Development of New Reactivators of Tabun Inhibited Acetylcholinesterase and the Evaluation of Their Efficacy by *in Vitro* and *in Vivo* Methods

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ABSTRACT

Tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate) belongs to highly toxic organophosphorus compounds misused as chemical warfare agents for military as well as terroristic purposes. It differs from other highly toxic organophosphates by its chemical structure and by the fact that tabun-inhibited acetylcholinesterase is extraordinarily difficult to reactivate. The antidotal treatment of tabun acute poisonings still represents a serious problem and the development of new, more effective AChE reactivators to achieve the satisfactorily effective antidotal treatment of acute poisonings with tabun still represents very important goal. Since 2003, we have prepared around 200 new AChE reactivators. Their potency to reactivate tabun-inhibited acetylcholinesterase has been subsequently evaluated using our *in vitro* screening test. Afterwards, promising compounds were selected and kinetic parameters and reactivation constants were determined. Then, the best reactivators were subjected to the *in vivo* studies (toxicity test, the evaluation of therapeutical, reactivating and neuroprotective efficacy).

According to the results obtained, all new oximes tested *in vivo* (K027, K048, K074, K075) were found to be relatively effective to eliminate acute lethal toxic effects in tabun poisoned mice and reactivate tabun-inhibited acetylcholinesterase in rats poisoned with tabun. In addition, newly developed oximes (K027, K048, K074, K075) combined with atropine seem to be sufficiently effective antidotes for a significant decrease in tabun-induced neurotoxicity in the case of sublethal poisonings of rats although they are not able to eliminate tabun-induced neurotoxicity completely. Our results also confirm that the reactivating efficacy of oximes evaluated by the methods *in vitro* correlates not only with the potency of oximes in reactivating tabun-inhibited acetylcholinesterase *in vivo* but also with the ability of oximes to protect rats poisoned with supralethal doses of tabun.

Keywords: tabun, acetylcholinesterase, K oximes, HI-6, obidoxime, atropine, rats, mice

1.0 INTRODUCTION

The current standard treatment for poisoning with organosphosphorous compounds called nerve agents usually consists of the combined administration of anticholinergic drugs (preferably atropine) and oximes (preferably pralidoxime or obidoxime). Anticholinergic drugs block the effects of overstimulation by acetylcholine accumulated at muscarinic receptor sites while oximes, compounds with nucleophilic bases,

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repair biochemical lesions by dephosphorylating tabun-inhibited acetylcholinesterase (AChE, EC 3.1.1.7) and restoring its activity (Marrs, 1993; Kassa, 2002).

Tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate) is an organophosphorus compound used as chemical warfare agent for military as well as terroristic purposes. It differs from other organophosphates in its chemical structure and that tabun-inhibited AChE is difficult to reactivate. Its deleterious effects are extraordinarily difficult to counteract because of the existence of a lone electron pair located on a dimethylamide group that makes nucleophilic attack almost impossible (Koplovitz et al., 1995; Jokanovic et al., 1996; Cabal and Bajgar, 1999). Another view on the resistence of tabun-inhibited AChE treatment was provided by Ekstrom et al in 2006. According to this study, the crystal structures of murine AChE showed that non-aged tabun conjugate induces structural changes in H447 and its hydrogen bonds. Moreover, the conformational change of P338 position partially closes the narrow AChE gorge. After aging reaction, the tabun molecule is coordinated in the AChE gorge and phosphoroamidoyl group is replaced by a water molecule. Due to these structural changes, the potency of AChE reactivators to split the bond between inhibitor and enzyme is lower in comparison with other nerve agents.

While anticholinergic drugs such as atropine are able to counteract the effects of tabun at peripheral cholinergic receptors (Bajgar and Patocka, 1977), commonly used reactivators of phosphorylated AChE based on monopyridinium (e.g. pralidoxime) and bispyridinium oximes (e.g. obidoxime, methoxime) are not able to counteract the adverse effects of tabun because of minimal reactivating efficacy (Koplovitz and Stewart, 1994). In addition, the reactivating efficacy of the oxime HI-6 which is relatively efficacious against effects of soman (Kassa, 1995), is not as efficient for tabun-inhibited AChE (Puu et al., 1986; Worek et al., 1998). Therefore, the replacement of commonly used oximes (pralidoxime, obidoxime) as well as H oximes (the oxime HI-6) with a more effective oxime has been a long-standing goal for the treatment of tabun poisoning. Our attention was focused on the development of new accessible, non-toxic and in vitro and in vivo potent AChE reactivator of tabun-inhibited AChE. Our development of new reactivators of tabun inhibited-AChE was divided into three steps:

