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ORIGINAL ARTICLE

Could oxime HI-6 really be considered as "broad-spectrum" antidote?

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Summary
The broad-spectrum reactivator is a valuable oxime able to reactivate acetylcholinesterase (AChE) inhibited by nerve agents and pesticides. At present there are many AChE reactivators (oximes) which are suitable candidates as broad-spectrum reactivators and among them is the oxime HI-6, highly enough thought of to have been recommended by many armies for use as a universal antidote. In this study, we wanted to establish whether the designation "broad-spectrum" is a an accurate description or if there are some lacks in reactivation of nerve agents or pesticides. For this purpose, the general *in vitro* test for the evaluation of AChE reactivators was used. Tabun, sarin, cyclosarin, soman, VX agent, Russian VX were used as nerve agents for testing, and chlorpyrifos, paraoxon, methyl-chlorpyrifos and dichlorvos (DDVP) were used as typical examples of organophoshorus pesticides. The results obtained showed that oxime HI-6 did not reactivate tabun- and DDVP-inhibited AChE, and, in the case of the other pesticides, only a high dose of oxime HI-6 was able to reactivate pesticide-inhibited AChE.

Key words: acetylcholinesterase; reactivator; oxime; nerve agent; pesticide; HI-6; broad-spectrum

INTRODUCTION

After the Tokyo sarin subway attack, it was clear that there exists potential for the misuse of chemical warfare agents, especially nerve agents, as the weapons of terrorist groups or individuals (Tu 2000). The preparation of appropriate antidotes has therefore become a priority. The current antidotes for nerve agent poisoning, are anticholinergics, acetylcholinesterase (AChE) reactivators and anticonvulsants. Atropine is currently the best anticholinergic drug, commonly used in antidote formulations. Diazepam or its pro-drug avizafone are the best candidates for treatment of convulsions. However in the case of the AChE reactivator, there are five commercially available oximes – pralidoxime (2-PAM; P2S), trimedoxime (TMB-4), obidoxime (toxogonin; Lüh-7), methoxime (MMC4; MMB-4)

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and oxime HI-6 (Kuča and Kassa 2004a, b; Žďárová Karasová et al. 2009). According to the literature, pralidoxime, although it is still used worldwide for the treatment of pesticide poisoning, seems to be already old-fashioned and has been replaced by obidoxime (Petroianu et al. 2007a, b, c). Obidoxime is generally used for the treatment of pesticide poisoning (Thiermann et al. 1999), however its use as an antidote against nerve agents is limited, especially in the case of cyclosarin and soman poisoning (Kassa et al. 2007a). Trimedoxime has a similar reactivation potency as obidoxime, it is recommended more as a treatment for tabun-poisoning (Kassa et al. 2007a). For these reasons, two other reactivators – methoxime and HI-6 are nowadays being thoroughly investigated throughout the world. This research can be divided into two parts – methoxime is under investigation in the US (Singh et al. 2007) and HI-6 is the subject of research in many other countries such as England, Germany, France, Canada, Czech Republic, etc. (Fig. 1). If the efficacy of both these oximes is compared by perusal of published articles, HI-6 has many more advantages than methoxime, and because of this, HI-6 is at present number one among the oxime reactivators (Kassa et al. 2007b, Kuča et al. 2007).

With all the above in mind, we wanted to establish if oxime HI-6 could be considered as a broad-spectrum reactivator, able to sufficiently reactivate AChE inhibited by all kinds of nerve agents and pesticides. Such a property is very important in view of the possible misuse of an unidentified nerve agent (i.e. where appropriate detectors of nerve agents are not available). Currently, a reactivation kit consisting of several oximes would be used to find the most active oxime which would then be recommended as an effective treatment. A so called broad-spectrum reactivator could save time in the identification of an antidote and intoxication could be treated immediately.

To evaluate the broad-spectrum reactivation potency of oxime HI-6, a broad range of nerve agents have to be tested. We therefore selected for our study six nerve agents (tabun, sarin, cyclosarin, soman, VX agent, Russian VX) and four organophosphorus pesticides [chlorpyrifos, paraoxon, methyl-chlorpyrifos and dichlorvos (DDVP)] (Fig. 2).

