidly progress to status epilepticus and contribute to profound brain damage (2–4).

Tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate) is one of the most resistant nerve agents. It differs from other highly toxic organophosphates by its chemical structure and by the fact that commonly used antidotes (atropine in combination with an oxime) are not able to sufficiently eliminate tabun-induced acute toxic effects. Its acute toxic 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, 6).

Severe intoxication with tabun usually leads to centrally mediated seizure activity and contribute to brain damage that is associated with long-lasting neurological and psychological injuries (7, 8). Therefore, the ability of antidotes to counteract acute neurotoxic effects of tabun and prevent tabun-poisoned organisms from irreversible lesions in the brain is very important for the successful antidotal treatment of acute tabun poisonings (2). However, all commonly used oximes are not able to sufficiently reactivate tabun-inhibited AChE and, in addition, their central reactivating efficacy is generally very low due to their low penetration through BBB (9–11).

Therefore, the development of new oxime or non-oxime reactivators of tabun-inhibited AChE has been a long-standing goal for the treatment of tabun poisoning. For this purpose, a novel dichlorinated bispyridinium oxime K870 with a double bond in the linker: *4-carbamoyl-1-[(2E)-4-{3.5-dichloro-4-[(hydroxyimino)methyl]-pyridinium-1-yl}but-2-en-1-yl]pyridinium dibromide* was synthesized to improve the efficacy of antidotal treatment in reactivating tabun-inhibited AChE and eliminating tabun-induced acute neurotoxic effects (12).

The aim of this study was to compare the potential neuroprotective and central reactivating effects of newly developed oxime K870 with the oxime HI-6 and pralidoxime in combination with an anticholinergic drug atropine in tabun-poisoned rats. The tabun-induced neurotoxic signs and symptoms were determined using a functional observational battery (FOB), the tabun-induced brain damage was investigated by the histopathological evaluation using hematoxylin-eosin staining.

MATERIAL AND METHODS

ANIMALS

Male Wistar albino rats (6-week-old, 240–270 g, VELAZ, Prague, Czech Republic) without genetic modification were kept in an accreditated animal facility (22 ± 2 °C and 50 ± 10% relative humidity, 12-h day-night cycle) and allowed access to standard food (VELAZ) and tap water *ad libitum*. Handling of the experimental animals was approved by the Ethics Committee of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic) and were conducted in accordance with the Animal Protection Law and Animal Protection Regulations.

CHEMICALS

Tabun was obtained from the Technical Institute in Brno (Czech Republic) and was 90% pure. Its purity was assayed by acidimetric titration. The basic solution of tabun (1mg/1mL) was prepared in propyleneglycol three days before starting the experiments. Actual solution of tabun was prepared from its basic solution with the help of saline immediately before its administration. Two oximes (pralidoxime, HI-6) were synthesized at the Department of Toxicology and Military Pharmacy of the Faculty of Military Health Sciences (University of Defence, Hradec Kralove, Czech Republic), the oxime K870 was synthesized at the Department of Chemistry of the Faculty of Science (University of Hradec Kralove, Czech Republic). Their purity was analyzed using HPLC technique with UV detection (310 nm) and they were more than 96% pure (13). All other drugs and chemicals of analytical grade were obtained commercially (Sigma-Aldrich, St. Louis, MO, USA) and used without further purification. The saline solution (0.9% NaCl, B Braun Melsungen AG, Melsungen, Germany) was used as a vehicle.

IN VIVO EXPERIMENTS

Animals were randomly divided into 6 groups (8 rats each): 1) a control group (administered with saline), 2) tabun-poisoned group ((220 μg/kg – 95% LD_{50}), 3) tabun- poisoned group treated with atropine sulphate alone (10 mg/kg), 4) tabun-poisoned group treated with atropine sulphate and K870 (100 mg/kg), 5) tabun-poisoned group treated with atropine sulphate and pralidoxime (197 mg/kg), 6) tabun-poisoned group treated with atropine sulphate and HI-6 (81 mg/kg).

