



## TREATMENT AND PROPHYLAXIS OF NERVE AGENT/ ORGANOPHOSPHATES INTOXICATION

Jiri Bajgar

*Department of Toxicology, Faculty of Military Health Sciences, University of Defense, Trebesska 1575, Hradec Kralove, Czech Republic*

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**Abstract.** Basic mechanism of action of organophosphates (OP)/nerve agents is based on acetylcholinesterase (AChE) inhibition and subsequent accumulation of neuromediator acetylcholine at the cholinergic synapses, either peripheral or central, causing cholinergic hyperstimulation and development of symptoms of poisoning, followed by metabolic dysbalance and, without effective prophylaxis/treatment leading to death.

The treatment of nerve agents poisoning consists of administration of parasympatholytics (preferably atropine), cholinesterase reactivators (oximes) and anticonvulsants (usually diazepam). The choice of reactivators is not so simple. Their administration alone is not effective but simultaneous administration with atropine potentiates their antidotal effects based on AChE reactivation at the cholinergic nerve synapses. AChE reactivation at the peripheral nervous system is indisputable; however, their passing the blood-brain-barrier facilitating their central effect is discussed. On the basis of our own and literature data, central reactivation efficacy of some oximes *in vivo* is demonstrated. Though the research is very intensive, unfortunately, up to now, there is not universal reactivator sufficiently effective against all nerve agents/OP. A possible direction solving this problem is discussed – it is the use of combination of more reactivators. The good reactivating and therapeutic effect of combination of trimedoxime and HI-6 against tabun poisoning in rats is demonstrated.

Prophylaxis against nerve agent intoxication is based on various approaches:

Keeping AChE, key enzyme for toxic action of OP/nerve agents intact (protection of cholinesterases) is a basic requirement for effective prophylaxis. Detoxification realised by administration of the enzymes splitting the OP or evaluating specific enzymes (cholinesterases) is another possibility (stoichiometric and catalytic scavengers). The antidotes currently used for the treatment of OP poisoning including reactivators are to be tested as prophylactics. This principle can be considered as a „treatment in advance”. The problem with use of reactivators is the timing, duration and achievement of sufficient levels of these antidotes after the administration. Transdermal administration of reactivators solves these difficulties. As a result of this research, prophylactic antidote TRANSANT (transdermal patch containing HI-6) was developed as the prophylactic mean and introduced into the Czech Armed Forces. Future development will be focused on scavengers (cholinesterases and other enzymes) acting before the binding of nerve agent to the target sites, and to other drugs either reversible cholinesterase inhibitors (e.g. huperzine A, physostigmine, acridine derivatives etc.)

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or other compounds. However, it will be necessary to study basic neuropharmacologic problems in general, i.e.

- binding of different ligands to cholinesterases for elucidation of cholinergic nerve transmission,
- prevention of neuronal cells death due to seizures,
- treatment of non-specific effects,
- the choice of reactivators/their combinations and parasympatholytics including their timing,
- evaluation of more informations dealing with long term effect of low doses,
- the gene expression profile after intoxication and its prophylaxis/treatment,
- new drugs including combination of scavengers with classic prophylactics/antidotes.

**Keywords:** nerve agent, organophosphates intoxication

## Introduction

Toxic effect of organophosphates (OP)/nerve agents is based on acetylcholinesterase (AChE, EC 3.1.1.7) inhibition and subsequent accumulation of neuromediator acetylcholine at the cholinergic synapses, either peripheral or central, leading to cholinergic hyperstimulation and development of symptoms of poisoning, followed by metabolic dysbalance and without effective treatment leading to death (Bajgar, 2004; Delfino et al., 2009; Fusek et al., 2009; Kassa et al., 2009; Marrs et al., 1996; Wright et al., 2009).