- Synthesis of the new AChE reactivators based on structure-activity relationship studies
- In vitro evaluation of new AChE reactivators potency
- In vivo evaluation of their therapeutic, reactivating and neuroprotetive efficacy

Since 2003, we have prepared around 200 new AChE reactivators. For this purpose, general organic chemistry approaches were used. Then, many results in synthesis and *in vitro* screening evaluation of tabun-inhibited AChE reactivators were obtained during last years. Based on these results, structural requirements needed for sufficient reactivation of tabun-inhibited AChE were determinated. There are five most important structural factors influencing the affinity of the AChE reactivators toward inhibited AChE and subsequent oxime reactivity (Kuca et al. 2006): presence of the quaternary nitrogen in the reactivator molecule, length of the connection chain between two pyridinium rings, presence of the oxime group, position of the oxime group at the pyridinium ring and number of oxime groups in the reactivator structure.

The structure-activity relationship study helps us to design new structures of promising reactivators of tabun-inhibited AChE: 1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium) propane dibromide (K027), 1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoyl pyridinium) butane dibromide (K048), 1,4-bis(4-hydroxyiminomethylpyridinium) butane dibromide (K074) and 1,4-bis(4-hydroxyimino- methylpyridinium) but-2-en dibromide (K075) were synthesized at our Department of Toxicology (Kuca et al., 2003a; 2003b; Kuca et al. 2005) to improve the efficacy of antidotal treatment in reactivating tabun-inhibited AChE and eliminating tabun-induced acute lethal toxicity including neurotoxicity. The evaluation of their potency to reactivate tabun-inhibited AChE using *in vitro* methods showed that the reactivating efficacy of all newly developed oximes is similar to the effectiveness of obidoxime. They seem to be better reactivators of tabun-inhibited AChE than HI-6 especially at concentrations corresponding to human-relevant concentrations (10^{-5} - 10^{-4} M) (Cabal et al., 2004; Kuca et

al. 2005). *In vitro* assessment of reactivating efficacy of oximes is usually followed by the evaluation of their reactivating efficacy *in vivo* and their therapeutic and neuroprotective efficacy against lethal nerve agent poisoning (Marrs, 1993; Kassa, 2002; Kassa et al. 2007). The aim of this study was to summarize the reactivating, therapeutic and neuroprotective efficacy of newly developed oximes (K027, K048, K074, K075) and currently available oximes (obidoxime, the oxime HI-6) against tabun using *in vitro* and *in vivo* methods.

2.0 MATERIAL AND METHODS

Male albino Wistar rats weighing 180-220g and NMRI mice weighing 15-17g were purchased from Konarovice (Czech Republic). They were kept in an air-conditioned room and allowed to access to standard food and tap water ad libitum. The rats and mice were divided into groups of eight animals (N=8). The experimental protocol was approved by the Ethics Committee of the Faculty of Military Health Sciences of University of Defense, Czech Republic. Tabun was obtained from Military Technical Institute in Brno (Czech Republic) and was 95% pure. Its purity was assayed by acidimetric titration. Obidoxime, the oxime HI-6 and newly developed oximes (K027, K048, K074, K075) of 98.5% purity were synthesized at the Department of Toxicology of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic). Their purity was analysed 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.) in rats and 10mL/kg b.w. in mice.

To evaluate the reactivating efficacy of newly developed and currently available oximes *in vitro*, the brain homogenate (0.5 ml) was treated with solution (0.5 ml) of tabun in appropriate concentration for 30 min. Afterwards, reaction solution was adjusted to 23 ml with 0.3 M sodium chloride. Then, 0.02 M solution of acetylcholine iodide (2 ml) was added to the mixture. The liberated acetic acid was titrated with 0.01 M sodium hydroxide on an RTS 822 titrator (Radiometer, Denmark) in the pH-stat mode (pH 8.0) at room temperature (25^{o}C). The slope of the linear part of the time dependence of the sodium hydroxide used represents the activity of the inhibited enzyme (in fact, the initial rate of the enzymatic reaction). Resulted inhibition of ChE was set to 95 %. Reactivation of inhibited ChE was performed immediately after its inhibition. A solution (1 ml) of the reactivator in appropriate concentration was added to the inhibited enzyme. After 10 min reactivation at 25^{o} C, the mixture was adjusted to 23 ml with 0.3 M sodium chloride solution. Then, 0.02 M solution of acetylcholine iodide (2 ml) was added and immediately afterwards the activity of the reactivated enzyme was determined by above described potentiostatic method (Kuca and Kassa 2003).