Tabun was administered intramuscularly (i.m.) at a sublethal dose (220 μg/kg – 95% LD_{50}). One minute following tabun poisoning, the rats were treated i.m. with atropine (10 mg/kg b.w.) alone or in combination with the oxime HI-6 (81 mg/kg), pralidoxime (197 mg/kg) or K870 (100 mg/kg) at maximal tolerated doses (MTD, unpublished observations). All substances were administered intramuscularly (i. m.) at a volume of 1 mL/kg body weight (b.w.).

The neurotoxicity of tabun was monitored using FOB at 2 and 24 h following tabun poisoning. The evaluated markers of tabun-induced neurotoxicity in experimental animals were compared with the parameters obtained from control rats given saline instead of tabun and antidotes at the same volume (1 mL/kg b.w.). FOB consists of 42 measurements of sensory, motor and autonomic nervous functions. Some of them are scored (14), the others are measured in absolute units. The description of this method including statistical analysis has been already published (14, 15).

MEASUREMENT OF ACHE ACTIVITY IN THE BRAIN

To evaluate the reactivating efficacy of the oximes in the brain, the rats were decapitated after FOB test (24 h 30 min after intoxication), totally exsanguinated and the brain was rapidly removed, cut in half along the mid-sagittal plane and one half of the brain was immediately frozen at the temperature −70 °C. All experiments were perfomed in the same part of the day (from 08:00 h to 10:00 h). The spectrophotometric method of AChE activity determination and percentage of reactivation calculation including the statistical analysis was described previously (16, 17).

HEMATOXYLIN-EOSIN STAINING AND HISTOPATHOLOGY EVALUATION

To evaluate histopathological changes after tabun poisoning, second half of brain was fixed with a 10% neutral buffered formalin (Bamed s.r.o., Ceske Budejovic, Czech Republic). The description of sample preparation and the method of hematoxylin-eosin staining as well as histopathology evaluation including statistical analysis has been previously published (14, 18).

RESULTS

THE EVALUATION OF NEUPROTECTIVE EFFICACY OF OXIMES STUDIED IN TABUN-POISONED RATS

The results of the experiments related to the measurement of tabun-induced neurotoxicity at 2 hours following tabun poisoning are divided into three parts (activity and neuromuscular measures, sensorimotor and excitability measures and autonomic measures) (15) and summarized in Table 1a–c. While five non-treated tabun-poisoned rats, two tabun-poisoned rats treated with atropine alone and one tabun-poisoned rat treated with atropine and pralidoxime died before the evaluation of tabun-induced neurotoxicity by FOB, all tabun-poisoned rats treated with atropine in combination with K870 or the oxime HI-6 survived till the end of experiment. The evaluation of tabun-induced neurotoxic signs and symptoms at 2 h following intoxication proved significant alteration of practically all observed parameters with the exception of urination, defecation and himdlimb grip strength (Table 1a–c). All types of antidotal treatment of tabun poisoning including atropine alone were able to prevent some tabun-induced signs of neurotoxicity observed at 2 h following tabun challenge

Tab. 1a The values of tabun-induced activity and neuromuscular neurotoxic markers measured at 2 hours following tabun challenge by the functional observational battery (No 1–2, 4–14 – scored values, No 3, 15–18 – values in absolute units). Statistical significance: * $p \leq 0.05$ (comparison with the control values). Applied abbreviations: RRF, air righting reflex from back position; RRV, air righting reflex from vertical position; x/M, average or modus value; ± s, standard deviation; A, atropine; n, number of surviving animals.