*Therapy* of nerve agents poisoning consists of administration of parasympatholytics (preferably atropine), cholinesterase reactivators (oximes) and anticonvulsants (usually diazepam) (Myhrer, 2009; Bajgar, 2004). The choice of reactivators is not so simple (Milatovic and Jokanovic, 2009; Jokanovic and Prostran, 2009; Kuca et al., 2009). Their administration alone is not effective but simultaneous administration with atropine potentiates their antidotal effects based on AChE reactivation at the cholinergic nerve synapses. AChE reactivation at the peripheral nervous system is indisputable; however, their passing the blood-brain-barrier facilitating their central reactivation efficacy in vivo was demonstrated (Bajgar et al., 2007a, 2007b; Kassa et al., 2006, 2007). Though the research is very intensive, unfortunately, up to now, there is not universal reactivator sufficiently effective against all nerve agents/OP (Kalasz et al., 2009; Kuca et al., 2007, 2009a; Musilek et al., 2007). A possible direction solving this problem is the use of combination of more reactivators. Reactivating effect of combination of trimedoxime and HI-6 against tabun poisoning in rats is demonstrated.

*Prophylaxis* against nerve agent intoxication is based on various approaches:

Keeping AChE, key enzyme for toxic action of OP/nerve agents intact (protection of cholinesterases) is a basic requirement for effective prophylaxis. Detoxification realised by administration of the enzymes splitting the OP or evaluating specific enzymes (cholinesterases) is another possibility (stoichiometric and catalytic scavengers). The antidotes currently used for the treatment of OP poisoning including reactivators are to be tested as prophylactics. This principle can be considered as a „treatment in advance” (Bajgar et al., 2009a,b). The problem with use of reactivators is the timing, duration and achievement of sufficient levels of these antidotes after the administration (Bajgar, 2004; Fusek et al., 2007). Transdermal administration of reactivators solves these difficulties. As a result of this research, prophylactic antidote TRANSANT (transdermal patch containing HI-6) was developed as the prophylactic mean and introduced into the Czech Armed Forces (Bajgar et al., 2004; Fusek et al., 2007). Comparison of different prophylactic approaches is demonstrated.

## Methodical part

Animals (female Wistar rats, weighing 180-220 g), nerve agents, therapeutic and prophylactic effectiveness, preparation of blood, diaphragm and brain parts, AChE activity determination and other experimental conditions were performed as described in more detailed way for prophylaxis (Bajgar, 2008) and treatment (Kassa et al., 2007). For better orientation, the data on doses and time schedule of experiments only are given below:

*Treatment with combination of reactivators*

**CONTROL GROUP:** The animals were injected with saline i.m. and 1 min later, they were injected once again with saline i.m. (0.1 ml/100 g). The decapitation and sampling was realized 30 min after the last saline injection.

**Treated groups**

**TABUN GROUP:** The animals were injected with tabun (i.m.) in a dose of  $1.5 \times LD_{50}$ , i.e. 300 µg/kg; 1 min later, the animals were injected with atropine (i.m., 21 mg/kg). 31 min after the intoxication, the animals were decapitated and the blood and tissues were collected for biochemical examinations.

**TRIMEDOXIME GROUP:** The animals were injected with tabun (i.m.) in a dose of  $1.5 \times LD_{50}$ , i.e. 300 µg/kg; 1 min later, the animals were injected with one injection (i.m.) of atropine (21 mg/kg) and trimedoxime chloride (5% of  $LD_{50}$  dose - 3.75 mg/kg). 31 min after the intoxication, the animals were decapitated and the brains, diaphragm and blood were used for biochemical examinations.

**HI-6 GROUP:** The animals were injected with tabun (i.m.) in a dose of  $1.5 \times LD_{50}$ , i.e. 300 µg/kg; 1 min later, the animals were injected with one injection (i.m.) of atropine (21 mg/kg) and HI-6 chloride (5% of  $LD_{50}$  dose - 19.5 mg/kg). 31 min after the intoxication, the animals were decapitated and the blood and tissues were collected for biochemical examinations.

**OXIME COMBINATION GROUPS:** The animals were injected with tabun (i.m.) in a dose of  $1.5 \times LD_{50}$ , i.e. 300 µg/kg; 1 min later, the animals were injected with one injection (i.m.) of atropine (21 mg/kg) and

- 5H+5TR - 5% + 5% of HI-6 and trimedoxime doses;
- 5H+2.5TR - 5% of HI-6 + 2.5% of trimedoxime doses;
- 2.5H+2.5TR - 2.5% + 2.5% of HI-6 + trimedoxime doses;
- 2.5H - 2.5% of HI-6 dose; 2.5 T - 2.5% of trimedoxime dose;
- 5TR+2.5H - 5% of trimedoxime + 2.5% of HI-6 doses.

