Defence Science Journal, Vol. 55, No. 2, April 2005, pp. 105-115

REVIEW PAPER

Cyclosarin-An Organophosphate Nerve Agent

G. Krejcova, K. Kuca, and L. Sevelova
Purkyne Military Medical Academy, Hradec Kralove, Czech Republic

ABSTRACT

Organophosphorus compounds ascribed to as nerve agents (sarin, soman, tabun, cyclosarin) are highly toxic, and are considered to be the most dangerous chemical compounds. All apparently share a common mechanism of cholinesterase inhibition and can cause similar symptoms. The standard therapy, in the case of organophosphorus poisoning, has the prophylactic use of reversibly acting AChE inhibitors and antidotal administration of AChE reactivators-oximes. Unfortunately, none of these oximes can be regarded as a broad spectrum antidote, ie, effective against all nerve agents. While the presently available oximes (pralidoxime, obidoxime) are not considered to be sufficiently effective against nerve agents, especially in the case of soman poisoning, the H oximes (HI-6, HLö7) appear to be very promising antidotes against nerve agents because these are able to protect the experimental animals from toxic effects and improve survival of animals poisoned with supralethal doses. A lot of research has been pursued on the treatment of sarin, soman, and tabun, but cyclosarin was not considered for such a study for a long time. Recently, attention of researchers has also turned to cyclosarin because of its potential use as a chemical warfare agent. Cyclosarin is highly toxic organophosphorus compound which is resistant to conventional oxime therapy. This paper reviews the latest position of cyclosarin in standpoint of medical treatment by various reactivators considering the ability of various oximes, HI-6, HS-6, BI-6, and K033 of their reactivation potency.

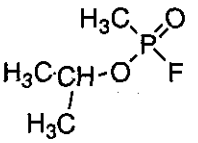
Keywords: Cyclosarin, nerve agent, chemical warfare agent, organophosphorous compounds, cholinesterase inhibition, AChE reactivators, antidots, oximes

1. INTRODUCTION

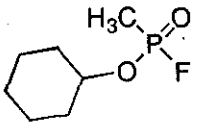
The organophosphates (OP) are used in agriculture and in veterinary practice. Potential for exposure to chemical warfare nerve agents such as sarin (GB; *O*-isopropylmethylfluorophosphonate), tabun (GA; *O*-ethyldimethylamidocyanophosphate), soman (GD; *O*-pinacolylmethylfluorophosphonate), VX (*O*-ethyl-S-(2-diisopropylaminoethyl)-methylthiophosphonate) and cyclosarin (GF; *O*-cyclohexylmethylfluorophosphonate) [Structure I] exists on the battlefield (eg, Iran-Iraq war, Desert Storm), or in the civilian sector as a threat by a terrorist group (eg, Tokyo subway incident), or as an accident as part of current demilitarisation efforts. All apparently share a common mechanism of cholinesterase inhibition and can cause similar symptoms. Because these share this mechanism, exposure to the same organophosphate by multiple routes or to multiple organophosphates by multiple routes, can lead to serious additive toxicity[1].

The organophosphates poison insects and mammals primarily by phosphorylation of the acetylcholinesterase enzyme (AChE, EC 3.1.1.7) at nerve endings (Structure II). The result is a loss

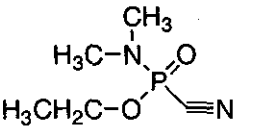
Revised 04 October 2004



SARIN



CYCLOSARIN



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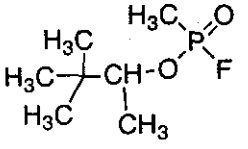

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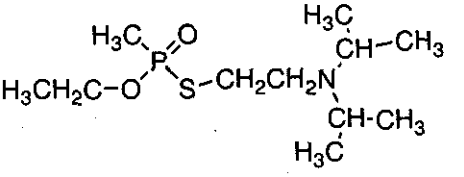
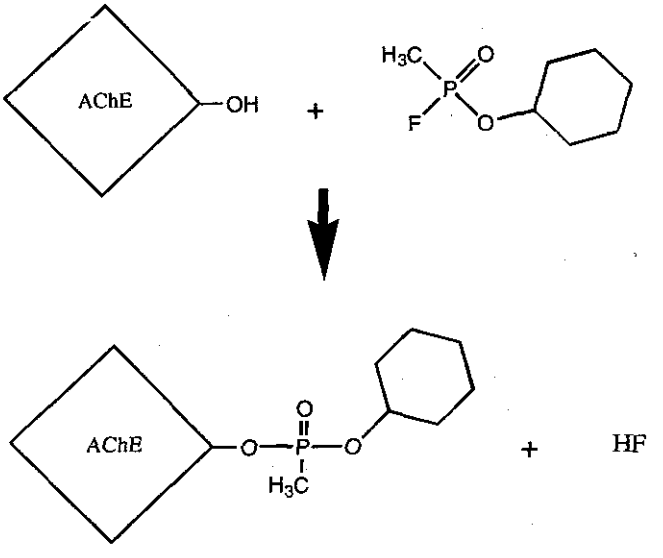