2 hours		Controls		Tabun + A + pralidoxime		Tabun + A + HI-6		Tabun + A + K870		Tabun + atropine		Tabun	
No	Marker	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s
1	posture	1.00		3.00		3.00		3.00		**3.00***		**7.00***	
2	muscular tonus	0.00		**-1.00***		0.00		0.00		0.00		**-2.00***	
3	rearing	10.13	6.73	**0.38***	1.06	**0.88***	1.36	**2.63***	2.88	**1.50***	2.00	**0.25***	0.71
4	hyperkinesis	0.00		**2.00***		**2.00***		0.00		0.00		**7.00***	
5	tremors	0.00		0.00		**3.00***		0.00		0.00		**5.00***	
6	clonic movements	0.00		0.00		0.00		0.00		0.00		**2.00***	
7	tonic movements	0.00		0.00		0.00		0.00		0.00		**5.00***	
8	gait	0.00		**1.00***		0.00		0.00		**1.00***		**7.00***	
9	ataxia	0.00		**1.00***		0.00		0.00		**1.00***		**2.00***	
10	paresis	0.00		0.00		0.00		0.00		0.00		**2.00***	
11	mobility score	1.00		1.00		1.00		1.00		1.00		**4.00***	
12	activity	4.00		3.00		3.00		4.00		4.00		**1.00***	
13	RRF	1.00		**2.00***		1.00		1.00		**2.00***		**7.00***	
14	RRV	1.00		**2.00***		1.00		1.00		**2.00***		**4.00***	
15	landing foot splay (mm)	9.70	1.61	5.73	3.97	7.05	3.09	8.76	1.88	8.34	1.20	**2.63***	3.81
16	forelimb grip strength (kg)	5.89	1.29	5.13	3.56	8.11	3.63	6.76	0.70	6.33	1.44	**2.21***	3.28
17	hindlimb grip strength (kg)	2.73	0.46	2.96	3.51	2.69	2.69	1.69	0.66	1.26	0.43	0.91	1.27
18	grip strength of all limbs (kg)	12.01	1.66	**3.86***	3.72	10.50	4.91	12.05	1.08	**9.55***	1.17	**4.18***	3.40
		n = 8		n = 7		n = 8		n = 8		n = 6		n = 3	

with the exception of a decrease in rearing activity, alteration of righting reflex, slight impairment of gait, the absence of pupil response to light, a decrease in forelimb and hindlimb grip strength, decrease in body temperature and respiration difficulties (Table 1a–c). Miosis was changed to mydriasis due to the effect of atropine. The ability of K870 to protect tabun-poisoned rats from tabun-induced acute neurotoxicity was slightly higher compared to other oximes studied. Pralidoxime was not able to eliminate fur abnormalities, unprovoked activity (arousal), a decrease in muscular tonus, the alteration of righting reflex, impairment of gait, absence of approach and tail-pinch reponse, a decrease in grip strength of all limbs while the other oximes (HI-6, K870) were able to do that. In addition, the oxime HI-6 was not able to eliminate hyperkinesis and tremors while the oxime K870 was able to do that (Table 1a–c).

Tab. 1b The values of tabun-induced sensorimotor and excitability neurotoxic markers measured at 2 hours following tabun challenge by the functional observational battery (scored values). Statistical significance: * p ≤ 0.05 (comparison with the control values).

2 hours		Controls		Tabun + A + pralidoxime		Tabun + A + HI-6		Tabun + A + K870		Tabun + atropine		Tabun	
No	Marker	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s
1	catch difficulty	2.00		2.00		2.00		2.00		2.00		**1.00***	
2	ease of handling	2.00		2.00		2.00		2.00		2.00		**1.00***	
3	arousal (GSC)	1.00		**2.00***		1.00		1.00		1.00		**4.00***	
4	tension	0.00		0.00		0.00		0.00		0.00		**2.00***	
5	vocalisation	0.00		0.00		0.00		0.00		0.00		**3.00***	
6	stereotypy	0.00		0.00		0.00		0.00		0.00		**5.00***	
7	bizzare behavior	0.00		0.00		0.00		0.00		0.00		**4.00***	
8	approach response	2.00		**1.00***		2.00		2.00		2.00		**1.00***	
9	touch response	2.00		2.00		2.00		2.00		2.00		**1.00***	
10	click response	2.00		2.00		2.00		2.00		2.00		**1.00***	
11	tail-pinch response	2.00		**1.00***		2.00		2.00		2.00		**1.00***	
		n = 8		n = 7		n = 8		n = 8		n = 6		n = 3	