MATERIAL AND METHODS

Oxime HI-6 was prepared in our department according to the synthesis described by Kuča et al. (2008). Its structure is shown in Fig. 1. The purity of this reactivator was detected using the TLC technique, the HPLC technique and NMR (Jun et al. 2007, 2008). All nerve agents were obtained from the Military Facility Brno (95% purity and higher). Pesticides were obtained from Sigma-Aldrich as analytical standards. All other chemicals used were of reagent grade (Sigma-Aldrich, Czech Republic).

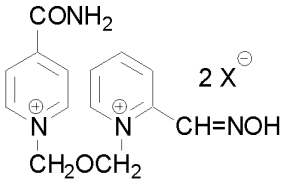

Fig. 1. **Structure of the oxime HI-6.**

Rat brains were chosen as the appropriate source of cholinesterases. Their preparation was as follows: Lightly ether-narcotized animals (the narcosis used did not influence cholinesterase activity – Novotný et al. 2009) were killed by bleeding from a carotid artery and then the brains were removed, washed with saline and homogenized using an Ultra-Turrax homogenizer in distilled water to make a 10% homogenate (w/v). The animals used in this study were handled under the supervision of the Ethics Committee of the Faculty of Military Medical Academy in Hradec Králové, Czech Republic.

In vitro testing of oxime HI-6 has been described in detail already earlier by Kuča and Cabal (2005). Briefly, the 10% rat brain homogenate in distilled water was used as a source of AChE. The brain homogenate (0.5 ml) was mixed with 20 µl of the isopropanol solution of an appropriate acetylcholinesterase inhibitor and distilled water (0.5 ml). The mixture was incubated at 25 °C for 30 minutes to achieve 95% inhibition of AChE (in case of somam – only five-minute incubation was used because of aging). The mixture was filled in an assay vessel to the volume 23 ml with distilled water, and sodium chloride (3M; 2.5 ml) was added. Finally, 2 ml of acetylcholine iodide (0.02M; substrate for enzymatic reaction) was added. The enzyme activity (analyzed by potentiometric titration of decomposed acetylcholine iodide) was measured at pH 7.6 and 25 °C using an autotitrator RTS 822 (Radiometer, Denmark).

The same procedure was repeated with the enzyme, which was incubated for 30 min with the appropriate AChE inhibitor and further treated for 10 min with an aqueous solution of the reactivator (0.2 ml – replacing same amount of distilled water). The activity of the intact AChE (a_0), inhibited AChE (a_i) and reactivated AChE (a_r) were calculated from