VX

Structure I: Nerve agents



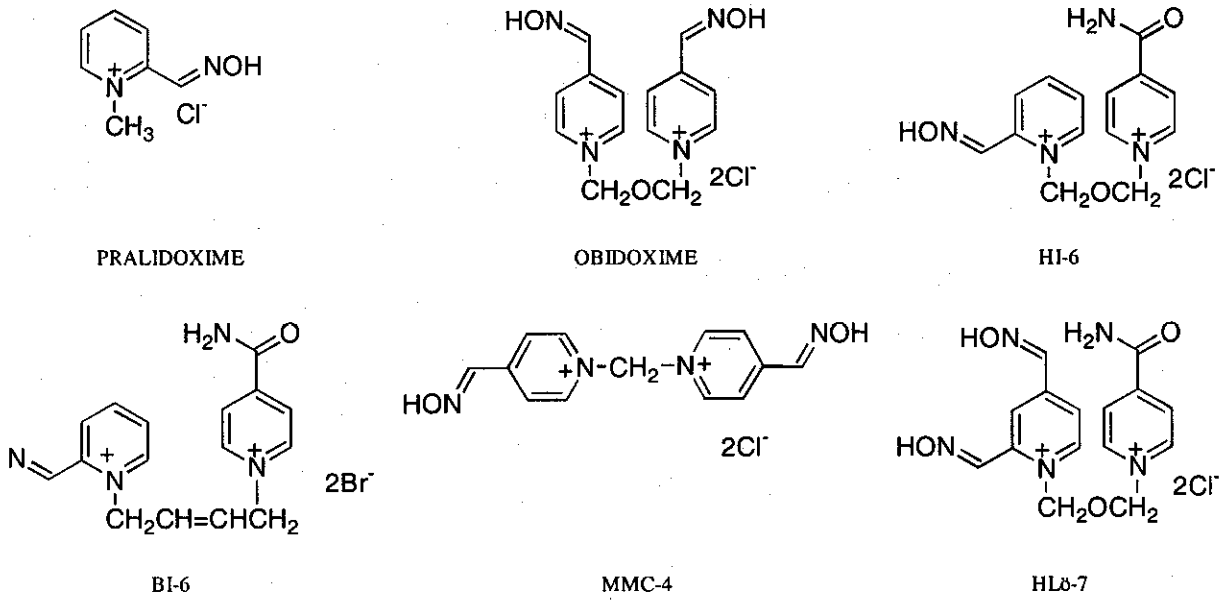
Structure II: Phosphorylation of the acetylcolinesterase enzyme at nerve endings

of the available AChE so that the effector organ becomes overstimulated by the excess acetylcholine (ACh) in the nerve ending. The enzyme is critical to normal control of nerve impulse transmission from nerve fibres to smooth and skeletal muscle cells, glandular cells, and autonomic ganglia, as well as within the central nervous system. Some critical proportion of the tissue enzyme mass must be inactivated by phosphorylation before symptoms and signs of poisoning become manifest[1].

In animals, generally all cholinesterase inhibitors produce autonomic signs of cholinergic overstimulation (salivation, lacrimation, miosis), hypothermia, mild tremors, and mouthasmacking (chewing motions), lowered motor activity, decreased tail-pinch response, and altered neuromuscular function[2] (gait changes and increased foot splay). Whereas the neuropathological lesions associated with organophosphates poisoning are well characterised, however, there is considerable lack of information on their behavioural effects, especially after chronic exposure. The importance of neurobehavioural studies lies in the fact that behaviour is considered as a functional end-product of the various sensory, motor, and integrative processes occurring in the nervous system.

This particular aspect, therefore, requires thorough investigation, more so because behavioural changes are now being regarded as a standard indicator of toxicity in human beings and animals, chronically exposed to low concentrations of potential neurotoxicants[3]. Prolonged epileptic seizures in a nerve agent casualty will produce profound, irreversible, brain damage that, in turn, will result in long-term deficits in cognitive function and behaviour. Combined prophylaxis and therapy regimen, however, does not prevent nerve agent-induced seizures[4], although the standard therapy in organophosphates poisoning consists of atropoine along with an AChE reactivators (Structure III).

Cyclosarin was not considered for such a study for a long time although research on the treatment of sarin, soman, and tabun was pursued[5-8]. Recently,



Structure III: Oximes

attention is also turned to cyclosarin* because of its potential use as a nerve agent and its properties are given in Table 1. The research interest was further enhanced during Operation Desert Shield and Operation Desert Storm with the possibility (later confirmed by the UN Special Commission) that cyclosarin also a constituent of the Iraqi chemical agent inventory[9,10].