The acute toxicity of oximes and their therapeutic efficacy against supralethal poisoning with tabun in mice was evaluated by the assessment of the LD_{50} values and their 95% confidence limit using probit-logarithmical analysis of death occurring within 24 hr after i.m. administration of tabun at 5 different doses with 8 animals per dose (Tallarida and Murray, 1987). To evaluate their therapeutic efficacy, the oximes were i.m injected. at equitoxic doses corresponding to 5% of their LD_{50} value in combination with atropine (21 mg/kg) 1 min after tabun administration. The influence of the nature of the antidotal treatment was expressed as protective ratio (LD_{50} value of treated mice/ LD_{50} value of untreated mice).

To evaluate the reactivating efficacy of the oximes, the rats were injected i.m. with either atropine (21 mg/kg) alone or atropine (21 mg/kg) in combination with one of the oximes studied in equimolar dose (50 μmol/kg) 5 min before intramuscular tabun poisoning in rats. The control rats were administered i.m. with saline instead of tabun and antidotes at the same volume. The prophylactic administration of antidotes was used because this procedure is suitable for a mechanistic study that compares the reactivating efficacy of various oximes. The technique should give better results than the treatment of animals after poisoning and reduce the influence of aging of nerve agent-AChE complex (Clement et al. 1992). The rats were decapitated and exsanguinated to obtain the blood 30 min following tabun poisoning at a dose corresponding to LD_{50}. The brains were removed and homogenized in distilled water to determine AChE

activity by a spectrophotometric method (Ellman et al. 1961). The reactivation rate (%) was calculated using the AChE activity values: {1- [((saline) – (oxime + atropine))/((saline) – (atropine control))]} x 100 (Clement et al. 1992). The AChE activity was expressed as μkat/kg or L (μmol substrate hydrolyzed/kg wet tissue or L blood/s). Statistical significance was determined by the use of Student`s t-test and differences were considered significant when $P < 0.05$. Statistical evaluation was determined with the relevant computer programs (Tallarida & Murray, 1987).

To evaluate neuroprotective efficacy of oximes, the rats were administered with tabun at a sublethal dose (170 μg/kg b.w. - 80% LD_{50}). One minute following tabun challenge, the rats were treated with atropine (21 mg/kg b.w.) in combination with obidoxime, the oxime HI-6, K027, K048, K074 or K075 at equimolar doses corresponding to 10 μmol/kg b.w. According to our *in vitro* results, this dose level causes almost maximal % reactivation of tabun-inhibited AChE (Kuca *et al*., 2005). The neurotoxicity of tabun was monitored using the Functional observational battery at 24 hours following tabun poisoning. The evaluated markers of tabun-induced neurotoxicity in experimental animals were compared with the parameters obtained from control rats, that saline was administered 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, the others are measured in absolute units (Frantik and Hornychova, 1995; Hornychova et al., 1995; Moser et al., 1997). 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 held in the hand. The exploratory activity, piloerection and other skin abnormalities were noted too. 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 three minutes during which the frequency of rearing responses was recorded. At the same time, gait characteristics were noted and ranked and arousal, stereotypy and bizarre behaviors and abnormal posture were evaluated. At the end of the third minute, the number of fecal boluses and urine pools on the absorbent pad was 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 clic 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 minutes per rat. The observer of behavior does not know about the design of experiments. The complete battery was performed by one observer with all animals. Motor activity data were collected shortly after finishing of the Functional observational battery, using an apparatus for testing of a spontaneous motor activity of laboratory animals (constructed in the Faculty of Military Health Sciences, Hradec Kralove, Czech Republic). The animals were placed for a short period (10 minutes) 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. Their statistical analyses were performed on a PC with a special interactive programme NTX (Frantík and Hornychova, 1995). The categorial 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, Barlett test for Equality of Variance, Williams test and Test for Distribution Functions (Roth et al*.,* 1962). The differences were considered significant when $P < 0.05$.

3.0 RESULTS

The screening test was used as the first step of evaluation of the large quantity of new oximes to reactivate tabun-inhibited AChE *in vitro.* As the results, we have obtained a percentage of reactivation. Only promising oximes together with standards were tested in the second step of *in vitro* investigation where their kinetic parameters of the reactivation were calculated. As indicated from the results (Table 1), all above-mentioned oximes seemed to be very promising and they were recommended for their *in vivo* investigation.

The acute i.m. toxicity of all oximes studied is summarized in Table 2. The results show that the acute toxicity of newly developed oxime K048 for mice corresponds to the acute toxicity of obidoxime, while another newly developed oxime K027 is less toxic and its LD_{50} value is approaching to LD_{50} value of the oxime HI-6 that is considered to be the least toxic for mammals among currently available oximes. On the other hand, the acute toxicity of other newly developed K oximes (K074, K075) for mice is markedly higher in comparison with other oximes studied. Their LD_{50} value is more than fifteen times lower than LD_{50} value of the oxime HI-6.

