ORIGINAL ARTICLE

A COMPARISON OF THE POTENCY OF NEWLY DEVELOPED OXIMES (K347, K628) AND CURRENTLY AVAILABLE OXIMES (OBIDOXIME, HI-6) TO COUNTERACT ACUTE NEUROTOXIC EFFECTS OF TABUN IN RATS

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Summary: The ability of newly developed oximes (K347, K628) to reduce tabun-induced acute neurotoxic signs and symptoms was compared with currently available oximes (obidoxime, HI-6) using a functional observational battery. The neuroprotective effects of the oximes studied (K347, K628, obidoxime, HI-6) combined with atropine on rats poisoned with tabun at a sublethal dose (220 μg/kg i.m.; 80 % of LD_{50} value) were evaluated. Tabun-induced neurotoxicity was monitored by a functional observational battery and automatic measurement of motor activity at 24 hours following tabun challenge. The results indicate that all tested oximes combined with atropine enable tabun-poisoned rats to survive 24 hours following tabun challenge. Both newly developed oximes (K347, K628) combined with atropine are able to decrease tabun-induced neurotoxicity in the case of sublethal poisonings but they do not eliminate all tabun-induced acute neurotoxic signs and symptoms. Their ability to decrease the tabun-induced acute neurotoxicity is higher than that of the oxime HI-6 and it is slightly slower than the neuroprotective efficacy of obidoxime. As the neuroprotective potency of both newly developed oximes (K347, K628) is not as high as the potency of obidoxime, they are not a suitable replacement for obidoxime for the treatment of acute tabun poisonings.

Key words: *Tabun; Functional observational battery; K347; K628; Obidoxime; HI-6; Rats*

Introduction

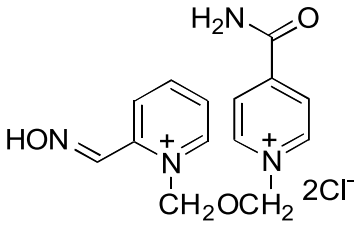
Organophosphorus nerve agents are considered to be the most dangerous chemical warfare agents. Their acute toxic effects are based on the phosphonylation of acetylcholinesterase (AChE, EC 3.1.1.7), leading to the irreversible inhibition of its active site and subsequent overstimulation of postsynaptic cholinergic receptors due to the accumulation of the neurotransmitter acetylcholine in the synapses of the central and peripheral nervous systems (21, 22).

The standard antidotal treatment of nerve agent poisoning usually includes an anticholinergic agent to block the overstimulation of cholinergic receptors and an oxime to reactivate nerve agent-inhibited AChE (4, 29). Compounds with a nucleophilic oximate anion have been discovered and are considered to be able to reactivate nerve agent-inhibited AChE by dephosphonylating the enzyme active site and restoring its activity. However, some nerve agents were found to be resistant to standard antidotal treatment. One of the most resistant nerve agent is tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate), the deleterious effects of which are extraordinarily difficult to antagonize because of the changes in hydrogen bonding and the conformational changes of AChE-tabun complex prior to an aging process in the AChE active site (1, 6).

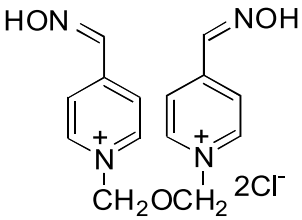
Tabun can produce centrally-mediated seizure activity that rapidly progresses to status epilepticus and contributes to profound brain damage (22, 29). The exposure of experimental animals to tabun in convulsions-inducing doses may result in irreversible lesions in the central nervous system (CNS) that can be manifested as behavioral effects in survivors that have convulsed (9). Therefore, the ability of antidotes to block the acute neurotoxic effects of tabun and prevent development of irreversible lesions in CNS is important for successful antidotal treatment. Generally, the oximes exert more potent effects in the peripheral compartment compared to central compartment due to their poor penetration into CNS. Nevertheless, the penetration of oximes into CNS and subsequent reactivation of nerve agent-inhibited AChE in the brain were demonstrated (3, 28). Although the rate of this reactivation is lower compared to that in the peripheral system, its role in CNS is important for survival from nerve agent exposure (11, 22).