Table 1. Properties of cyclosarin

Molecular formula	$C_7H_{14}FO_2P$
Molecular weight	180.140
Freezing point (°C)	–30
Boiling point (°C)	239
Liquid density (20 °C)	1.133
Vapour density	6.200
Vapour pressure (20 °C)	0.044 mm Hg
Volatility (20 °C) (mg m^{-3})	438
Flash point (°C)	94
Estimated miosis level (mg min m^{-3})	0.200
Median lethal dose (mg min m^{-3})	2500 by skin (vapour) or 350 (liquid); 35 inhaled (man)
Anticholinesterase potency (pI_{50})	10.100
Ageing half-time (h)	40

2. *IN VITRO* REACTIVATION OF CYCLOSARIN-INHIBITED AChE

In vitro reactivation of the AChE is commonly used as the screening method for pre-evaluation of the new potential AChE reactivators before the *in vivo* experiments[11-13]. Advantage of the *in vitro* method is the rather quick evaluation of reactivation potency of the AChE reactivators within a short period. The reactivating efficacy of oximes *in vitro* usually corresponds to their efficacy[14] *in vivo*. On the other hand, Lucic[15], *et al.* reported that this rule is not always true. They described samples of the AChE reactivators, which were without biological activity *in vitro*, however, were potent *in vivo*.

An important factor, which can influence reactivation of the AChE, is the choice of the suitable animal species. Till today, many species of laboratory animals (mouse, guinea pig, rabbit, rat, and monkey) have been tested as the suitable source of the AChE similar to the human AChE[16,17].

*Its combination with another nerve agent, sarin, named GB/GF, has come to the attention of scientists involved in medical protection research, since this combined agent was confirmed to be in chemical weapon stockpiles in some countries.

Human erythrocyte AChE is used as a source reported by Dr Worek[18] in Germany. It is generally considered, that quaternary salts do not penetrate blood-brain barrier[19,20]. However, this general rule does not hold good in many cases. Bajgar[21], *et al.* and Kassa[22] have opined that quaternary pyridinium salts can penetrate the blood-brain barrier. Recently, Sakurada[20], *et al.* using *in vivo* rat brain microdialysis technique with HPLC/UV demonstrated an ability of pralidoxime (2-PAM; 2-hydroxyiminomethyl-1-methylpyridinium chloride) to penetrate the blood-brain barrier[21,22].

Another factor, which can influence the results obtained in reactivation process, is the choice of the AChE assay, of which the most common methods are colorimetric[23] and potentiostatic titration[24-26]. Research works, which focus on the reactivation of cyclosarin inhibited AChE, are not much informative. Some of these are aimed at the preparation or testing of the new AChE reactivators[27-30], and others aimed at the choice of appropriate species[17,18]. A list of the currently tested reactivators of cyclosarin-inhibited AChE and conditions of the reactivation process are given in Table 2.

Table 2. List of the currently tested reactivators of cyclosarin-inhibited AChE and conditions of the reactivation process

Oxime	Species	Conditions	Method	References
Pralidoxime	Human erythrocytes AChE	Oxime concentration (30 μM); inhibition time (2 min); reactivation time (0–60 min)	Ellman	(31)
	Rat brain AChE	Oxime concentration (10^{-5}–10^{-2} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(30)
	Rat brain AChE	Oxime concentration (10^{-3}–10^{-1} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(32)
	Human erythrocytes AChE	Oxime concentration (10 μM, 100 μM, and 1000 μM); inhibition time (10 min); reactivation time (0–250 min)	Ellman	(18)
	Rat brain AChE	Oxime concentration (10^{-3} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(30)
	Human, guinea-pig, rabbit, rat erythrocytes AChE	Oxime concentration (100 μM); inhibition time (15 min); reactivation time (1–10 min)	Ellman	(17)
	Rat brain AChE	Oxime concentration (10^{-3}–10^{-1} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(11, 14)
	Human erythrocytes AChE	Oxime concentration (10–30 μM); inhibition time (30 min); reactivation time (5–60 min)	Ellman	(31)
Obidoxime	Human erythrocytes AChE	Oxime concentration (30 μM); inhibition time (2 min); reactivation time (0–60 min)	Ellman	(31)
	Rat brain AChE	Oxime concentration (10^{-5}–10^{-2} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(27)
	Human erythrocytes AChE	Oxime concentration (10 μM, 100 μM and 1000 μM); inhibition time (10 min); reactivation time (0–250 min)	Ellman	(18)
	Rat brain AChE	Oxime concentration (10^{-5}–10^{-2} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(11, 14)
	Human, guinea-pig, rabbit, rat erythrocytes AChE	Oxime concentration (100 μM); inhibition time (15 min); reactivation time (1–10 min)	Ellman	(17)
	Rat brain AChE	Oxime concentration (10^{-3} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(30)
	Human erythrocytes AChE	Oxime concentration (10 μM and 30 μM); inhibition time (30 min); reactivation time (5–60 min)	Ellman	(31)