The therapeutic efficacy of all oximes studied in combination with atropine against tabun is presented in Table 3. Tabun – poisoned mice showed wide spectrum of clinical signs of poisoning including muscarinic (lacrymation, salivation, chewing, miosis) and niconitic (tremor, tonic-clonic convulsions) signs within a few minutes. In the case of antidotal treatment, the clinical sings appeared later and their intensity was diminished compared to untreated poisoning regardless of the type of oxime. They died within 20-30 minutes after poisoning with tabun if they were not treated with antidotes. All antidotal mixtures used for the antidotal treatment of acute tabun poisoning were potent to significantly decrease acute toxicity of tabun with the exception of the oxime HI-6 in combination with atropine. While all newly developed oximes (K027, K048, K074, K075) were able to significantly decrease the acute toxicity of tabun almost 2-fold and their therapeutic efficacy was a little higher than the therapeutic efficacy of obidoxime, the decrease in acute toxicity of tabun induced by HI-6 was not significant.

The ability of oximes to reactivate tabun-inhibited AChE in rat blood and diaphragm *in vivo* is shown in Table 4. All newly developed oximes seemed to be effective reactivators of tabun-inhibited AChE. While the reactivating effectiveness of all newly developed oximes in the peripheral compartment (blood) is similar and corresponds to the efficacy of obidoxime, K074 appeared to be the best reactivator of tabun-inhibited AChE in the central compartment (brain) among all oximes studied. The oxime HI-6 has significantly lower potency in reactivating tabun-inhibited AChE in peripheral as well as central compartments.

The ability of newly synthesized oximes K027 and K048 to eliminate tabun-induced neurotoxicity at 24h following tabun poisoning is shown in Table 5. The observation of neurotoxic signs indicated that many functional disorders of poisoned organisms outlasted at least 24 hours not only in non-treated tabun-poisoned rats but also in tabun-poisoned rats treated with atropine combined with the oxime HI-6. Tabun caused passive behavior of rats during handling and catching and a decrease in muscular tonus. The exploratory activity (rearing) was significantly decreased, gait and mobility were somewhat impaired and the level of unprovoked activity was reduced. Involuntary clonic movements were also observed. In addition, no reaction during a reflex testing consisting of recording each rat`s response to the frontal approach of the blunt end of a pen or a touch of the pen to the posterior flank was observed. No ability of pupils to constrict in response to light was demonstrated either. A significant decrease in the distance between hindpaws after a jump, forelimb and hindlimb grip strength, food receiving and spontaneous horizontal as well as vertical motor activity were also observed at 24 h following tabun challenge. Both newly developed oximes (K027, K048) in combination with atropine was able to eliminate many tabun-induced signs of neurotoxicity observed at 24 hours following tabun challenge with the exception of passive behavior of rats during handling and catching, a decrease in the exploratory activity, body temperature, food receiving and spontaneous motor activity. In addition, the oxime K027 was not able to eliminate a decrease in the distance between hindpaws after a jump and forelimb grip strength. On the other hand, the oxime HI-6 in combination with atropine was not able to eliminate or at least to decrease the intensity of most of above mentioned tabun-induced signs of neurotoxicity. Obidoxime in combination with atropine was almost as effective as newly developed oximes with the exception of approach response and hindlimb grip strength.

The potency of other newly synthesized oximes (K074 and K075) to eliminate tabun-induced neurotoxicity at 24h following tabun poisoning is shown in Table 6. The newly developed oxime K075 in combination with atropine was able to prevent many tabun-induced signs of neurotoxicity observed at 24

hours following tabun challenge with the exception of a decrease in the distance between hindpaws after a jump, forelimb grip strength, body temperature, food receiving and spontaneous motor activity. Another newly developed oxime K074 seems to be less efficacious than K075 because it was not able to eliminate tabun-induced miosis, a decrease in muscular tonus, passive behavior of rats during handling and catching and a decrease in rat`s response to the frontal approach of the blunt end of a pen. Obidoxime in combination with atropine was almost as effective as K075 with the exception of miosis. On the other hand, the oxime HI-6 in combination with atropine was not able to prevent or at least to decrease the intensity of some above mentioned tabun-induced signs of neurotoxicity.

