1,3–BIS(2–HYDROXYIMINOMETHYLPYRIDINIUM) PROPANE AS THE POTENTIAL REACTIVATOR OF THE ACETYLCHOLINESTERASE INHIBITED BY NERVE AGENTS

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Summary: The oxime K005 [1,3–bis(2–hydroxyiminomethylpyridinium) propane dibromide] for the reactivation of the enzyme acetylcholinesterase (AChE) inhibited by cyclosarin and VX was tested. Its reactivation potency was not better in comparison with the currently used AChE reactivator – pralidoxime. On the other hand, the oxime K005 has its maximum reactivation ability at the concentration 10^{-4} M, which could be achieved for human use. The maximum concentration of pralidoxime was reached at the concentrations 10^{-1} M (for cyclosarin) and 10^{-3} M (for VX) but these concentrations are not available for the use in vivo.

Key words: Acetylcholinesterase; Reactivation; Inhibition; Oximes; Reactivator; Pralidoxime; Cyclosarin; VX; In vitro; K005; Nerve agents; Organophosphates

Introduction

Acetylcholinesterase (AChE; EC 3.1.1.7) reactivators and parasympatolytics are currently used as the antidotes for the treatment of the organophosphate compounds (OPCs) intoxications (10). OPCs are largely used in agriculture (insecticides), in industry and technology (softening agents) and in military technology (chemical warfare agents) (4). OPCs affect AChE as irreversible inhibitors. They phosphorylate or phosphonylate the serine hydroxyl group at the active site of the enzyme (6). Due to the inhibition, AChE cannot be physiologically functional (10).

Pralidoxime [1–methyl–2–hydroxyiminomethylpyridinium chloride], obidoxime [1,3–bis(4–hydroxyiminomethylpyridinium)-2-oxa-propane dichloride] and H-oxime HI-6 [1–(2–hydroxyiminomethylpyridinium)-3–(4–carbamoylpyridinium)-2-oxa-propane dichloride] are currently considered to be the main representatives of the AChE reactivators (5). These compounds have in their molecules incorporated characteristic features: the presence of the quaternary nitrogen (in the case of obidoxime and HI-6 even two quaternary nitrogen atoms), functional aldoxime group and in the case of bisquaternary pyridinium aldoximes, the connecting chain of the specific length and shape (9).

None from the above mentioned AChE reactivators are able to satisfactorily reactivate AChE inhibited by all kinds of OPCs, especially by nerve agents such as tabun, sarin or cyclosarin (5).

The reactivation insufficiency is caused by the differences in the structure of the AChE inhibitors (3). According to the insufficient reactivation potency of all currently used reactivators, scientists in many laboratories throughout the world synthesize and test new AChE reactivators, with the aim to treat intoxications by all known OPCs (11).

In our Department we have synthesized 1,3-bis(2-hydroxyiminomethylpyridinium) propane dibromide (called K005) as the potential reactivator of the AChE inhibited by nerve agents (7). In this work we have tested the potency to reactivate AChE inhibited by highly toxic nerve agents such as cyclosarin and VX.

Material and Methods

Chemical

Compound K005 [1,3-bis(2-hydroxyiminomethylpyridinium) propane dibromide] was prepared earlier at our department (7) by the modification of the synthesis described by Berry et al. (1). Pralidoxime [1-methyl-2-hydroxyiminomethylpyridinium chloride] was used for comparison as the currently used AChE reactivator. Purities of the tested AChE reactivators were detected using HPLC. Nerve agents (cyclosarin and VX) were obtained from the Military Facility Brno (97% purity). All other chemicals used were of reagent grade (Aldrich).

Enzyme

Rat brain acetylcholinesterase was chosen as the source of the enzyme. Its preparation was as follows. Lightly ether-narcotized animals were killed by bleeding from a carotid artery and the brains were removed, washed with saline and homogenized in an Ultra-Turrax homogenizer in a distilled water to make a 10 % homogenate.

Tab. 1: Kinetic parameters for the reactivation of cyclosarin inhibited AChE.

Oxime	K_R [μM]	k_R [min^{-1}]	k_r [min^{-1}M^{-1}]
2-PAM	12000	0.040	3
K005	5	0.010	2196

Tab. 2: Kinetics parameters for the reactivation of VX inhibited AChE.

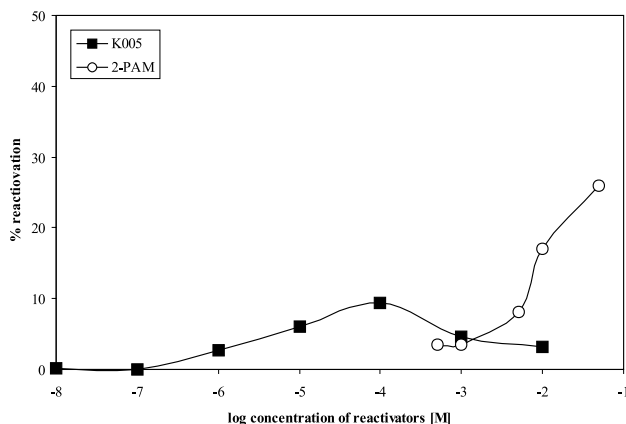| Oxime	K_R [μM]	k_R [min^{-1}]	k_r [min^{-1}M^{-1}]
2-PAM	127	0.047	370
K005	1350	0.132	98