Methoxime	Rat brain AChE	Oxime concentration (10^{-3} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(32)
	Rat brain AChE	Oxime concentration (10^{-7}–10^{-3} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(11, 14)
	Rat brain AChE	Oxime concentration (10^{-3} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(30)
HI-6	Human erythrocytes AChE	Oxime concentration (30 μM); inhibition time (2 min); reactivation time (0–60 min)	Ellman method	(31)
	Rat brain AChE	Oxime concentration (10^{-7}–10^{-3}M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(27)
	Human erythrocytes AChE	Oxime concentration (10 μM, 100 μM, and 1000 μM); inhibition time (10 min); reactivation time (0–250 min)	Ellman method	(18)
	Human, guinea-pig, rabbit, rat erythrocytes AChE	Oxime concentration (40 μM); inhibition time (15 min); reactivation time (1–10 min)	Ellman method	(17)
	Rat brain AChE	Oxime concentration (10^{-7}–10^{-2} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(30)
	Human erythrocytes AChE	Oxime concentration (1 μM, 2,5 μM, and 5 μM); inhibition time (10–15 min); reactivation time (0–25 min)	Ellman method	(23)
	Rat brain AChE	Oxime concentration (10^{-7}–10^{-3} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(11, 14)
	Human erythrocytes AChE	Oxime concentration (10 μM and 30 μM); inhibition time (30 min); reactivation time (5–60 min)	Ellman method	(31)
HLö-7	Human erythrocytes AChE	Oxime concentration (10 μM and 30 μM); inhibition time (30 min); reactivation time (5–60 min)	Ellman method	(31)
	Rat brain AChE	Oxime concentration (10^{-7}–10^{-3} M); inhibition time(30 min); reactivation time (10 min)	Potentiostatic titration	(11, 14)
	Human erythrocytes AChE	Oxime concentration (10 μM, 100 μM, and 1000 μM); inhibition time (10 min); reactivation time (0–250 min)	Ellman method	(18)
	Human, guinea-pig, rabbit, rat erythrocytes AChE	Oxime concentration (40 μM); inhibition time (15 min); reactivation time (1–10 min)	Ellman method	(17)
BI-6	Rat brain AChE	Oxime concentration (10^{-7}–10^{-2} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(30)
	Rat brain AChE	Oxime concentration (10^{-7} to 10^{-3} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(11, 14)
TMB-4	Rat brain AChE	Oxime concentration (10^{-3} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(32)
	Human erythrocytes AChE	Oxime concentration (10 μM, 100 μM, and 1000 μM); inhibition time (10 min); reactivation time (0–250 min)	Ellman method	(18)
	Rat brain AChE	Oxime concentration (10^{-3} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(30)

K005	Rat brain AChE	Oxime concentration (10^{-8}–10^{-2} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(28, 29)
K033	Rat brain AChE	Oxime concentration (10^{-8}–10^{-2} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(28, 29)
HS-6	Rat brain AChE	Oxime concentration (10^{-7}–10^{-2} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(30)
K048	Rat brain AChE	Oxime concentration (10^{-7}–10^{-2} M); inhibition time (30 min); reactivation time (10 min)	Potentiostatic titration	(27)

3. *IN VIVO* REACTIVATION OF CYCLOSARIN-INHIBITED AChE

The assessment of the reactivating and therapeutic efficacy of oximes *in vivo* is necessary for estimating the benefits of a new antidote in the treatment of human poisoning with organophosphorus compounds[14]. The reactivating efficacy is determined by measuring AChE activity in different body parts after intoxication with cyclosarin. The therapeutic efficacy of oximes against supralethal poisoning with cyclosarin is determined by the evaluation of the ED_{50} values.

3.1 *In Vivo* Reactivation in Rats

Kassa, *et al.* tested reactivating efficacy of obidoxime [Toxogonin®; 1,3-bis-(4-hydroxyiminomethylpyridinium)-2-oxa-propane dibromide], pralidoxime, methoxime [MMC-4; 1,1-bis-(4-hydroxyiminomethylpyridinium)methane dibromide], HI-6 [1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxa-propane dichloride], HLö-7 [1-(2,4-dihydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium)-2-oxa-propane dibromide] and BI-6 [1-(2-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium)-but-2-ene dibromide] (100 μmol/kg) in brain and diaphragm of rats. The oxime therapy was administered 5 min before intramuscular application of cyclosarin (80 μg/kg). BI-6 and methoxime were significantly better reactivators of cyclosarin-inhibited AChE than pralidoxime and obidoxime but not so efficacious as HI-6 and HLö-7. The potency of pralidoxime and obidoxime to reactivate cyclosarin-inhibited AChE in rat diaphragm was very low. In this study, the therapeutic efficacy of the same oximes was determined. The H-oximes including BI-6 are sufficiently effective against supralethal cyclosarin poisoning at doses suggested for human beings (approx. 2 % of their LD_{50} values). Pralidoxime is not able to completely protect rats against the supralethal cyclosarin poisoning. Obidoxime is able to protect cyclosarin-poisoned rats but only at a higher dose than that suggested for human beings (3.5 mg/kg). Methoxime is significantly more efficacious than obidoxime and pralidoxime, but its ED_{50} value is also higher than the dose suggested for human beings[14] (14 mg/kg). After intramuscular administration of cyclosarin in a sublethal dose (54 μg/kg), the subsequent (after 30 s) antidotal therapy was more effective when HI-6 (100 μ mol/kg) was used, because reactivation of AChE in all body parts under study (frontal cortex, pontomedullar region, hypothalamus, hippocampus, basal ganglia and diaphragm) was more marked than in the case of obidoxime (100 μ mol/kg) as antidotal treatment[22]. When HI-6 was compared with obidoxime in eliminating of stressogenic effect of cyclosarin in rats, it was found that obidoxime had practically no effect, while HI-6 decreased plasma corticosteron level, and liver tyrosine aminotransferase activity increased after cyclosarin poisoning[32].