4.0 DISCUSSION

Currently used obidoxime is able to partly reactivate tabun-inhibited AChE and eliminate tabun-induced acute neurotoxicity following i.m. administration of tabun at a lethal dose, nevertheless, its therapeutic, reactivating and neuroprotective efficacy is not satisfactory (Kassa and Krejcova, 2003). The oxime HI-6 has been produced and introduced by some countries for the antidotal treatment of severe acute poisonings with soman because of its higher reactivation and therapeutical efficacy compared to currently used oximes such as pralidoxime and obidoxime (Dawson, 1994; Kassa and Cabal, 1999a). Nevertheless, it was demonstrated to be significantly less efficacious to reactivate tabun-inhibited AChE and eliminate tabun–induced acute neurotoxicity than obidoxime (Kassa and Krejcova, 2003). The unsatisfactory efficacy of the above mentioned oximes to eliminate tabun-induced acute neurotoxicity is possible to explain by very low the potency of oximes in reactivating tabun-inhibited AChE *in vitro* as well as *in vivo* (Puu et al., 1986; Jokanovic et al., 1996; Worek et al., 1998). Therefore, new oximes have been developed to increase the reactivating potency as well as neuroprotective efficacy of antidotal treatment of acute tabun poisonings.

New AChE reactivators able to satisfactorily reactivate AChE inhibited by tabun and counteract tabun-induced acute neurotoxicity have been still developed. According to our former results, bisquaternary AChE reactivators with functional oxime group in the position four at the pyridinium ring are the most potent reactivators of tabun-inhibited AChE (Cabal et al., 2004). Other very important structural feature is the number of methylene groups linking chain between two quaternary pyridinium rings in the molecule of reactivators (Kuca and Kassa, 2003). Above mentioned data on a reactivator structure allowed us to postulate requirements on the structural parameters of new reactivators of tabun-inhibited AChE.

Our results demonstrate that all newly developed oximes (K027, K048, K074, K075) appear to be more effective to reactivate tabun-inhibited AChE and eliminate tabun-induced acute toxicity in rats than the oxime HI-6 and they are as effective as obidoxime. In addition, there is a small difference between the efficacy of K oximes to reactivate tabun-inhibited AChE and eliminate tabun-induced acute toxicity with the exception of reactivation of tabun-inhibited AChE in the brain by K074. The reason for their relatively high efficacy is probably a special chemical structure of their molecule. The stereochemical arrangement of oximes can play a role in the difference in therapeutic efficacy of oximes against tabun (Cabal and Bajgar, 1999).

In conclusion, there is not any broad spectrum oxime able to satisfactorily counteract acute toxic effects of all nerve agents. The oxime HI-6 is the most efficacious oxime to reactivate soman or cyclosarin-inhibited AChE and to protect soman or cyclosarin-exposed mammals from their acute toxic effects (Kassa and Cabal, 1999a,b; Kassa, 2002), nevertheless, it is not efficacious to protect tabun-exposed animals from tabun-induced neurotoxicity (Kassa and Krejcova, 2003). Obidoxime is suitable oxime for the reactivation of sarin or VX-inhibited AChE (Kassa, 2002) but it is not able to sufficiently protect soman or cyclosarin-exposed mammals from their acute toxic effects (Kassa and Cabal, 1999a,b). Newly developed K oximes seem to be the very effective reactivators of VX agent-inhibited AChE (Kuca and Kassa, 2004) but they are not suitable oximes for the treatment of cyclosarin poisonings because they are not sufficiently effective to reactivate cyclosarin-inhibited AChE and eliminate cyclosarin-induced toxic effects (Musilek et al. 2006; Kassa et al. 2007). On the other hand, they seem to be sufficiently effective and suitable oximes for the antidotal treatment of acute tabun poisoning of mammals due to their reactivating, therapeutic and neuroprotective effects although the differencies of reactivating and

neuroprotective efficacy between newly developed and some currently available oximes (obidoxime) is not so high to think about replacement of currently used oximes by them for the treatment of acute tabun poisoning.

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Table 1. In vitro reactivation of tabun-inhibited AChE

Oxime	*Enzyme*	K_R [μM]	k_R [min^{-1}]	k_r [$min^{-1}. M^{-1}$]
Pralidoxime	Rat	575	0.006	10
Methoxime	Rat	- *	- *	- *
Trimedoxime	Rat	460	0.079	172
Obidoxime	Rat	3	0.020	6250
HI-6	Rat	6	0.007	1111
K027	Rat	54	0.015	273
K048	Rat	93	0.032	348
K074	Rat	29	0.056	1931
K075	Rat	19	0.056	2947
Pralidoxime	Human	- *	- *	- *
Methoxime	Human	2512	0.05	20
Trimedoxime	Human	1585	0.08	50
Obidoxime	Human	1412	0.06	42
HI-6	Human	- *	- *	- *
K027	Human	2512	0.05	20
K048	Human	251	0.06	239
K074	Human	1995	0.08	40
K075	Human	200	0.02	100

K_R - dissociation constant of inhibited enzyme-reactivator complex;
k_R - the first-order rate constant of reactivation;
k_r - the second-order rate constant of reactivation

* appropriate constants cannot be calculated due to extremely low potency of this oxime to reactivate tabun-inhibited acetylcholinesterase

Table 2 LD_{50} values of oximes following i.m. administration in mice.