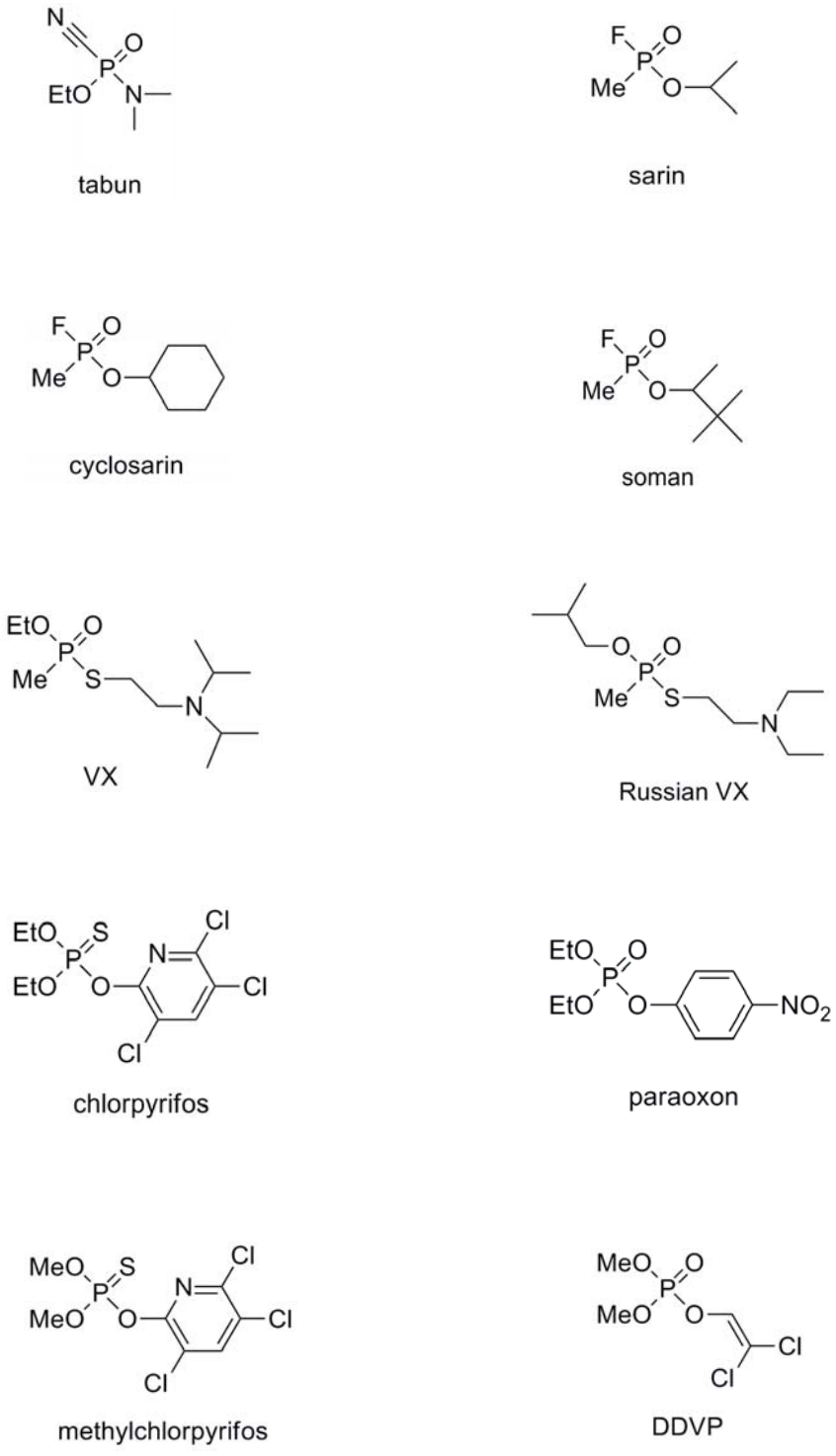

Fig. 2. **Structures of used nerve agents and pesticides.**

the amount of NaOH solution (0.01 M) versus time; NaOH reacted with acetic acid released from decomposed acetylcholine iodide. The percentage of reactivation (%) was calculated from the measured data according to the formula:

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

RESULTS

All the results obtained are summarized in Table 1 and for better visualization also in Fig. 3. Oxime HI-6 was able to reactivate AChE inhibited by all nerve agents and pesticides. However, the reactivation percentage ranged from 2% to 71% depending on the nerve agent or pesticide used and depending on the concentration of the oxime. As is known from the literature, 10% of the reactivation potency is enough to save the life of an intoxicated organism (Bajgar et al. 2007), so that reactivation values under this limit cannot be considered satisfactory, and therefore, we can say that the oxime is not sufficient for the reactivation of AChE inhibited by a given inhibitor.

Given this limitation, oxime HI-6 is not able to reactivate tabun and DDVP-inhibited AChE. In all other cases it exceeded 10% reactivation.

The concentration of the oxime is another factor to be considered as influencing the reactivation process in the living organism. High oxime concentrations are not allowed because of the risk of overdosing (Bartošová et al. 2006). Overdosing of AChE reactivators has, unfortunately, the same effect as the AChE inhibitors (Pohanka et al. 2007).

From this point of view, reactivation potency at low oxime concentrations should be considered as more likely. Therefore, our attention should be directed on the concentration 10^{-5} M. In this case, also paraoxon- and methyl-chlorpyrifos-inhibited AChE are not reactivated by oxime HI-6.

Table 1. **Reactivation of nerve agents- and pesticide-inhibited AChE using oxime HI-6.**

Nerve agent	Reactivation [%] (10^{-5} M)	SD	Reactivation [%] (10^{-3} M)	SD
Tabun	4	0	2	0
Sarin	49	6	47	4
Cyclosarin	28	3	52	6
Soman*	16	2	23	3
VX	14	1	28	3
Russian VX	53	7	42	5
Chlorpyrifos	11	1	20	2
Paraoxon	0	0	35	5
Methyl-chlorpyrifos	0	0	13	2
DDVP	2	0	3	0