Generally, the commonly used reactivators of phosphonylated AChE based on monopyridinium (e.g. pralidoxime) and bispyridinium oximes (e.g. obidoxime, the oxime HI-6)

are not able to counteract sufficiently the acute toxic effects of tabun because of their low reactivating efficacy (13, 18, 26, 30). Therefore, the replacement of commonly used oximes (pralidoxime, obidoxime, HI-6) with a more effective oxime has been a long-standing goal for the treatment of tabun poisoning (5, 24). New oximes, K347 (1-benzyl-2-hydroxyiminomethylpyridinium bromide) and K628 [4-carbamoyl-4'-hydroxyiminomethyl-1,1'-(1,3-phenylenedimethyl)-bispyridinium dibromide] (Fig. 1) have been synthesized at our Department of Toxicology (25) to improve the efficacy of antidotal treatment. The evaluation of their potency to reactivate tabun-inhibited AChE using *in vitro* methods showed that the reactivating efficacy of both newly developed oximes roughly corresponds to the effectiveness of obidoxime and it is better than the potency of HI-6 to reactivate tabun-inhibited AChE (25). The aim of this study was to compare the neuroprotective efficacy of two newly developed oximes (K347, K628) with currently available oximes (obidoxime, the oxime HI-6) in combination with an anticholinergic drug atropine in tabun-poisoned rats. The tabun-induced neurotoxic signs were determined using a functional observational battery, a non-invasive and relatively sensitive type of neurological examination for a wide range of neurobiological functions including measurements of sensory, motor and autonomic nervous functions (7).

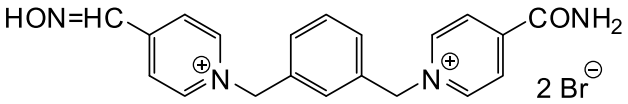


HI-6



Obidoxime

K347



K628

Fig. 1: Chemical structure of oximes studied.

Materials and Methods

Male albino Wistar rats weighing 200–230 g were purchased from VELAZ (Prague, Czech Republic). They were kept in an air-conditioned room (22 ± 2 °C and 50 ± 10 % relative humidity, with lights from 7.00 to 19.00 hr) and allowed access to standard food and tap water *ad libitum*. The rats were divided into groups of 8 animals. Handling of the experimental animals was performed in compliance with relevant laws and institutional guidelines and under the supervision of the Ethics Committee of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic).

Tabun was obtained from the Military Technical Institute in Brno (Czech Republic) and was 96 % pure as assayed by acidimetric titration. All oximes studied were of 98.5 % purity and were synthesized at the Department of Toxicology of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic). 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 (i.m.) at a volume of 1 mL/kg body weight (b.w.).

Tabun was administered at a sublethal dose (220 μg/kg b.w. – 80 % LD_{50}). One minute following tabun poisoning, the rats were treated with atropine (21 mg/kg b.w.) in combination with the oxime HI-6, obidoxime, K347 or K628 at equitoxic doses corresponding to 5 % of their LD_{50} values. The neurotoxicity of tabun was monitored using the functional observational battery at 24 hr 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.

The functional observational battery consists of 47 measurements of sensory, motor and autonomic nervous functions. Some of them are scored (Table 1), the others are measured in absolute units (7, 8). The first evaluation was obtained when tabun-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 hand-held. The exploratory activity, piloerection and other skin abnormalities were noted. Salivation and nose secretion were also registered and scored. Then, the rats were placed on a flat surface which 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

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*	defense	flight	escape	aggrression	
Ease of handling				very easy	*easy*	moderately difficult	difficult			
Muscular tonus	atonia	hypotonia	*normal*	hypertonia	rigidity	fasciculations				
Lacrimation			*none*	slight	severe	crusta	coloured crusta			
Palpebral closure				*open*	slightly	half-way	completely shut	ptosis		
Endo-exophthalmus		endo	*normal*	exo						
Piloerection			*no*	yes						
Skin abnormalities			*normal*	pale	erythema	cyanosis	pigmented	cold	injury	
Salivation			*none*	sllight	severe					
Nose secretion			*none*	slight	severe	coloured				
Clonic movements			*normal*	repetitive	non-rhythmic	mild tremors	severe tremors	myoclonic	clonic	
Tonic movements			*normal*	contraction of extensors	opisthotonus	emprostho-tonus	explosive jumps	tonic convulsions		
Gait			*normal*	ataxia	overcompen-sation of hindlimbs movement	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					
Stereotypy			*none*	head	body weaving	grooming weaving	circling	others		
Bizarre behavior			*none*	head	body	self-mutilation	abnormal movements	others		
Approach response				no reaction	*normal*	slow reaction	energetic reaction	exaggerated reaction		
Touch response				no reaction	*normal*	slow reaction	energetic reaction	exaggerated reaction		
Click response				no reaction	*normal*	slow reaction	energetic reaction	exaggerated reaction		
Tail-pinch response				no reaction	*normal*	slow reaction	energetic reaction	exaggerated reaction		
Pupil size		miosis	*normal*	mydriasis						
Pupil responze			no reaction	*normal reaction*						
Righting reflex				*normal*	slightly uncoordin.	lands on side	lands on back			