Antidote	LD_{50} (mg/kg) ± 95% confidence limit
HI-6	671.3 (627.4 – 718.3)
Obidoxime	188.4 (156.3 – 208.0)
K027	**577.0 (539.0 – 617.8)**
K048	**233.5 (217.8 – 250.3)**
K074	**23.3 (20.6-26.4)**
K075	**43.0 (38.6-47.8)**

Table 3 The potency of oximes in combination with atropine to eliminate acute lethal effects of tabun in mice.

* significantly different from non-treated group at the level of $P < 0.05$,
x significantly different from the group treated with obidoxime or the oxime HI-6 at the level of $P < 0.05$.

Treatment	LD_{50} (µg/kg) ± 95% IS	Protective ratio
----	318.0 (305.5 – 331.0)	----
Obidoxime + atropine	451.6 (418.8 – 487.5)*	1.42
HI-6 + atropine	343.4 (336.9 – 349.5)*	1.08
K027 + atropine	**534.2 (494.1 – 575.2)*x**	**1.68**
K048 + atropine	**556.5 (514.4 – 590.8)*x**	**1.75**
K074 + atropine	**551.7 (490.3 – 620.7)*x**	**1.74**
K075 + atropine	**635.3 (587.9 – 686.6)*x**	**2.00**

Table 4 Rate of reactivation of tabun-inhibited AChE by oximes in rat blood and brain *in vivo*. AChE activity (μkat/L or μkat/kg)

TREATMENT	Blood	Brain
Atropine	2.23 ± 0.59	82.8 ± 20.5
Atropine + obidoxime (% reactivation)	**6.43 ± 0.46 (24.3*)**	**126.5 ± 17.1 (20.7*)**
Atropine+ HI-6 (% reactivation)	3.28 ± 0.61 (6.1)	96.9 ± 21.3 (6.7)
Atropine+ K027 (% reactivation)	**5.14 ± 0.77 (16.9*)**	**129.3 ± 23.8 (22.1*)**
Atropine+ K048 (% reactivation)	**5.94 ± 0.91 (21.5*)**	115.4 ± 20.6 (15.5)
Atropine+ K 074 (% reactivation)	**5.48 ± 1.04 (18.8*)**	**265.5 ± 30.3 (86.8*)**
Atropine+ K 075 (% reactivation)	**5.61 ± 0.75 (19.6*)**	112.1 ± 9.5 (13.9)

*significantly different from atropine-treated group at the level of $P < 0.05$. The untreated control value for rat blood AChE was 19.50 μkat/L and for brain AChE activity 293.3 μkat/kg.

Table 5 The values of tabun-induced neurotoxic markers measured at 24 hours following tabun challenge by the Functional observational battery (No 1-11, 13-36, 44 - scored values, No 12, 37-43, 45-47 - values in absolute units). Statistical significance: * P < 0.05; ** P < 0.01; *** P < 0.001 (comparison with the control values)