3.2 *In Vivo* Reactivation in Mice

Therapeutic efficacy of obidoxime and HI-6 in two doses (15 mg/kg; 30 mg/kg) was studied in mice intramuscularly intoxicated with cyclosarin. HI-6 was found to be better than obidoxime in both doses[33]. Also pralidoxime (50 mg/kg) was compared in therapeutic efficacy with HI-6 (50 mg/kg) in mice poisoned s.c. with cyclosarin. Both oximes were applied i.p. in combination with atropine (10 mg/kg) and benactyzine (10 mg/kg). The protective ratio of HI-6 was seven-fold higher than of pralidoxime[12].

3.3 *In Vivo* Reactivation in Guinea-pigs

Anticonvulsant potency of some anticholinergic and benzodiazepine drugs was tested in guinea-pigs after intoxication with cyclosarin. Guinea-pigs were pretreated with pyridostigmine (0.026 mg/kg i.m.) 30 min before cyclosarin (2 LD_{50}; 114 μg/kg s.c.), followed 1 min later by pralidoxime (25 mg/kg i.m.) and atropine (2 mg/kg i.m.). Anticholinergics (atropine, trihexyphenidyl, biperiden) or benzodiazepines (diazepam, midazolam) were administered i.m., 5 min after seizure onset. All the drugs were capable of terminating seizure activity, with midazolam and trihexyphenidyl being significantly more potent than the other drugs, and midazolam being more rapid in controlling seizure than atropine, trihexyphenidyl or diazepam[34,35].

3.4 *In Vivo* Reactivation in Rhesus Monkeys

The rhesus monkeys were challenged with 5×LD_{50} cyclosarin (233 μg/kg, i.m.) following pretreatment with pyridostigmine (0.3–0.7 mg/kg per 24 h) and treated with atropine (0.4 mg/kg, i.m.) and either 2-PAM (25.7 mg/kg, i.m.) or HI-6 (37.8 mg/kg, i.m.) at the onset of clinical signs or at 1 min after exposure.

The therapeutic regimen applied was completely effective against cyclosarin, saving all the 10 animals in each group. The cyclosarin-treated groups had minimal nervous system changes with no significant lesion difference resulting from the different oxime therapies in comparison with non-treated group in which neuronal degeneration/necrosis and spinal cord hemmorrhage were observed[9]. When serum and biochemical changes were monitored, it was observed that the early release of serum enzymes which indicated damage to striated or cardiac muscles. There were mild elevations of sodium and potassium levels 2 days after exposure, inferring the presence of hypoxia, metabolic acidosis, slight plasma hyperosmolality, or a combination of these conditions. By 1 week after exposure, the initially elevated levels of creatine kinase, aspartate transaminase, lactate dehydrogenase, and alanine transaminase declined essentially to baseline levels, implying that injury to these tissues was not permanent[36].

4. THERAPEUTIC EFFICACY

A combined regimen of prophylaxis and oxime therapy is now generally considered the most effective medical approach for dealing with the threat of nerve agent poisoning of military personnel[37]. Pretreatment with carbamate ChE inhibitors such as pyridostigmine, shields a fraction of ChE in the periphery from irreversible inhibition by the nerve agents. However, pyridostigmine-induced increase in the level of ACh can itself cause symptoms of poisoning. Therefore, it would be useful to counteract the effects of the accumulated ACh using anticholinergic drugs such as benactyzine or trihexyphenidyl. In addition, the combination of pyridostigmine with anticholinergic drugs allows the dose of pyridostigmine that is otherwise limited by symptoms caused by elevated level of ACh to be increased and results in higher prophylactic efficacy than that observed for pyridostigmine alone[38]. In the event of poisoning, immediate therapeutic treatment with an anticholinergic drug such as atropine sulphate, antagonizes the effect of excess ACh at muscarinic receptor sites, and an oxime is used to reactivate any unaged inhibited enzyme. The most important oximes are pralidoxime, obidoxime, methoxime, HI-6, and HLö7. However, clinical experience has indicated that oximes are not always very efficient reactivators of AChE depending on the type of nerve agent intoxication. Therefore, none of these oximes can be regarded as a broad spectrum antidote, ie, effective against all the nerve agents[39]. The use of carbamate pretreatment in conjunction with atropine and oxime therapy has been shown to significantly increase the survival rate of experimental animals exposed to multiple LD_{50} doses of some organophosphate nerve agents[40,41].