Tab. 1c The values of tabun-induced autonomic neurotoxic markers measured at 2 hours following tabun challenge by the functional observational battery (No 1–7, 10–11, 13 – scored values, No 8–9, 12 – values in absolute units). Statistical significance: * p ≤ 0.05 (comparison with the control values).

2 hours		Controls		Tabun + A + pralidoxime		Tabun + A + HI-6		Tabun + A + K870		Tabun + atropine		Tabun	
No	Marker	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s
1	lacrimation	0.00		0.00		0.00		0.00		0.00		**4.00***	
2	palpebral closure	1.00		1.00		1.00		**2.00***		1.00		**5.00***	
3	endo/ exophtalmus	0.00		0.00		0.00		0.00		0.00		**1.00***	
4	fur abnormalities	0.00		**7.00***		0.00		0.00		0.00		**7.00***	
5	skin abnormalities	0.00		0.00		0.00		0.00		0.00		**4.00***	
6	salivation	0.00		0.00		0.00		0.00		0.00		**2.00***	
7	nose secretion	0.00		0.00		0.00		0.00		0.00		**3.00***	
8	urination	2.25	3.06	6.25	4.43	3.13	3.72	2.38	3.16	3.13	3.56	1.88	3.72
9	defecation	0.00		0.00		0.00		0.00		0.00		0.00	
10	pupil size	0.00		**2.00***		**2.00***		**1.00***		**2.00***		**-2.00***	
11	pupil response	1.00		**0.00***		**0.00***		**0.00***		**0.00***		**0.00***	
12	body temperature (°C)	37.50	0.22	**36.66***	1.47	**36.42***	1.38	**35.74***	0.37	**35.94***	0.53	**36.17***	1.76
13	respiration	0.00		**-1.00***		**-1.00***		0.00		**-1.00***		**-2.00***	
		n = 8		n = 7		n = 8		n = 8		n = 6		n = 3	

Tab. 2a The values of tabun-induced activity and neuromuscular neurotoxic markers measured at 24 hours following tabun challenge by the functional observational battery (No 1–2, 4–14 – scored values, No 3, 15–18 – values in absolute units). Statistical significance: * p ≤ 0.05 (comparison with the control values). Applied abbreviations: RRF, air righting reflex from back position; RRV, air righting reflex from vertical position; x/M, average or modus value; ± s, standard deviation; A, atropine; n, number of surviving animals.

24 hours		Controls		Tabun + A + pralidoxime		Tabun + A + HI-6		Tabun + A + K870		Tabun + atropine		Tabun	
No	Marker	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s
1	posture	1.00		1.00		1.00		1.00		1.00		**7.00***	
2	muscular tonus	0.00		0.00		0.00		0.00		0.00		**-2.00***	
3	rearing	0.75	0.71	3.25	2.76	3.38	6.32	0.63	1.06	3.88	7.61	0.25	0.46
4	hyperkinesis	0.00		0.00		0.00		0.00		**3.00***		**7.00***	
5	tremors	0.00		0.00		0.00		0.00		**3.00***		**5.00***	
6	clonic movements	0.00		0.00		0.00		0.00		0.00		**2.00***	
7	tonic movements	0.00		0.00		0.00		0.00		0.00		**5.00***	
8	gait	0.00		0.00		0.00		0.00		**1.00***		**7.00***	
9	ataxia	0.00		0.00		0.00		0.00		**2.00***		**2.00***	
10	paresis	0.00		0.00		0.00		0.00		0.00		**2.00***	
11	mobility score	1.00		1.00		1.00		1.00		**2.00***		**4.00***	
12	activity	4.00		4.00		**3.00***		**3.00***		**2.00***		**1.00***	
13	RRF	1.00		1.00		1.00		1.00		1.00		**7.00***	
14	RRV	1.00		1.00		1.00		1.00		1.00		**4.00***	
15	landing foot splay (mm)	9.51	0.86	**5.23***	3.91	**6.99***	3.19	**6.56***	0.94	8.20	1.70	**1.95***	3.70
16	forelimb grip strength (kg)	6.34	1.27	6.63	4.30	8.61	5.64	8.24	1.35	7.31	1.32	**1.89***	3.72
17	hindlimb grip strength (kg)	3.14	1.32	1.91	1.32	2.10	1.17	**1.84***	0.39	2.25	0.91	**0.53***	0.99
18	grip strength of all limbs (kg)	11.23	0.97	10.41	6.67	10.80	4.74	10.09	1.34	12.04	1.37	**2.76***	5.19
		n = 8		n = 6		n = 6		n = 6		n = 4		n = 2	