24 hours:		Controls		T+Atr + Obidoxime		T+Atr + K048		T+Atr + K027		T+Atr + 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		1,00		1,00		1,00		3,00		3,00	
2	catch difficulty	2,00		**1,00***		**1,00***		**1,00***		**1,00*****		**1,00*****	
3	ease of handling	2,00		**1,00***		**1,00***		**1,00***		**1,00*****		**1,00*****	
4	muscular tonus	0,00		0,00		0,00		0,00		**-2,00*****		**-2,00*****	
5	lacrimation	0,00		0,00		0,00		0,00		0,00		0,00	
6	palpebral closure	1,00		1,00		1,00		1,00		1,00		1,00	
7	endo/exophtalmus	0,00		0,00		0,00		0,00		0,00		0,00	
8	fur abnormalities	0,00		0,00		0,00		0,00		0,00		0,00	
9	skin abnormalities	0,00		0,00		0,00		0,00		0,00		0,00	
10	salivation	0,00		0,00		0,00		0,00		0,00		0,00	
11	nose secretion	0,00		0,00		0,00		0,00		0,00		0,00	
12	rearing	23,38	8,19	**6,63*****	5,71	**12,50****	4,50	**6,50*****	6,28	**10,00****	7,21	12,83	10,30
13	urination	0,00		0,00		0,00		0,00		0,00		0,00	
14	defecation	0,00		0,00		0,00		0,00		0,00		0,00	
15	hyperkinesis	0,00		0,00		0,00		0,00		0,00		0,00	
16	tremors	0,00		0,00		0,00		0,00		0,00		0,00	
17	clonic movements	0,00		0,00		0,00		0,00		0,00		**2,00***	
18	tonic movements	0,00		0,00		0,00		0,00		0,00		0,00	
19	gait	0,00		0,00		0,00		0,00		**1,00****		**7,00***	
20	ataxia	0,00		0,00		0,00		0,00		**1,00****		**2,00***	
21	gait score	0,00		0,00		0,00		0,00		**1,00*****		**2,00***	
22	mobility score	1,00		1,00		1,00		1,00		1,00		1,00	
23	arousal (GSC)	1,00		1,00		1,00		1,00		**2,00*****		**4,00****	
24	activity	4,00		2,00		4,00		3,00		3,00		2,00	
25	tension	0,00		0,00		0,00		0,00		0,00		0,00	
26	vocalisation	0,00		0,00		0,00		0,00		0,00		0,00	
27	stereotypy	0,00		0,00		0,00		0,00		0,00		0,00	
28	bizzare behavior	0,00		0,00		0,00		0,00		0,00		0,00	
29	approach response	2,00		**2,00***		2,00		2,00		2,00		**1,00*****	
30	touch response	2,00		2,00		2,00		2,00		2,00		**1,00*****	
31	click response	2,00		2,00		2,00		2,00		2,00		1,00	
32	tail-pinch response	2,00		2,00		2,00		2,00		2,00		2,00	
33	pupil size	0,00		**1,00***		0,00		0,00		**2,00*****		**-2,00****	
34	pupil response	1,00		**0,00****		**0,50***		1,00		0,50		**0,00****	
35	RRF	1,00		1,00		1,00		1,00		1,00		1,00	
36	RRV	1,00		1,00		1,00		1,00		1,00		**3,00***	
37	landing foot splay (mm)	109,69	14,69	**81,56*****	17,82	96,00	13,27	**86,00***	18,59	**68,06*****	31,49	**54,75*****	36,09
38	forelimb grip strength (kg)	5,53	0,85	**3,79*****	0,98	4,83	1,00	**4,36***	0,88	**3,93*****	0,77	**4,28***	0,74
39	hindlimb grip strength (kg)	1,24	0,24	**0,96***	0,18	1,21	0,23	1,01	0,15	**0,87****	0,19	0,98	0,54
40	grip strength of all limbs (kg)	14,54	1,79	**11,35***	3,31	13,88	2,51	13,89	1,88	10,94	4,09	11,02	2,96
41	food receiving (%)	100,00	0,00	**74,50*****	6,21	**77,50*****	3,21	**77,00*****	8,90	**70,50***	30,50	**48,75*****	30,44
42	body weight (g)	186,00	14,74	186,63	25,86	197,13	19,97	190,38	19,42	191,71	17,57	182,67	24,33
43	body temperature (°C)	37,41	0,23	37,21	0,39	**36,86*****	0,35	**36,89*****	0,55	37,01	0,55	37,18	0,42
44	respiration	0,00		0,00		0,00		0,00		0,00		0,00	
45	vertical activity	362,00	90,71	**174,13*****	80,67	**225,75***	105,02	260,75	122,36	**139,75*****	164,13	**181,25****	145,50
46	horizontal activity	92,75	15,72	**22,50*****	18,58	**36,75*****	29,84	**29,75*****	14,73	**23,38*****	40,66	**27,38*****	33,17
47	total motor activity	454,75	96,99	**196,63*****	97,59	**262,50*****	122,22	**290,50***	123,64	**163,13*****	199,64	**208,63*****	175,94
		n=8		n=8		n=8		n=8		n=7		n=6	

Table 6 The values of tabun-induced neurotoxic markers measured at 24 hours following tabun challenge by the Functional observational battery (No 1-11, 14-36, 44 - scored values, No 12-13, 37-43, 45-47 - values in absolute units). Statistical significance: * P < 0.05; ** P < 0.01; *** P < 0.001 (comparison with the control values)