This combined prophylaxis and oxime therapy regimen, however, does not appear to ameliorate nerve-agent induced, centrally mediated seizure activity and concomitant motor convulsions[42]. Seizure activity in organophosphate intoxication creates a problem for the medical management of exposed subjects. Additionally, the seizure activity rapidly progresses to status epilepticus[43] and contributes to the profound brain damage and cardiac pathology, that develops as a consequence of exposure to

these highly toxic compounds[44,45]. Therefore, concomitant administration of an adjunct compound selected for its anticonvulsant activity (eg diazepam) is required to improve the presently utilised regimen of carbamate pretreatment plus atropine and oxime therapy[37].

5. CONCLUSION

The world political scene urges more detailed research on toxic organophosphate compounds and the development of treatment methods of their toxic effects. A comprehensive study on the treatment of cyclosarin toxicity has not been made. Kassa[14] reported that there is no single, broad-spectrum oxime suitable for the antidotal treatment of poisoning with all organophosphate agents[14]. If more than one oxime is available, the choice depends primary on the identity of the responsible organophosphates. While the presently available oximes (pralidoxime, obidoxime) are not considered to be sufficiently effective against nerve agents[17], especially in the case of soman poisoning[5], the H oximes (HI-6, HLö7) appear to be very promising antidotes against nerve agents because they are able to protect experimental animals from toxic effects and improve survival of animals poisoned with supralethal doses[11]. As a result, the effects of these reactivators in counteracting cyclosarin toxicity have to be examined. According to the latest *in vitro* studies, oximes HI-6, HS-6, BI-6, and K033 seem to be promising because of their reactivation potency[27,29,30,46].

ACKNOWLEDGEMENTS

This study was supported by a grant from the Ministry of Defence, No. 9079101301–Czech Republic.

REFERENCES

1. Reigart, J.R. & Roberts, J.R. Recognition and management of pesticide poisonings, Ed 5. Oregon State University Press, Corvallis, USA, 1999. 243p.

2. Moser, V.C. Comparisons of the acute effects of cholinesterase inhibitors using a neurobehavioural screening battery in rats. *Neurotoxicology Teratology*, 1995, **17**(6), 617-25.

3. White, R.P. & Procter, S.P. Research and clinic criteria for development of neurobehaviour test batteries. *J. Occup. Med.*, 1985, **34**, 140-4

4. Filliat, P.; Baubichon, D.; Burckhart, M.F.; Perno Marino, I.; Foquin, A.; Masqueliez, C.; Perricho C.; Carpantier, P. & Lallement, G. Memo impairment after soman intoxication in ra Correlation with central neuropatholog Improvement with anticholinergic an antiglutamatergic therapeutics. *Neurotoxicolog* 1999, **20**, 535-50.

5. Kassa, J.; Krejcova, G. & Samnaljev, I. A comparis of the neuroprotective efficacy of pharmacologoic pretreatment and antidotal treatment in soma poisoned rats. *Acta Medica* (Hradec Králové 2003, **46**(3), 101-07.

6. Krejcova, G. & Kassa, J. Neuroprotective efficac of pharmacological pretreatment and antidot treatment in tabun-poisoned rats. *Toxicolog* 2003, **185**, 129-39.

7. Kassa, J. & Krejcova, G. The impairment spatial memory following low-level sarin inhalatic exposure and antidotal treatment in rats. *Ac Medica* (Hradec Kralove), 2002, **45**(4), 149-5

8. Krejcova, G.; Kassa, J. & Vachek, J. Effect atropine and the oxime HI-6 on low-level sari induced alternation of performance of rats a *t*-maze. *Acta Medica* (Hradec Kralove), 200 **45**(3), 107-10.

9. Koplovitz, I.; Gresham, V.C.; Dochterman, L.W Kaminskis, A. & Stewart, J.R. Evaluation the toxicity, pathology, and treatment cyclohexylmethylphosphonofluoridate (CMPI poisoning in rhesus monkeys. *Arch. Toxicolog* 1995, **66**(9), 622-28.

10. Potential military chemical/biological agents ar compounds. U.S. Army Field Manual 3- (NAVFAC P-467, AFR 355-'7), 12 Decemb 1990. Washington, DC, US Government Printir Office.

11. Kassa, J. & Cabal, J. A comparison of t efficacy of acetylcholinesterase reactivators again

cyclohexyl methylphosphonofluoridate (GF agent) by *in vitro* and *in vivo* methods. *Pharmacology Toxicology*, 1999, **84**, 41-45.

12. Kassa, J. & Cabal, J. A comparison of the efficacy of a new asymmetric bispyridinium oxime BI-6 with currently available oximes and H oximes against soman by *in vitro* and *in vivo* methods. *Toxicology*, 1999, **132**, 111-18.

13. Kassa, J. & Cabal, J. A comparison of the efficacy of a new asymmetric bispyridinium oxime BI-6 with presently used oximes and H oximes against sarin by *in vitro* and *in vivo* methods. *Hum. Exp. Toxicol.*, 1999, **18**, 560-65.