noted and ranked, and arousal, stereotypy and bizarre behaviors and abnormal posture were evaluated. At the end of the third min, the number of fecal boluses and urine pools on the adsorbent pad was registered. Reflex testing comprising 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 also used. The response 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, body temperature and finally hindlimb landing foot splay. The whole battery of tests required approximately 6–8 min per rat. The observer of the behavior did not know about the design of the experiments. Motor activity data were collected shortly after finishing the functional observational battery, using an apparatus for testing of a spontaneous motor activity of laboratory animals (constructed at the Faculty of Military Health Sciences, Hradec Kralove, 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.

Data collected with the functional observational battery and motor activity assessment include categorial, ordinal and continuous values. Statistical analyses were performed on a PC with a special interactive programme NTX (7). The categorial and ordinal values were formulated as contingency tables and judged consecutively by the Chi-squared test of homogeneity, the Concordance-Discordance test and the Kruskal-Wallis test, respectively. The continual data were assessed by successive statistical tests: the CI for Delta, the Barlett test for Equality of Variance, the Williams test and the Test for Distribution Functions (27). The differences were considered significant when $p < 0.05$.

Results

All tabun-poisoned rats survived till the end of experiment (24 hr following the intoxication) regardless of the type of oxime used. Non-treated tabun-poisoned rats survived for 24 hr after tabun challenge, too.

The results of the experiments related to the measurement of tabun-induced neurotoxicity at 24 hr following tabun poisoning are divided into three parts (activity and neuromuscular measures, sensorimotor and excitability measures and autonomic measures – 23) and summarized in Tab. 2a–c. Observation of neurotoxic signs indicated that many functional disorders in the tabun-poisoned rats lasted for at least 24 hr. Tabun produced passive behavior of rats during handling and retention, miosis and a decrease in muscular tone at 24 hr following tabun administration. Exploratory

Tab. 2a: The values of tabun-induced activity and neuromuscular neurotoxic markers measured at 24 hr following tabun challenge by the functional observational battery (No 1–2, 4–14 – scored values, No 3, 15–21 – values in absolute units). Statistical significance: *p < 0.05 (comparison with the control values).

24 hours		Controls		Tabun – A + K347		Tabun – A + K628		Tabun – A + obidoxime		Tabun – A + HI-6		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***		1,00		**3,00***		**3,00***	
2	muscular tonus	0,00		**-1,00***		0,00		**-1,00***		**-1,00***		**-1,00***	
3	rearing	13,38	7,15	**2,75***	3,69	**1,88***	2,10	**1,13***	1,89	**1,75***	1,49	**3,00***	2,67
4	hyperkinesis	0,00		0,00		0,00		0,00		0,00		0,00	
5	tremors	0,00		0,00		0,00		0,00		0,00		0,00	
6	clonic movements	0,00		0,00		0,00		0,00		0,00		0,00	
7	tonic movements	0,00		0,00		0,00		0,00		0,00		0,00	
8	gait	0,00		**1,00***		**1,00***		0,00		**1,00***		**1,00***	
9	ataxia	1,00		**2,00***		**2,00***		0,00		**2,00***		**2,00***	
10	gait score	0,00		0,00		0,00		0,00		0,00		0,00	
11	mobility score	1,00		1,00		1,00		1,00		1,00		1,00	
12	activity	4,00		**1,00***		**1,00***		**1,00***		**1,00***		**1,00***	
13	RRF	1,00		1,00		1,00		1,00		1,00		1,00	
14	RRV	1,00		1,00		1,00		1,00		1,00		1,00	
15	landing foot splay (mm)	100,63	13,00	**77,50***	17,98	**78,75***	16,65	**72,75***	12,80	**79,00***	12,60	**73,50***	19,76
16	forelimb grip strength (kg)	5,73	0,51	5,21	0,49	5,16	2,12	5,13	0,45	**4,86***	0,79	**5,06***	0,31
17	hindlimb grip strength (kg)	1,36	0,32	**0,83***	0,23	**0,84***	0,37	**1,06***	0,16	**0,70***	0,23	**1,03***	0,10
18	grip strength of all limbs (kg)	15,94	1,35	15,27	2,54	13,88	5,99	15,69	2,84	**11,20***	4,57	**11,88***	2,64
19	vertical activity	234,38	112,00	**56,25***	51,22	**27,50***	33,79	130,00	92,91	**26,63***	34,12	**49,25***	48,27
20	horizontal activity	42,00	27,44	**2,50***	3,63	**1,63***	3,11	**9,50***	7,09	**2,25***	4,56	**5,13***	5,00
21	total motor activity	276,38	123,77	**58,75***	54,02	**29,13***	36,68	**139,50***	98,25	**28,88***	38,34	**54,38***	42,47
		n=8		n=8		n=8		n=8		n=8		n=8	