24 hours:		**Controls**		**GA + A + HI - 6**		**GA + A + Obidoxime**		**GA + A + K074**		**GA + A + K075**		**GA**	
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***		1,00		1,00		3,00		**3,00***	
2	catch difficulty	2,00		**1,00*****		2,00		**1,00*****		2,00		**1,00*****	
3	ease of handling	2,00		**1,00*****		2,00		**1,00*****		2,00		**1,00*****	
4	muscular tonus	0,00		**´-1,00***		0,00		**´-2,00****		0,00		**´-2,00*****	
5	lacrimation	0,00		0,00		0,00		0,00		0,00		**4,00*****	
6	palpebral closure	1,00		1,00		1,00		1,00		1,00		1,00	
7	endo/exophtalmus	0,00		0,00		0,00		0,00		0,00		0,00	
8	fur abnormalities	0,00		0,00		0,00		0,00		0,00		0,00	
9	skin abnormalities	0,00		0,00		0,00		0,00		0,00		0,00	
10	salivation	0,00		0,00		0,00		0,00		0,00		0,00	
11	nose secretion	0,00		0,00		0,00		0,00		0,00		**3,00*****	
12	rearing	11,75	4,68	**2,00*****	2,20	**1,75*****	0,89	**2,63*****	1,85	5,63	4,34	5,67	7,74
13	urination	1,00	1,69	2,63	7,03	0,00	0,00	1,75	3,24	0,75	0,71	4,00	5,90
14	defecation	0,00		0,00		0,00		0,00		0,00		0,00	
15	hyperkinesis	0,00		0,00		0,00		**3,00***		**3,00***		0,00	
16	tremors	0,00		0,00		0,00		0,00		2,00		0,00	
17	clonic movements	0,00		0,00		0,00		0,00		0,00		1,00	
18	tonic movements	0,00		0,00		0,00		0,00		0,00		0,00	
19	gait	0,00		**1,00****		**5,00****		**1,00*****		**5,00****		**7,00*****	
20	ataxia	0,00		**1,00*****		**1,00****		**1,00****		**1,00***		**1,00****	
21	gait score	0,00		**1,00*****		0,00		0,00		0,00		**1,00***	
22	mobility score	1,00		1,00		1,00		1,00		2,00		**3,00*****	
23	arousal (GSC)	1,00		**3,00****		**2,00****		**2,00*****		**2,00****		**4,00*****	
24	activity	4,00		**1,00*****		**1,00*****		**1,00*****		2,00		**1,00*****	
25	tension	0,00		0,00		0,00		0,00		0,00		0,00	
26	vocalisation	0,00		0,00		0,00		0,00		0,00		0,00	
27	stereotypy	0,00		0,00		0,00		0,00		0,00		0,00	
28	bizzare behavior	0,00		0,00		0,00		0,00		0,00		0,00	
29	approach response	2,00		**1,00*****		2,00		**1,00***		2,00		**1,00***	
30	touch response	2,00		**1,00*****		2,00		1,00		2,00		**1,00*****	
31	click response	2,00		2,00		2,00		3,00		2,00		**3,00****	
32	tail-pinch response	2,00		2,00		2,00		2,00		2,00		**1,00****	
33	pupil size	0,00		**´-2,00*****		**´-2,00***		**´-2,00*****		0,00		**´-2,00*****	
34	pupil response	1,00		**0,00*****		**0,50****		**0,50*****		**0,50*****		**0,00*****	
35	RRF	1,00		1,00		1,00		1,00		1,00		**3,00****	
36	RRV	1,00		1,00		1,00		1,00		1,00		**3,00****	
37	landing foot splay (mm)	121,13	13,72	**87,38*****	21,99	**101,44***	21,29	**85,25*****	12,48	**93,13*****	21,44	**50,75*****	33,08
38	forelimb grip strength (kg)	8,55	1,57	**5,34*****	0,58	**6,48***	1,94	**5,39*****	1,31	**5,94*****	1,49	**5,58*****	1,45
39	hindlimb grip strength (kg)	1,23	0,15	0,99	0,32	**0,99***	0,20	**0,71*****	0,25	1,01	0,30	**0,40*****	0,20
40	grip strength of all limbs (kg)	19,38	2,43	**13,10***	6,46	16,21	4,72	**12,75*****	4,39	**14,21****	4,03	**8,07*****	3,05
41	food receiving (%)	100,00	0,00	**72,50***	29,40	**65,00*****	26,73	**50,00*****	0,00	**48,00*****	2,14	**7,50*****	4,63
42	body weight (g)	259,88	25,18	278,63	20,32	264,50	34,83	253,75	22,80	253,00	16,76	248,17	11,70
43	body temperature (°C)	37,40	0,50	36,91	0,44	**36,91***	0,26	36,92	0,50	**36,64****	0,40	**36,23*****	0,35
44	respiration	0,00		0,00		0,00		0,00		0,00		0,00	
45	vertical activity	391,88	127,39	**126,38*****	127,79	**181,00****	83,61	**152,00*****	97,33	**183,25*****	95,99	**110,75*****	102,96
46	horizontal activity	93,75	44,55	**15,88*****	22,02	**23,13*****	24,79	**18,13*****	13,82	**28,38***	47,94	**10,50*****	11,78
47	total motor activity	485,63	160,93	**142,25*****	147,31	**204,13*****	103,76	**170,13*****	107,17	**211,63*****	140,60	**121,25*****	112,77
		n=8		n=8		n=8		n=8		n=8		n=6	