14. Kassa, J. Review of oximes in the antidotal treatment of poisoning by organophosphorus nerve agents. *J. Toxicol.–Clin. Toxicol.*, 2002, **40**, 803-16.

15. Lucic, A.; Radic, B.; Peraica, M.; Mesic, M.; Primozic, I. & Binenfeld, Z. Antidotal efficacy of quinuclidinium oximes aganst soman poisoning. *Arch. Toxicology*, 1997, **71**, 467-70.

16. Berry, W.K. Some species differences in the rates of reaction of diaphragm particulate acetylcholinesterase with tetraethyl pyrophosphate and pralidoxime. *Biochemical Pharmacology*, 1971, **20**, 1333-334.

17. Worek, F.; Reiter, G.; Eyer, P. & Szinicz, L. Reactivation kinetics of acetylcholinesterase from different species inhibited by highly toxic organophosphates. *Arch. Toxicology*, 2002, **76**, 523-29.

18. Worek, F.; Eyer, P. & Szinicz, L. Inhibition, reactivation and ageing kinetics of cyclohexylmethylphosphonofluoridate-inhibited human cholinesterases. *Arch. Toxicology*, 1998, **72**, 580-87.

19. Koplovitz, I.; Gresham, V.C.; Dochterman, L.W.; Kaminskis, A. & Stewart, J.R. Evaluation of toxicity, pathology, and treatment of cyclohexyl methylphosphonofluoridate (CMPF) poisoning in rhesus monkeys. *Arch. Toxicology*, 1992, **66**, 622-28.

20. Sakurada, K.; Matsubara, K.; Shimizu, K.; Shiono, H.; Seto, Y.; Tsuge, K.; Yoshino, M.; Sakai, I.; Mukoyama, H. & Takatori, T. Pralidoxime iodide (2-PAM) penetrate across the blood-brain barrier. *Neurochemical Research*, 2003, **28**, 1401-07.

21. Bajgar, J.; Jakl, A. & Hrdina, V. The influence of obidoxime on acetylcholinesterase activity in different parts of the mouse brain following isopropylmethyl phosphonofluoridate intoxication. *Eur. J. Pharmacol.*, 1972, **19**, 199-02.

22. Kassa, J. A comparison of the reactivating efficacy of oxime HI-6 and obidoxime on cyclosin-inhibited acetylcholinesterase in the diaphragm and various parts of the rat brain. *Ces. a Slov. Farm.*, 1998, **47**, 47-50.

23. Ellman, G.L.; Courtney, K.D.; Anders, V. & Featherstone, R.R. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 1961, **7**, 88-95.

24. Kuèa, K.; Bielavský, J.; Cabal, J. & Kassa, J. Synthesis of a new reactivator of tabun-inhibited acetylcholinesterase. *Bioorg. Med. Chem. Lett.*, 2003, **13**, 3545-547.

25. Kuèa, K.; Bielavský, J.; Cabal, J. & Bielavská, M. Synthesis of a potential reactivator of acetylcholinesterase 1-(4-hydroxyiminomethyl-pyridinium)-3-(carbamoylpyridinium)-propane dibromide. *Tetrahedron Letters*, 2003, **44**, 3123-125.

26. Patoèka, J. & Bielavský, J. Kinetic and thermodynamic parameters of reactivation of *O*-isopropyl-methylphosphorylated rat brain-acetylcholinesterase by asymmetric bis-quaternary 4-pyridine aldoximes. *Collect. Czech. Chem. Commun.*, 1975, **48**, 1794-800.

27. Kuèa, K. & Kassa, J. A comparison of the ability of a new byspyridinium oxime-1-(4-hydroxyiminomethylpyridinium)-4-(4-

carbamoylpyridinium)butane dibromide and currently used oximes to reactivate nerve agent-inhibited rat brain acetylcholinesterase by methods. *J. Enzym. Inhib. Med. Chem.*, 2003, **18**, 529-35.

28. Kuèa, K.; Pícha, J.; Cabal, J. & Liška, F. Synthesis of three monopyridinium oximes and evaluation of their potency to reactivate acetylcholinesterase inhibited by nerve agents. *J. Appl. Biomed.*, 2004, **2**, 51-56.

29. Kuèa, K.; Cabal, J.; Patoèka, J. & Kassa, J. Synthesis of bisquaternary symmetric-c,d-bis(2-hydroxyiminomethylpyridinium)alkane dibromides and their reactivation of cyclosarin-inhibited acetylcholinesterase. *Lett. Org. Chem.*, 2004, **1**, 84-86.

30. Kuèa, K. & Patoèka, J. Reactivation of cyclosarin-inhibited rat brain acetylcholinesterase by paridinium-oximes. *J. Enzyme Inhib. Med. Chem.*, 2004.

31. Worek, F.; Backer, M.; Thiermann, H.; Szinicz, L.; Klimmek, R. & Eyer, P. Reappraisal of indications and limitations of oxime therapy in organophosphate poisoning. *Hum. Experi. Toxicol.*, 1997, **16**, 466-72.