Tab. 2b: The values of tabun-induced sensorimotor and excitability neurotoxic markers measured at 24 hr 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 + K347		Tabun – A + K628		Tabun – A + obidoxime		Tabun – A + HI-6		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		**1,00***		**1,00***	
2	ease of handling	2,00		2,00		2,00		2,00		**1,00***		**1,00***	
3	arousal (GSC)	1,00		**2,00***		**3,00***		1,00		**2,00***		**2,00***	
4	tension	0,00		0,00		0,00		0,00		0,00		0,00	
5	vocalisation	0,00		0,00		0,00		0,00		0,00		0,00	
6	stereotypy	0,00		0,00		0,00		0,00		0,00		0,00	
7	bizarre behavior	0,00		0,00		0,00		0,00		0,00		0,00	
8	approach response	2,00		2,00		2,00		2,00		2,00		2,00	
9	touch response	2,00		2,00		2,00		2,00		2,00		2,00	
10	click response	2,00		2,00		2,00		2,00		2,00		2,00	
11	tail-pinch response	2,00		2,00		2,00		2,00		2,00		2,00	
		n=8		n=8		n=8		n=8		n=8		n=8	

Tab. 2c: The values of tabun-induced autonomic neurotoxic markers measured at 24 hr following tabun challenge by the functional observational battery (No 1–7, 10–11, 15 – scored values, No 8–9, 12–14 – values in absolute units). Statistical significance: *p < 0.05 (comparison with the control values).

24 hours		Controls		Tabun – A + K347		Tabun – A + K628		Tabun – A + obidoxime		Tabun – A + HI-6		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		0,00	
2	palpebral closure	1,00		1,00		1,00		1,00		1,00		1,00	
3	endo/exophtalmus	0,00		0,00		0,00		0,00		0,00		0,00	
4	fur abnormalities	0,00		0,00		0,00		0,00		0,00		0,00	
5	skin abnormalities	0,00		0,00		0,00		0,00		0,00		0,00	
6	salivation	0,00		0,00		0,00		0,00		0,00		0,00	
7	nose secretion	0,00		0,00		0,00		0,00		0,00		0,00	
8	urination	1,13	4,16	2,00	3,16	4,88	4,67	0,25	0,71	3,00	4,99	3,63	5,90
9	defecation	0,00		0,00		0,00		0,00		0,00		0,00	
10	pupil size	0,00		**–2,00***		**–1,00***		**–2,00***		**–2,00***		**–2,00***	
11	pupil response	1,00		**0,00***		**0,00***		**0,00***		**0,00***		**0,00***	
12	food receiving (%)	100,00	0,00	**24,50***	2,67	**41,75***	17,58	**49,50***	11,22	**20,00***	0,00	**27,50***	2,67
13	body weight (g)	238,13	18,82	211,88	21,69	199,13	81,20	207,88	22,15	211,00	16,67	218,63	13,43
14	body temperature (°C)	37,23	0,12	36,80	0,48	37,09	0,36	36,97	0,25	**35,64***	0,65	**35,38***	0,29
15	respiration	0,00		0,00		0,00		0,00		0,00		0,00	
		n=8		n=8		n=8		n=8		n=8		n=8	