Fig. 1: Concentration-reactivation relationship for the reactivation of cyclosarin inhibited AChE.
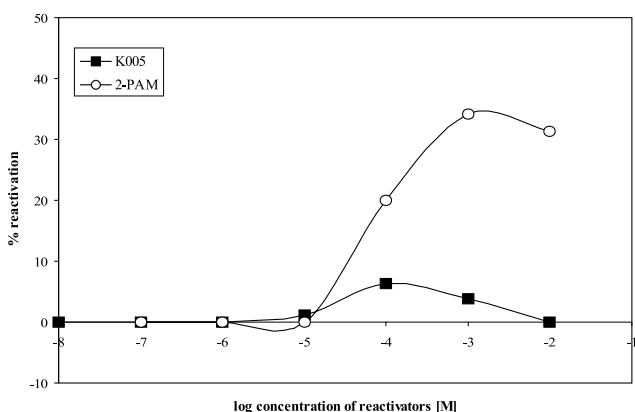

Fig. 2: Concentration-reactivation relationship for the reactivation of VX inhibited AChE.

In vitro measurement

Reactivation effectivities of the oximes were tested *in vitro* on the model of AChE inhibited by cyclosarin using standard reactivation test with electrometric instrumentation (8). The AChE homogenate (0.5 ml) was mixed with 0.5 ml of 0.01 μM cyclosarin in dry isopropanol and incubated for 30 min (25 °C). Then 2.5 ml of 3M NaCl was added and supplied by distilled water to a volume of 23 ml. After that, 2 ml of 0.02 M acetylcholine bromide was added and enzyme activity was assayed titrimetrically at pH 8.0 and 25 °C on the Autotitrator RTS 822 (Radiometer, Denmark).

The activities of intact (a_o) and GF-inhibited (a_i) AChE were determined. When GF-inhibited AChE was incubated 10 min with solution of reactivator, the activity of reactivated AChE (a_r) was obtained. The activity values a_0, a_i and a_r were calculated from the slopes of the initial part of titration curves. Each value represents arithmetic mean from two independent measurements.

Results

Affinity to the intact enzyme

Constant K_{DIS} characterizes the affinity of the AChE reactivators to the intact enzyme. The lower is its value, the higher is its affinity to the intact enzyme. In our case, compound K005 has value of the dissociation constant four times lower (53 μM) compared to pralidoxime (210 μM). Thus, its affinity to the intact enzyme is higher.

Reactivation of cyclosarin inhibited AChE

The results obtained for the cyclosarin inhibited AChE are summarized in Table 1. Values of the constant k_R (characterizing the decomposition of the inhibited enzyme-reactivator complex) and k_r (characterizing velocity of overall reaction) favour compound K005. On the other hand, K_R (characterizing the affinity of the enzyme to the inhibited AChE) is advantageous for the pralidoxime. The relationship between the concentration of the oxime (concentration range from 10^{-8} to 10^{-1} M) and reactivation potency of the oximes is shown in Figure 1. The concentration with the maximum reactivation potency for the oxime K005 is at the concentration 10^{-4} M (10 %). On the other hand, pralidoxime is able to reactivate cyclosarin-inhibited AChE in vitro in very high concentrations, and therefore, it would be toxic for the use in vivo.

Reactivation of VX inhibited AChE

Kinetics parameters characterizing the ability of the oxime K005 and pralidoxime to reactivate VX-inhibited AChE are summarized in Table 2. Both reactivators were able to reactivate VX-inhibited AChE. Value of the constant K_R characterizing affinity to the inhibited enzyme favours pralidoxime. Its afinity to the inhibited enzyme is almost 11 times higher compared to the oxime K005. Constant k_R characterizing the decomposition of the inhibited enzyme-reactivator complex is about three times higher for the oxi-

me K005. On the other hand, constant k_r characterizing the velocity of the overall reaction favours pralidoxime and its value is about 3.5 times higher compared to oxime K005.

The concentration-reactivation relationship is demonstrated in the Figure 2. 10^{-3} M concentration of the pralidoxime is necessary to reach 34 % reactivation of VX inhibited AChE. In the case of the oxime K005, maximum reactivation potency (6.35 %) is reached at the concentration 10^{-4} M.

Discussion

Oxime K005 does not reach better reactivation potency than pralidoxime in the case of cyclosarin and VX-inhibited AChE. On the other hand, concentration with maximum reactivation potency was reached at the concentration lower than that in the case of the pralidoxime. These concentrations could be safe for human use (8).

Reactivation of inhibited AChE depends on the inhibitors used and on the chemical structure of the reactivators (3,5,8). Our results confirm these observations. There are differences in the course of the reactivation curves of the oxime K005 or pralidoxime for inhibition by cyclosarin or VX.

Shape of the molecules of the AChE reactivators affects reactivation process, too (2,9). According to our results, there were significant differences in the reactivation potency between oxime K005 and pralidoxime. Pralidoxime has in its molecule one quaternary nitrogen only while oxime K005 has two quaternary niotrogens. The presence of the quaternary nitrogen in the molecule is an important factor for reactivation process (12).

In conclusion, we have tested oxime K005 [1,3-bis(2-hydroxyiminomethylpyridinium) propane dibromide] for the reactivation of the enzyme AChE inhibited by cyclosarin and VX. Its reactivation potency was not better in comparison with currently used AChE reactivator – pralidoxime. On the other hand, oxime K005 has maximum reactivation ability at the concentration 10^{-4} M, which could be safe for human use. Maximum concentration of pralidoxime was reached at the concentrations 10^{-1} M (for cyclosarin) and 10^{-3} M (for VX). Nevertheless, these concentrations are not available for the use in vivo.

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