

ORIGINAL ARTICLE

ON THE UNIVERSALITY OF OXIME HLö-7 – ANTIDOTE FOR CASE OF THE NERVE AGENT POISONING

Kamil Kuca^{1,2✉}, Kamil Musilek^{2,3}, Jana Karasova^{2,4}, Daniel Jun², Ondrej Soukup^{1,2}, Miroslav Pohanka^{1,2}, Kallol Kumar Ghosh⁵, Martina Hrabínova¹

¹ Center of Advanced Studies, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

² University Hospital, Hradec Kralove, Czech Republic

³ University of Hradec Kralove, Faculty of Science, Department of Chemistry, Hradec Kralove, Czech Republic

⁴ Department of Public Health, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

⁵ School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur (C.G.), India

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Summary

Searching for the universal oxime, which could be able to reactivate acetylcholinesterase inhibited by various nerve agents is still topic of high interest. In this contribution, oxime HLö-7, that was thoroughly discussed in the last decade, is evaluated. Its universality was tested *in vitro* using the rat brain homogenate as a source of the cholinesterases. The main members of the nerve agent family (tabun, sarin, soman, cyclosarin and VX) were used for this purpose. As shown, oxime HLö-7 was able to reactivate cholinesterases inhibited by all tested nerve agents with the exception of tabun. Hence, it could not be designated as the broad-spectrum reactivator.

Key words: oxime; HLö-7; reactivator; nerve agent; acetylcholinesterase; antidote

INTRODUCTION

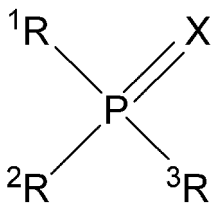
The nerve agents are considered to be a potential threat connected to terroristic attack because of their high toxicity, immediate toxic effect and relatively easy production [1,2]. According to the present knowledge, antidotes against nerve agents consist of anticholinergics, cholinesterase reactivators and

anticonvulsives [1,3]. If anticholinergics are considered, atropine sulphate seems to be drug of the first choice. In case of anticonvulsives, diazepam or avizafone were well described. In the field of AChE reactivators, there are still doubts, which reactivator from the broad family of structurally different compounds should be used as a universal one [4]. Among them, five oximes (pralidoxime, trimedoxime, obidoxime, methoxime and asoxime) are discussed as the standard reactivators [5,6]. However, none of them is able to satisfactorily counteract intoxication caused by every nerve agent. It means that there is no single reactivator available in the case of intoxication by the whole known nerve agents (tabun, sarin, soman, cyclosarin, VX) (Figure 1).

✉ University of Defence, Faculty of Military Health Sciences, Center of Advanced Studies, Třebešská 1575, 500 01 Hradec Králové, Czech Republic

✉ kucakam@seznam.cz

☎ +420 973253028

				
Compound	¹ R	² R	³ R	X
tabun	O-Et	N-Me ₂	CN	O
sarin	O-isopropyl	Me	F	O
soman	O-(3,3-dimethylbut-2-yl)	Me	F	O
cyclosarin	O-cyclohexyl	Me	F	O
VX	S-[(2-diisopropyl)aminoethyl]	Me	O-Et	O

Note: Et - ethyl; Me - methyl.

Figure 1. Chemical structure of selected nerve agents.

Owing to the above mentioned facts, many novel structurally different AChE reactivators were synthesized within last twenty years [7-11]. Among them, several drug candidates were highlighted – oxime BI-6, oxime HLö-7, oxime K027, oxime K048 and oxime K203 [5,12-17]. If the results obtained for these compounds are considered,

oxime HLö-7 seems to be a hot candidate for desired universal antidote (Figure 2) [18]. To summarize its universality, the evaluation of its reactivation activity using simple *in vitro* test was chosen [19]. Tabun, sarin, cyclosarin, soman and VX were used as the important members of the nerve agent family.

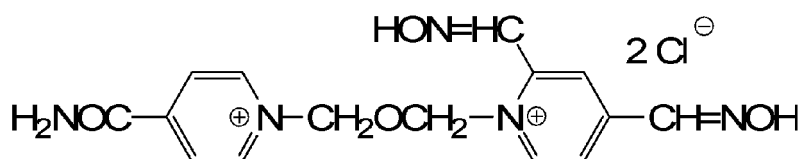


Figure 1. Chemical structure of oxime HLö-7.