and rearing activity were significantly decreased and gait was somewhat impaired. The pupils of the tabun-poisoned rats did not constrict in response to light because of tabun-induced miosis. A significant decrease in limb grip strength, food receiving, body temperature and spontaneous horizontal as well as vertical motor activity were also observed at 24 hr following tabun challenge (Tab. 2a–c). While the potency of the oxime HI-6 to eliminate tabun-induced acute neurotoxic effects was negligible, both newly developed oximes (K347, K628) in combination with atropine were able to prevent some tabun-induced signs of neurotoxicity observed at 24 hr following tabun challenge with the exception of a decrease in muscular tone, exploratory and rearing activity, miosis, gait impairment, a decrease in hind-limb grip strength, food receiving and spontaneous horizontal as well as vertical motor activity (Tab. 2a-c). Obidoxime in combination with atropine was able additionally to eliminate gait impairment and a decrease in spontaneous vertical motor activity (Tab. 2a).

Discussion

Generally, the ability of currently available oximes to eliminate tabun-induced acute neurotoxic effects is relati-

vely low. Pralidoxime, a currently available oxime for the treatment of poisonings with highly toxic organophosphates (4), seems to be practically ineffective in preventing tabun-induced neurotoxicity (15) although its penetration into the CNS is higher compared to commonly used bispyridinium oximes such as obidoxime, trimedoxime or HI-6 (3, 28). Obidoxime and trimedoxime are able to partly eliminate tabun-induced acute neurotoxicity following i.m. administration of tabun at a lethal dose, nevertheless, their neuroprotective efficacy is not satisfactory (14, 15, 17). The oxime HI-6, developed and introduced by some countries for the antidotal treatment of severe acute soman poisonings (4, 12), was found to be significantly less efficacious to counteract tabun–induced acute neurotoxicity than obidoxime (15). The unsatisfactory efficacy of the above mentioned oximes in eliminating tabun-induced acute neurotoxicity can be explained by the low potency of oximes in reactivating tabun-inhibited AChE *in vitro* and *in vivo* (10, 20, 26, 30). Therefore, new oximes have been developed to increase the reactivating potency as well as the neuroprotective efficacy of the antidotal treatment of acute tabun poisonings.

Based on the structure-activity relationship study, there are five most important structural factors influencing the affinity of the AChE reactivators toward nerve agent-inhibited AChE and subsequent oxime reactivity: the presence of the quaternary nitrogen in the reactivator molecule, the length of the connection chain between two pyridinium rings, the presence of the oxime group, the position of the oxime group at the pyridinium ring and the number of oxime groups in the reactivator structure. The above mentioned data on a reactivator structure allowed us to postulate requirements for the structural parameters of new reactivators of tabun-inhibited AChE (19). For tabun poisonings, at least one oxime in position four on the heteroaromatic ring is necessary for substantial reactivating, therapeutic and neuroprotective potency whilst an oxime in position two has a low or no capability to counteract acute toxicity of tabun (19). Additionally, the optimal linker length suitable for tabun intoxication varies from 3 to 4 carbon-carbon bonds (2).

Our results demonstrate that both newly developed oximes (K347, K628) are partly able to reduce tabun-induced acute neurotoxic signs and symptoms. The difference between the neuroprotective efficacy of both newly developed oximes is not significant although K347 is a monopyridinium oxime and K628 is a bispyridinium oxime. Thus, the difference between the penetration of monopyridinium and bispyridiniun oximes into CNS is not enough to cause the significant difference between their neuroprotective effectiveness.

The results confirm that there is not a single, broad-spectrum oxime suitable for the antidotal treatment of poisonings with all organophosphorus agents (16). The neuroprotective efficacy of both newly developed oximes (K347, K628) is slightly lower than the neuroprotective efficacy of obidoxime and it is markedly higher compared to the oxime HI-6. Their neuroprotective efficacy corresponds to their potency in reactivating tabun-inhibited AChE (25). As the neuroprotective potency of both newly developed oximes is slightly lower compared to the effectiveness of the currently available obidoxime, they are not a suitable replacement for obidoxime in the treatment of acute tabun poisonings.

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