Tab. 2b The values of tabun-induced sensorimotor and excitability neurotoxic markers measured at 24 hours following tabun challenge by the functional observational battery (scored values). Statistical significance: * p ≤ 0.05 (comparison with the control values).

24 hours		Controls		Tabun + A + pralidoxime		Tabun + A + HI-6		Tabun + A + K870		Tabun + atropine		Tabun	
No	Marker	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s	x/M	-/+s
1	catch difficulty	2.00		2.00		2.00		2.00		2.00		**1.00***	
2	ease of handling	2.00		2.00		**3.00***		2.00		2.00		**1.00***	
3	arousal (GSC)	1.00		1.00		1.00		1.00		**2.00***		**4.00***	
4	tension	0.00		0.00		0.00		0.00		0.00		**2.00***	
5	vocalisation	0.00		0.00		0.00		0.00		0.00		**3.00***	
6	stereotypy	0.00		0.00		0.00		0.00		0.00		**5.00***	
7	bizzare behavior	0.00		0.00		0.00		0.00		0.00		**5.00***	
8	approach response	1.00		1.00		1.00		1.00		1.00		1.00	
9	touch response	2.00		2.00		2.00		2.00		**1.00***		**1.00***	
10	click response	2.00		2.00		2.00		2.00		2.00		**1.00***	
11	tail-pinch response	2.00		**1.00***		2.00		2.00		2.00		**1.00***	
		n = 8		n = 6		n = 6		n = 6		n = 4		n = 2	

The results of the experiments related to the measurement of tabun-induced neurotoxicity at 24 hours following tabun poisoning are divided into three parts (activity and neuromuscular measures, sensorimotor and excitability measures and autonomic measures) (15) and summarized in Table 2a–c. Six non-treated tabun-poisoned rats, four tabun-poisoned rats treated with atropine alone and two tabun-poisoned rats treated with the oxime K870, HI-6 or pralidoxime in combination with atropine died before the evaluation of tabun-induced neurotoxicity by FOB. The evaluation of tabun-induced neurotoxic signs at 24 h following intoxication proved significant alteration of practically all observed parameters with the exception of rearing, urination, defecation and approach response (Table 2a–c).

All studied oximes in combination with atropine were able to prevent almost all tabun-induced signs of neurotoxicity observed at 24 h following tabun challenge with the exception of a slight decrease in activity, body temperature, landing foot splay and hindlimb grip strength. On the other hand, the ability of atropine alone to eliminate or at least reduce tabun-induced signs of acute neurotoxicity was lower compared to all oximes studied in combination with atropine. Atropine alone was not be able to eliminate hyperkinesis, tremors, slight impairment of gait in the form of ataxia, no reaction during a reflex testing consisting of recording each rat's response to the touch of the pen to the posterior flank and a decrerase in body temperature (Table 2a–c).