MATERIAL AND METHODS

Chemicals

Oxime HLö-7 (chemical name: 1-(4-carbamoylpyridinium)-2,4-bis(hydroxyiminomethylpyridinium)-2-oxapropane dichloride) used in this study was previously synthesized at the Department of Toxicology of the Faculty of Military Health

Sciences in Hradec Kralove using similar synthetic approach as in the case of other oximes [7-9]. Its purity was checked prior to the experiment using HPLC and NMR. The nerve agents (tabun, sarin, cyclosarin, soman and VX) were purchased from the Military Facility (Brno, Czech Republic). They were of 95 % purity. All other chemical used in this study were purchased (Sigma-Aldrich; Czech Republic) and were of reagent grade.

In vitro methodology

Reactivation activity of oxime HLö-7 was tested *in vitro* on rat brain homogenate (10 %; wet weight/saline) using a modification of standard reactivation test [19]. The brain homogenate (0.5 ml) was mixed with isopropanol solution of nerve agent (20 µl; 95% inhibition of cholinesterases was reached) and incubated at 25 °C for 30 minutes. Then, 3 M sodium chloride solution (2.5 ml) was added. Afterwards, the reaction mixture was filled to the volume 23 ml with distilled water. Finally, 0.02 M acetylcholine iodide solution (2 ml) was added. The enzyme activity was measured at pH 7.6

and the 25 °C temperature on an autotitrator Radiometer RTS 822 (Copenhagen, Denmark). Activities of intact AChE (a_0) and inhibited AChE (a_i) were calculated from the 0.01 M NaOH solution consumption on time. After 30 min incubation of AChE with nerve agent (in case of soman only 5 minutes were used to omit the aging), oxime HLö-7 was added to reactivation mixture and the mixture was incubated for further 10 minutes. Activity of reactivated AChE (a_r) was also calculated from the NaOH solution consumption on time. Each measurement was repeated in triplicate. The percentage of reactivation (%) was calculated from measured data using formula:

$$x = \left(1 - \frac{a_r - a_i}{a_0 - a_i} \right) \cdot 100 \quad [\%]$$

RESULTS AND DISCUSSION

Results obtained in this study are summarized in the Table 1. Among the tested nerve agents, oxime HLö-7 was not able to reactivate only tabun-inhibited cholinesterases. This fact was already described earlier [20]. Notably, many promising oximes are unable to reactivate tabun-inhibited AChE [21]. First description of oximes impotency to reactivate tabun-inhibited AChE was given by Wilson et al. [22]. The low reactivation potency of oximes in case of tabun is caused by the presence of the lone electron pair on the tabun amide group. Relatively new description of oxime insufficiency in case of tabun gave Ekstrom et al. [23].

In case of other tested nerve agents (sarin, cyclosarin, soman and VX), favorable results were achieved. If the sarin results are considered, good reactivation was reached at higher oxime

concentration, which is not relevant for human therapeutic use. At the concentration 10^{-5} M (attainable therapeutically in human plasma), only poor reactivation occurred. These results are in the contrary with those published earlier [20]. In case of cyclosarin and VX, the promising results were obtained at both concentrations. These results are in the very good agreement with those published by our group and others earlier [18,20]. Surprisingly good results were also achieved for soman reactivation. Such favorable results were reached because of the different incubation time (5 min instead of 30 min) of homogenate with soman compared with other tested nerve agents. This modification was used because of the aging of soman-inhibited AChE that is resistant to reactivation after relatively short time. In the selected time (5 min), only small portion of the soman-inhibited AChE is aged.

Nerve agent / oxime concentration	% of Reactivation	
	10^{-5} M	10^{-3} M
Tabun	2	3
Sarin	7	25
Cyclosarin	24	31
Soman	24	86
VX	47	32

Table 1. Reactivation activity of oxime HLö-7

From the structure-activity relationship point of view, oxime HLö-7 behaves much more as reactivators with oxime group in position 2 than those with oxime group in the position 4. This statement becomes from the known facts, that tabun-inhibited AChE is easily reactivable by compounds with the oxime group in position 4 (oxime HLö-7 in this study did not fulfill this rule) [3]. On the contrary, cyclosarin inhibited AChE is reactivable by reactivators with the oxime group in position 2 (oxime HLö-7 in this study fulfills this rule) [24]. As known from the literature, other nerve agents used in this study may be reactivated generally easily *in vitro* (except the aged soman) regardless the oxime position in the reactivator's structure [3]. The reactivation difference between both oxime positions is caused by different pKa of both oxime groups in HLö-7.

CONCLUSION

This contribution showed that oxime HLö-7 could not be considered as the universal reactivator. Its universality is not higher in comparison with oxime HI-6 [25].

Due to this fact, the replacement of the most currently promising oxime HI-6 by oxime HLö-7 is not needed/recommended. Perhaps, development of some more universal oxime with broader potency could be a future solution. On the contrary, such broader activity could be attained by the two oximes combination [26].

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