**Statistical evaluation:**

Enzyme activities determined by biochemical method (Ellman et al., 1961) were expressed as a mean  $\pm$ SD or % of control values and statistical differences were tested by t-test. The reactivation (%) was determined using the AChE activity values:

$$[1 - (a_o - a_r) / (a_o - a_i)] \times 100$$

where

- $a_o$  is activity in control group (with administration of saline),
- $a_r$  is activity in tabun-intoxicated group treated with atropine and reactivator,
- $a_i$  is activity in tabun-intoxicated group treated with atropine only.

*Prophylaxis*

The groups of 6-10 rats were pretreated with pyridostigmine alone (PYRIDOSTIGMINE), with HI-6 alone (TRANSANT), with pyridostigmine, benactyzine and trihexyphenidyle ((PANPAL), with pyridostigmine, benactyzine, trihexyphenidyle and HI-6 (PANPAL + TRANSANT), or with equine BuChE (EqBuChE; thanks are due to Dr. B. Doctor, WRAIR, USA for supplying the enzyme).

The animals in group were injected im with drug or its combination and 20 min later, the animals were intoxicated with nerve agent or OP (im) in different doses and the ratios of LD50 values with and without pretreatment were determined and designated as prophylactic index.

Nerve agents used were VX, sarin, soman, tabun, Russian VX, cyclosarin; OP were DDVP and EDMM, respectively.

**Doses of drugs used:**

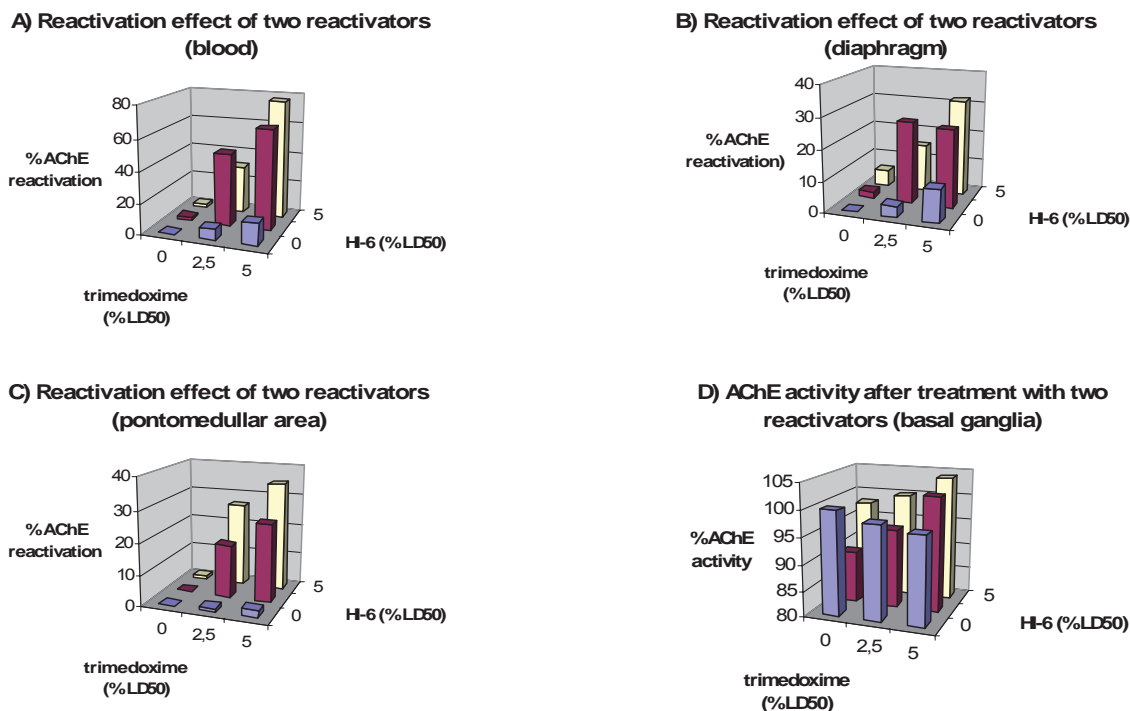
- HI-6 15 mg/kg, im;
- Benactyzine 9 mg/kg, im;
- Trihexyphenidyle 6 mg/kg, im;
- Pyridostigmine 1 mg/kg, im;
- BuChE 250 mU/kg, ip.

