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ORIGINAL ARTICLE

Oxime K027: novel low-toxic candidate for the universal reactivator of nerve agent- and pesticide-inhibited acetylcholinesterase

Kamil Kuca[1,2,3], Kamil Musilek[2,3], Daniel Jun[1,2], Miroslav Pohanka[1], Kallol Kumar Ghosh[4], and Martina Hrabinova[1]

[1]*Center of Advanced Studies, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic,* [2]*Department of Toxicology, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic,* [3]*Department of Chemistry, Faculty of Sciences, J.E. Purkinje University, Usti nad Labem, Czech Republic, and* [4]*School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur (C.G.), India*

Abstract

Oxime K027 is a low-toxic bisquaternary compound originally developed as a reactivator of acetylcholinesterase (AChE) inhibited by nerve agents. The reactivation potency of K027 has been tested as a potential reactivator of AChE inhibited by tabun, sarin, cyclosarin, soman, VX, Russian VX, paraoxon, methylchlorpyrifos, and DDVP. The results show that oxime K027 reactivated AChE inhibited by almost all tested inhibitors to more than 10%, which is believed to be enough for saving the lives of intoxicated organisms. In the case of cyclosarin- and soman-inhibited AChE, oxime K027 did not reach sufficient reactivation potency.

Keywords: *K027; acetylcholinesterase; nerve agent; pesticide; reactivator; oxime; antidote; universal*

Introduction

Highly neurotoxic organophosphorus-type (OP) chemical warfare agents ("nerve agents") and OP pesticides have been used by terrorists and during military conflicts, emphasizing the necessity for the development of potential antidotes. Numerous new oximes have been synthesized and tested in recent decades. However, an effective therapy by a single oxime to all the known nerve agents and pesticides is still lacking.

Intoxication with nerve agents or pesticides leads to inhibition of acetylcholinesterase (AChE) by phosphorylation of its active site serine residue. The subsequent accumulation of the neurotransmitter acetylcholine and overstimulation of cholinergic receptors results in a generalized cholinergic crisis, including breakdown of neuromuscular function. Generally, the reactivation of AChE, i.e. the removal of the phosphoryl or phosphonyl moiety from the active site of the enzyme, is considered to be the main mechanism of action of oximes[2].

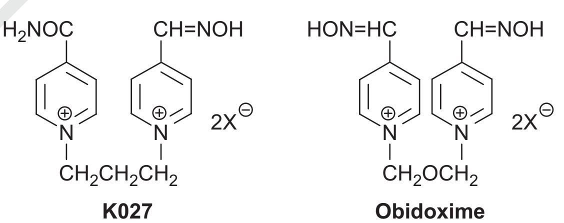

Figure 1. Chemical structures of oxime K027 and obidoxime.

In 2003, the oxime K027 (1-(4-hydroxyiminomethyl–pyridinium)-3-(4-carbamoylpyridinium) propane dibromide) was synthesized (Figure 1)[1]. The K027 reactivator was tested *in vitro* for the first time as a promising oxime (with several limitations) for reactivation of nerve agent-inhibited AChE[3–11]. Then, its acute toxicity was determined, and it was found that the toxicity is very low (LD_{50} > 1200 mg/kg; rat)[12]. After this finding, many *in vivo* studies were conducted throughout the world, and a large number of promising results were obtained[2,13–23]. Probably the most promising

Address for Correspondence: Kamil Kuca, Center of Advanced Studies, Faculty of Military Health Sciences, University of Defense, Trebesska 1575, Hradec Kralove, 50001 Czech Republic. E-mail: kucakam@pmfhk.cz

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finding was in the United Arab Emirates by the group of Petroianu. They found that oxime K027 can reactivate AChE inhibited by many kinds of organophosphorus pesticides *in vitro*. Subsequently, they confirmed their *in vitro* results using *in vivo* studies. According to this group, oxime K027 seems to be, at present, the most promising candidate to replace obidoxime (Figure 1) in the treatment of organophosphorus pesticide poisonings[24–36].

As mentioned above, in the case of nerve-agent reactivation, the obtained results were not so promising. In particular, cyclosarin- and soman-inhibited AChE is resistant to K027 reactivation. Similar results were confirmed also using *in vivo* studies[2,17,18].

Owing to the fact that all the results published on this oxime were prepared in different models (different animals, different techniques, different AChE inhibitors, etc.), in this study, the potency of this compound to reactivate a larger group of organophosphorus AChE inhibitors was tested. For this purpose, *in vitro* study was selected to compare only the reactivation process.