* only 5 min inhibition because of aging

DISCUSSION

Many scientific departments have in recent times been focused on the synthesis of promising new oximes with increased reactivation potency in the hope of discovering broad-spectrum reactivators. During last five years, more than thirty articles have been published on the topic of this synthesis (Chennamaneni et al. 2005, Kim et al. 2005, 2006, Pícha et al. 2005, Musílek et al. 2006a, b, 2007a, b, Oh et al. 2006, 2008, Ohta et al. 2006, Yang et al. 2007). Unfortunately, none has achieved the preparation of the so-called broad-spectrum oxime. As a result of this study, oxime HI-6 also cannot be considered as a broad-spectrum compound. In the case of pesticides, HI-6 is almost ineffective (Petroianu et al. 2006a, b, Lorke et al. 2007, 2008a, b, 2009). According to the data in the literature, obidoxime clinically used exerts more promising results in this field (Bond et al. 2008). Moreover, a new candidate for this purpose is now under investigation – oxime K027 (Petroianu et al. 2006a, b, 2007a, b, c, Lorke et al. 2007, 2008a, b, 2009, Tekes et al. 2006, Gyenge et al. 2007, Nurulain et al. 2009).

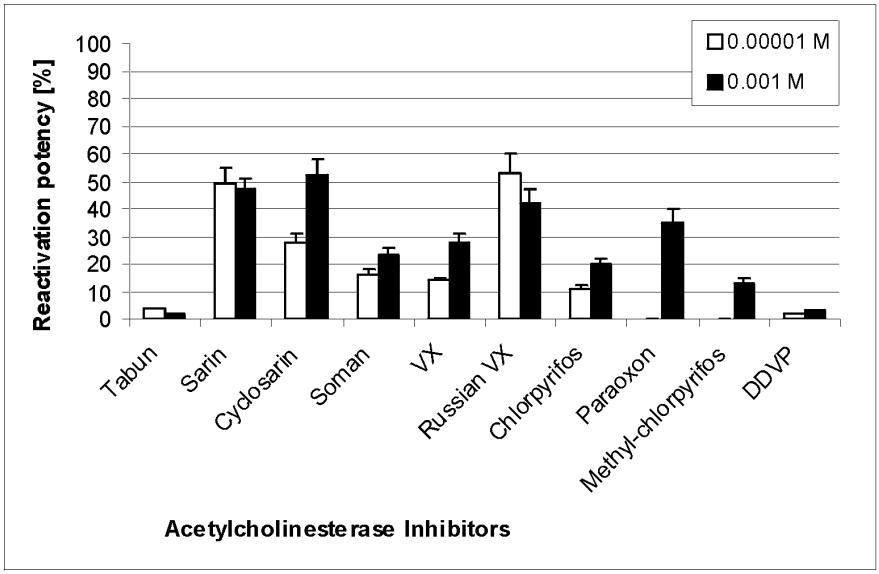

Fig. 3. **Efficacy of oxime HI-6 in two concentrations (10^{-3} M and 10^{-5} M) in reactivation of different AChE inhibitors** (three independent measurements)**.**

From the point of view of reactivation of nerve agents, the reactivation efficacy of the oxime HI-6 is more promising, except in the case of tabun-inhibited AChE (Kassa et al. 2006). However, Hamilton with his co-workers established in 1989 that HI-6 could influence receptors, and thus, it saved the lives of intoxicated monkeys although no reactivation occurs (Hamilton and Lundy 1989). These data are in conflict with the data obtained on rats, where HI-6 had no benefit (Kassa et al. 2006). This discrepancy could have been caused by the species differences which are well known (Kuča et al. 2005, Wiesner et al. 2007). Thus, HI-6 could be considered as a universal reactivator for the treatment of nerve agent intoxication, with the exception of tabun. If the data obtained for HI-6 are compared with data published in earlier literature for oxime HLö-7 (the reactivator developed and tested as a replacement for HI-6), the results are very similar. Therefore, consideration of HLö-7 as HI-6 replacement is not necessary (Kuča et al. 2006).

According to the results obtained, HI-6 could be recommended as a broad-spectrum reactivator for nerve agents among the presently available oximes. On the other hand, HI-6 is not a good candidate for the treatment of pesticide intoxication. In this case, new candidates should be considered to replace the relatively toxic obidoxime and trimedoxime or pralidoxime.

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