**Results****Treatment**

Therapeutic efficacy of all combinations caused survival of all experimental animals. However, clinical status of animals treated with atropine and trimedoxime was better than that treated with atropine only or atropine with HI-6. Reactivation efficacy was different, too. Following treatment with atropine, reactivation was not observed. When combination of atropine and HI-6 was used, low reactivation (less than 5%) was demonstrated. When combination of atropine and trimedoxime was used, the reactivation was also relatively low but higher than that observed for HI-6 (5-16%). Combination of two reactivators caused much more higher AChE reactivation, both at the periphery

(blood, diaphragm) and brain (pontomedullar area and frontal cortex). AChE activity detected in the basal ganglia was not practically affected either by tabun or by the treatment, independently on the reactivator used (Fig. 1A-D).

varied in the range from 0.95 to 1.1; slightly better prophylaxis was caused by isolated administration of PYRIDOSTIGMINE able to protect rats against 1-2xLD<sub>50</sub> doses of nerve agents and 5xLD<sub>50</sub> dose of DDVP. EqBuChE was more effective and prophy-



**Figure 1.** AChE reactivation (%) following intoxication with tabun and treatment with atropine and two reactivators (trimedoxime and HI-6) in the blood (A), diaphragm (B) and pontomedullar area (C) and AChE activity (%) (D) following intoxication with tabun and treatment with atropine and two reactivators (trimedoxime and HI-6) in the basal ganglia in rats. The results are means only, because of SD varied in the range ± 5.6 – 13.5 %.

### Prophylaxis

Prophylactic efficacy of isolated administration of HI-6 (TRANSANT) 30 min prior the intoxication with particular agent was practically without any effects (Table 1 and 2) - prophylactic indexes

lactic indexes varied from 5.5 (for VX) to 3.9 (for soman). Prophylactic efficacy of combination of pyridostigmine with benactyzine and trihexyphenidyle (PANPAL) against sarin was comparable with that of EqBuChE and slightly less (against soman)

proPHyLaCtic Combination	VX	sarin	soman	tabun
PANPAL	15,9±2.44	3,4±0.51	2,0±0.55	2,2±0.67
TRANSANT	1,0±0.23	0,95±0.21	1,0±0.23	1,1±0.43
PANPAL+TRANSANT	18,7±2.3	5,8±1.03	4,1±1.04	6,8±2.12
EqBuChE	5,5±1.0	4,3±0.97	3,9±1.00	4,8±1.33
PYRIDOSTIGMINE	1,5±0.88	1,8±0.67	1,3±0.78	2,0±0.45

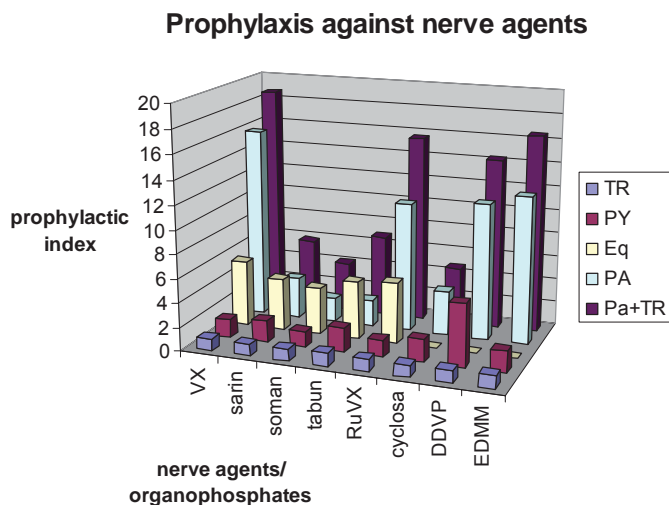
**Table 1.:** Comparison of prophylactic efficacy (± SD) of different prophylactics (expressed as prophylactic index) against some nerve agents in rat