Materials and methods

All pesticides (paraoxon, dichlorvos (DDVP), methylchlorpyrifos) used in this study were obtained from Sigma-Aldrich. Nerve agents were obtained from the Military Technical Institute (Brno, Czech Republic). All inhibitors were of 95% purity and higher. The purity was evaluated by acidimetric titration prior to the experiment. Oxime K027 was synthesized at our department using a standard synthetic approach[1]. Its purity was checked using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) techniques prior to the experiment[37]. All other drugs and chemicals of analytical grade were obtained commercially and used without further purification.

The reactivation efficacy of oxime K027 was assayed *in vitro* in a model of AChE inhibited by selected AChE inhibitors using a standard reactivation test[38]. As a source of AChE, a homogenate from rat brains (rats of Wistar strain; individuals weighing 200–240 g without sex preference) was used. The animals were killed in ether narcosis by cutting the carotids. Narcosis did not influence the enzyme activity[39]. The brains were removed, rinsed in saline, and then homogenized in an Ultra-Turrax homogenizer in distilled water to make a 10% homogenate. AChE homogenate (0.5 mL) was mixed with 20 µL of solution of nerve agent (in dry isopropyl alcohol) and incubated at 25°C for 30 min to reach 95% inhibition. Then, 2.5 mL of 3M NaCl was added, followed by distilled water to a final volume of 23 mL. Finally, 2 mL of 0.02 M acetylcholine iodide was added and the enzyme activity was assayed titrimetrically at pH 8.0 and 25°C using an Autotitrator RTS 822 (Radiometer, Copenhagen).

The activity of non-inhibited enzyme was measured in the same way (without the nerve agent or pesticide). Reactivation of the enzyme inhibited by the nerve agent was performed immediately after the inhibition. A solution of the reactivator of given concentration (1.0 mL) was added to the inhibited enzyme, and 10 min afterward, the activity of the reactivated enzyme was determined using the same method as described for the previous experiment. The percentage of reactivation was calculated from the following equation:

$$\%\,\text{reactivation} = 100 - 100(a_o - a_r)/(a_o - a_i)$$

where a_o is the activity of the intact enzyme, a_i is the activity of the OP-inhibited enzyme, and a_r is the activity of the OP-inhibited enzyme after incubation with the oxime reactivator.

Results and discussion

As indicated (Table 1), oxime K027 reactivated AChE inhibited by all tested nerve agents except cyclosarin and soman. In the case of pesticides, it was able to reactivate AChE inhibited by all tested members of this family. This is in very good agreement with results shown in former studies[24–36]. Oxime K027 was tested at two different concentrations, which had already been selected earlier in the study of Kuca and Cabal to capture the promising compounds[7,40]. The higher concentraton (10^{-3} M) is not relevant for the majority of oximes because of their relatively high toxicity. However, for non-toxic compounds, it could be achievable. As mentioned above, oxime K027 toxicity is very low. Moreover, if considering the lower concentration (10^{-5} M), this is reachable also in the brain of an intoxicated organism. It is known that 1–10% of AChE reactivators, although they are quaternary compounds, can penetrate the blood–brain barrier and so act centrally[24,26,41].

The minimal reactivation activity needed for saving the life of an intoxicated organism is considered to be 10%[41]. According to this knowledge, at the lower concentration, oxime K027 was not able to reactivate AChE inhibited by the tested inhibitors except that inhibited by paraoxon and methylchlorpyrifos.

This contribution summarizes the reactivation activity of oxime K027 on several cholinesterase inhibitors in order to demonstrate the universality of this oxime. The results obtained show that oxime K027 cannot be considered a universal compound if discussing the lower concentration. To elucidate its broad-spectrum potency, further investigations should be conducted, especially on human tissue. Moreover, its potency should be compared with results obtained recently with other oximes[42–46].

Table 1. Reactivation of nerve agent- and pesticide-inhibited AChE by oxime K027.

	Reactivation (%)	
OP inhibitor	10^{-5} M	10^{-3} M
Tabun	1	11
Sarin	2	22
Cyclosarin	0	0
Soman	0	6
VX	7	70
Russian VX	3	39
Paraoxon	21	59
DDVP	5	26
Methylchlorpyrifos	23	45

The present results point to the development of new oximes, which could enrich the group of current commercially available reactivators.

Declaration of interest

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