32. Kassa, J. & Bajgar, J. Comparison of the efficacy of HI-6 and obidoxime against cyclohexyl methylphosphonofluoridate (GF) in rats. *Hum. Exper. Toxicol.*, 1995, **14**(11), 923-28.

33. Kassa, J. & Bajgar, J. Therapeutic efficacy of obidoxime or HI-6 with atropine against intoxication with some nerve agents in mice. *Acta Medica* (Hradec Kralove), 1996, **39**(1), 27-30.

34. Shih, T.M. & McDonough, J.H. Efficacy of biperiden and atropine as anticonvulsant treatment for organophosphorus nerve agent intoxication. *Arch. Toxicology*, 2000, **74**(3), 165-72.

35. Shih, T.M.; Duniho, S.M. & McDonough, J.H. Control of nerve agent-induced seizures is critical for neuroprotection and survival. *Toxicol. Appl. Pharmacol.*, 2003, **188**(2), 69-80.

36. Young, G.D. & Koplovitz, I. Acute toxicity of cyclohexylmethylphosphonofluoridate (CMPF) in rhesus monkeys: Serum biochemical and hematologic changes. *Arch. Toxicology, 1995*, **69**(6), 379-83.

37. Dunn, M.A. & Sidell, F.R. Progress in medical defence against nerve agents. *JAMA*, 1989, **262**(5), 649-52.

38. Kassa, J. & Bajgar, J. The influence of pharmacological pretreatment on efficacy of HI-6 oxime in combination with benactyzine in soman poisoning in rats. *Hum. Exper. Toxicol.*, 1996, **15**(5), 383-88.

39. Helden, H.P.M.; Busker, R.W.; Melchers, B.P.C. & Bruijnzeel, P.L.B. Pharmacological effects of oximes: how relevant are they? *Arch. Toxicology* 1996, **70**(12), 779-86.

40. Dirnhuber, P.; French, M.C.; Green, D.M.; Leadbeater, L.; Stratton, J.A. The protection of primates against soman poisoning by pretreatment with pyridostigmine. *J. Pharm. Pharmacol.*, 1979, **31**(5), 295-99.

41. Lennox, W.J.; Harris, L.W.; Talbot, B.G. & Anderson, D.R. Relationship between reversible acetylcholinesterase inhibition and efficacy against soman lethality. *Life Sciences*, 1985, **37**(9), 793-98.

42. Shih, T.M.; Koplovitz, I. & McDonough, J.H. Evaluation of anticonvulsant drug for soman-induced seizure activity. *J. Am. Coll. Toxicol.*, 1996, **15**(Suppl. 2), 46-60.

43. McDonough, J.H. & Shih, T.M. Pharmacological modulation of soman-induced seizures. *Neurosci. Biobehav. Rev.*, 1993, **17**(2), 203-15.

44. McDonough, J.H.; Jaax, N.K.; Crowley, N.A.; Mays, M.Z. & Modrow, H.E. Atropine and/or diazepam therapy protects against soman-induced neural and cardiac pathology. *Fund. Appl. Toxicol.*, 1989, **13**(2), 256-76.

45. McDonough, J.H.; Dochterman, L.W.; Smith, C.D. & Shih, T.M. Protection against nerve agent-induced neuropathology, but not cardiac pathology, is associated with the anticonvulsant action of drug treatment. *Neurotoxicology*, 1995, **16**(1), 123-32.

46. Kuèa, K.; Patoèka, J. & Cabal, J. Reactivation of organophosphate-inhibited acetylcholinesterase activity by a,w-bis-(4-hydroxyiminomethyl-pyridinium)alkanes *in vitro*. *J. Appl. Biomed.*, 2003, **1**, 207-11.

Contributors

Ms Gabriela Krejcova has been working as university teacher and research fellow since 2000. She is also pursuing her PhD from the Dept of Toxicology, Purkyni Medical Military Academy, Hradec Kralove for the past four years. Her areas of research include: Neurotoxicology and behavioural science.

Mr Kamil Kuca was a student in the Dept of Organic Chemistry at the Institute of Chemical Technology, Prague, from 1996-2001. He has been working as university teacher and research fellow since 2001. He is also pursuing his PhD from the Dept of Toxicology, Purkyni Medical Military Academy, Hradec Kralove for the past four years. His areas of research include: Synthesis of the new acetylcholinesterase reactivators, *in vitro* reactivation of acetylcholinesterase inhibited by nerve agents, relationship between the structure of acetylecholinesterase reactivators and their ability to reactivate nerve agents poisoning, acetylcholinesterase inhibitors in Alzheimer disease treatment, and synthesis of the detergents.

Ms Lucie Sevelova has been working as university teacher and research fellow since 2000. She is also pursuing her PhD from the Dept of Toxicology, Purkyni Medical Military Academy, Hradec Kralove for the past four years. Her areas of research include: Toxicology and acute inhalation toxicity.