Tab. 2c The values of tabun-induced autonomic neurotoxic markers measured at 24 hours following tabun challenge by the functional observational battery (No 1–7, 10–11, 13 – scored values, No 8–9, 12 – values in absolute units). Statistical significance: * $p \le 0.05$ (comparison with the control values).

24 hours		Controls		Tabun + A + pralidoxime		Tabun + A + HI-6		Tabun + A + K870		Tabun + atropine		Tabun	
No	**Marker**	**x/M**	**-/+s**	**x/M**	**-/+s**	**x/M**	**-/+s**	**x/M**	**-/+s**	**x/M**	**-/+s**	**x/M**	**-/+s**
1	lacrimation	0.00		0.00		0.00		0.00		0.00		**4.00***	
2	palpebral closure	1.00		1.00		1.00		1.00		1.00		**5.00***	
3	endo/ exophtalmus	0.00		0.00		0.00		0.00		0.00		**1.00***	
4	fur abnormalities	0.00		0.00		0.00		0.00		0.00		**7.00***	
5	skin abnormalities	0.00		0.00		0.00		0.00		0.00		**4.00***	
6	salivation	0.00		0.00		0.00		0.00		0.00		**2.00***	
7	nose secretion	0.00		0.00		0.00		0.00		0.00		**3.00***	
8	urination	0.00		0.00		0.00		0.00		0.00		0.00	
9	defecation	v0.00		0.00		0.00		1.00		0.00		0.00	
10	pupil size	0.00		0.00		0.00		0.00		0.00		**-2.00***	
11	pupil response	1.00		1.00		1.00		1.00		1.00		**0.00***	
12	body temperature (°C)	37.55	0.17	**36.48***	0.36	36.67	0.52	36.80	0.45	**36.41***	0.12	**36.90***	0.57
13	respiration	0.00		0.00		0.00		0.00		**-1.00***		**-2.00***	
		n = 8		n = 6		n = 6		n = 6		n = 4		n = 2	

THE EVALUATION OF REACTIVATING EFFICACY OF OXIMES STUDIED IN THE BRAIN OF TABUN-POISONED RATS

The ability of studied oximes to eliminate or reduce tabun-induced signs and symptoms of neurotoxicity does not correspond to their ability to reactivate tabun-inhibited AChE in the brain that was low. The highest reactivating efficacy was shown for the oxime K870 (5.5%) while the ability of pralidoxime to reactivate tabun-inhibited AChE in the brain was slightly lower compared to the oxime K870 (4.2%). The reactivating efficacy of the oxime HI-6 was not found. The differences among tested oximes were not significant (Table 3).

Tab. 3 Percentage of reactivation of tabun-inhibited AChE by oximes in rat brain *in vivo*.

TREATMENT	AChE activity (mkat/kg)
	Brain
Saline control	102.4 ± 2.44
Atropine control	39.56 ± 10.97[a]
Atropine + **K870** (% reactivation[b])	43.02 ± 10.91 (5.5)
Atropine + **pralidoxime** (% reactivation)	42.17 ± 6.35 (4.2)
Atropine + **HI-6** (% reactivation)	31.18 ± 6.40 (0)

[a] Means ± S.E.M., n = 4–8.
[b] % reactivation was determined using the AChE activity values: {1 – [((saline control) – (oxime + atropine))/((saline control) – (atropine control))]} × 100.

HISTOPATHOLOGICAL EVALUATION OF TABUN-INDUCED BRAIN DAMAGE

Significantly increased histopathological damage scores were only found in amygdaloid body (p = 0.005) of tabun-poisoned rats without treatment when compared to control animals. In this group, shrunken eosinophilic neurons were present, especially in basolateral and central nuclei of this brain region in 1 of 2 surviving animals. Administration of atropine alone or its mixture with K870, pralidoxime or HI-6 significantly reduced tabun-induced histopathological damage of amygdaloid body (p = 0.016, 0.008, 0.008, and 0.008).

No other significant changes were observed.