proPHYLACTIC COMBINATION	RVX	cyklosin	DDVP	EDMM
PANPAL	10,8±2.12	3,7±0.94	11,4±2.03	12,3±1.76
TRANSANT	1,01±0.38	0,98±0.45	1,0±0.43	1,02±0.33
PANPAL+TRANSANT	15,6±2.12	4,7±1.37	14,3±2.21	16,5±2.18
EqBuChE	5,1±1.32	-	-	-
PYRIDOSTIGMINE	1,4±0.76	1,9±0.65	5,2±1.21	1,8±0.65

**Table 2.:** Comparison of prophylactic efficacy (± SD) of different prophylactics (expressed as prophylactic index) against some nerve agents and OP in rat

in comparison with EqBuChE; its prophylactic efficacy against V agents was very high. The best prophylactic effect was observed for combination of PANPAL and TRANSANT. The results with prophylaxis are summarized in figure 2.

intoxicaton, our results in vivo were surprising: on the contrary of results in vitro (Worek et al., 2007), where the effect of combination of two reactivators was practically without interaction, our results suggested that in vivo potentiation can be present.



**Figure 2.** Prophylactic efficacy of different prophylactics against nerve agents/OP in rats. The results are means only. TR – TRANSANT; PY – pyridostigmine; Eq – equine butyrylcholinesterase; PA – PANPAL; Pa+TR – combination of PANPAL and TRANSANT.

## Discussion

At the light of its particular inhibitory effects on peripheral and central cholinesterases, tabun, compared to the other nerve agents such as soman and sarin, seems to be the optimal candidate to be tested as a model for the action of two reactivators. More detailed studies dealing with pharmacodynamics/pharmacokinetics will be necessary as discussed in different comprehensive reviews (Bajgar et al., 2007b; Jokanovic and Prostran, 2009; Kalasz et al., 2009; Milatovic and Jokanovic, 2009; Voicu, 2009). There are oximes without reactivation efficacy to tabun-inhibited AChE (HI-6) and others reactivating AChE inhibited by this agents (Bajgar, 2004; Kassa et al., 2005, 2006; Worek et al., 2007).

In preventing the lethality of tabun, atropine and trimedoxime was effective compared with other reactivators (obidoxime was comparatively effective; however, HI-6 was practically ineffective (Kassa et al., 2006).

In all these comparisons, it is necessary to point out that inhibition of cholinesterases in the blood is an indicator of exposure but it is not connected with the mechanism of action of nerve agents: it has been demonstrated that inhibition of erythrocyte AChE in rabbits does not influence normal functions of the organism (Kassa and Bajgar, 1999; Kassa et al., 1997). As for as treatment of tabun

This situation is good for the treatment; in vivo, good therapeutic efficacy of combination of HI-6 and obidoxime was demonstrated earlier in mice (Clement et al., 1987) and in rats (Maksimovic et al., 1987). The problem is a type of autoinjector used for the first aid treatment – HI-6 is unstable in solution and therefore, wet dry autoinjector can be used. In this connection, new 3-chambered Autoinjector is under development (Kuca et al., 2009b), allowing to administer simultaneously HI-6 (lyophilized), atropine (in solution) and diazepam; similar approach can be applied for combination of two oximes (one added into atropine solution).

Future development will be focused on scavengers (cholinesterases and other enzymes) acting before the binding of nerve agent to the target sites; however, there are some problems with immune properties of these enzymes (and a high cost). Other drugs - either reversible cholinesterase inhibitors (e.g. huperzine A, physostigmine, acridine derivatives etc.) or other compounds are studied (Bajgar et al., 2009b). However, it will be necessary to study basic neuropharmacologic problems in general, i.e. binding of different ligands to cholinesterases for elucidation of cholinergic nerve transmission, prevention of neuronal cells death due to seizures, treatment of non-specific effects, the choice of reactivators/their combinations and parasym-

tholytics including their timing, evaluation of more informations dealing with long term effect of low doses, the gene expression profile after intoxication and its prophylaxis/treatment, and effects of new drugs including combination of scavengers with classic prophylactics/antidotes.

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