DISCUSSION

Severe poisoning with nerve agents including tabun can cause long-term overstimulation of the central muscarinic receptors leading to increased glutamatergic activity and subsequent excitotoxic damage of the brain (19, 20). Therefore, the antidotes with sufficient neuroprotective efficacy are important for the successful antidotal treatment of acute tabun poisonings.

It was found that atropine alone is not able to prevent tabun-induced seizures and subsequent neurotoxic effects including the brain damage following sublethal or lethal poisoning with tabun due to its low central antimuscarinic activity (21, 22). Thus, atropine should be combined with AChE reactivator in the antidotal treatment of tabun poisonings to improve its neuroprotective efficacy. However, the ability of commonly used oximes to eliminate tabun-induced acute neurotoxic effects is insufficient because of low reactivation of tabun-inhibited AChE and limited penetration through BBB (2, 9, 23–26). Therefore, new oximes with higher potency to reactivate tabun-inhibited AChE and to counteract tabun-induced acute neurotoxicity are still searched in order to increase the efficacy of antidotal treatment of acute tabun poisonings. Unfortunately, the presently available database on reactivators presented in the past few years gives no indication of a candidate which is clearly superior to the classic oximes.

The design of newly developed oximes should respect not only the goal to increase their reactivating efficacy via higher affinity to AChE but also the goal to increase their BBB penetration as much as possible. It was demonstrated that proper length between covalently connected proper peripheral site ligand and a non-ionic part containing nucleophilic aldoxime in the structure of AChE resulted in higher reactivation potency (27). The oxime K870 (dichlorinated bispyridinium AChE reactivator) has been developed based on aforementioned approach (12, 28–30).

Our results demonstrate that the neuroprotective efficacy of studied oximes is comparable. At 2 h after tabun administration, the ability of the oxime K870 to eliminate tabun-induced neurotoxic signs and symptoms was slightly higher than the neuroprotective efficacy of pralidoxime and similar to the efficacy of the oxime HI-6. No significant differences among all three groups (atropine with one of the tested oxime) at 24 h after tabun poisoning were found, either. On the other hand, the neuroprotective efficacy of atropine alone was markedly lower compared to the combination of atropine with one of studied oximes, especially at 24 h after tabun poisoning. This finding corresponds to the literature data (21). The differences in the neuroprotective efficacy of studied oximes do not correspond to their reactivating efficacy in the brain. Although the ability of K870 to reactivate tabun-inhibited AChE was the highest, the potency of all oximes studied to reactivate tabun-inhibited brain AChE was generally very low. This finding is supported by the fact that at least two tabun-poisoned rats died within 24 h after tabun challenge in all experimental groups. As the neuroprotective efficacy of all oximes studied is not possible to explain by their central reactivating efficacy that was very small, their neuroprotective efficacy could be also caused by their direct pharmacological effects such as inhibition of acetylcholine release, interaction with presynaptic cholinergic nerve terminals and/or with postsynaptic receptors (31–33).

Above mentioned data roughly correlate with histopathological evaluation. In comparison with our previous study (14), histopathological picture of tabun-poisoned rats without antidotal treatment was scarse. Significant damage was only found in amygdaloid body, which represents nuclei susceptible to excitotoxic damage (19). Nevertheless, these results are highly affected by a very low number of surviving animals that underwent the assessment. Regarding treated tabun-poisoned rats, no statistically significant differences were observed among all experimental groups as in case of the evaluation of neuroprotective and reactivating efficacy of all oximes studied.

CONCLUSIONS

It was found that neuroprotective and central reactivating efficacy of K870 did not prevail the effectiveness of currently available oximes studied and, therefore, it is not a suitable replacement for commonly used oximes in the antidotal treatment of acute tabun poisonings. Thus, a new structured, stepwise approach and a comprehensive set of *in vitro* and *in vivo* studies are required for the successful identification and downselection of new candidate reactivators with better entering into the active site of AChE-tabun complex and higher penetration through BBB.

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