ORIGINAL ARTICLE

IN VITRO REACTIVATION OF ACETYLCHOLINESTERASE INHIBITED BY CYCLOSARIN USING BISQUATERNARY PYRIDINIUM ALDOXIMES K005, K033, K027 AND K048

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Summary: We have tested four new bisquaternary pyridinium acetylcholinesterase (AChE; EC 3.1.1.7) reactivators – K005 (1,3-bis(2-hydroxyiminomethylpyridinium) propane dibromide), K033 (1,4-bis(2-hydroxyiminomethylpyridinium) butane dibromide), K027 (1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium) propane dibromide) and K048 (1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium) butane dibromide) as the potential reactivators of AChE inhibited by cyclosarin. Their reactivation potencies were studied using standard in vitro reactivation test. Rat brain homogenate was used as the source of the enzyme. Oxime K033 seems to be the most potent reactivator of cyclosarin-inhibited AChE. Its reactivation potency is significantly higher than the efficacy of all other tested AChE reactivators.

***Key words**: Cyclosarin; In vitro; Reactivation; Inhibition; Acetylcholinesterase; K005; K033; K027; K048; Oximes; Nerve agents; GF agent; Cyclosin*

Introduction

Sarin (GB; O-isopropylmethylfluorophosphonate), tabun (GA; O-ethyldimethylamidocyanophosphate), soman (GD; O-pinacolylmethylfluorophosphonate), cyclosarin (GF; O-cyclohexylmethylfluorophosphonate) and VX (O-ethyl-S-(2-diisopropylaminoethyl)-methylthiophosphonate) belong among the best known nerve agents (20).

These compounds cause longterm inactivation of acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8) by phosphorylating a serine residue at the active site of the enzyme (8,18).

The presently used antidotes against nerve agents intoxications, such as pralidoxime or obidoxime (functional antidotes) in combination with atropine (causal antidote), do not appear as suitable antidotes against poisonings with all kinds of nerve agents (6,7,9,19). For example, pralidoxime has no reactivation ability in treatment of cyclosarin and tabun-inhibited AChE (11,17). Obidoxime is not able to reactivate AChE inhibited by cyclosarin and its ability to reactivate tabun inhibited AChE is poor (1).

In this work, we are interested in the comparison of four new potential AChE reactivators – K005 (1,3-bis(2-hydroxyiminomethylpyridinium) propane dibromide), K033 (1,4-bis(2-hydroxyiminomethylpyridinium) butane dibromide), K027 (1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium) propane dibromide) and K048 (1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium) butane dibromide) developed during last year at the Department of Toxicology Purkyne Medical Military Academy (Figure 1). Their synthesis (10,11,13) and reactivation potencies (12,14,15,16) were described earlier in some chemical, biochemical and toxicological journals.

This work summarizes reactivation potency of these drugs as treatment of cyclosarin intoxications in vitro.

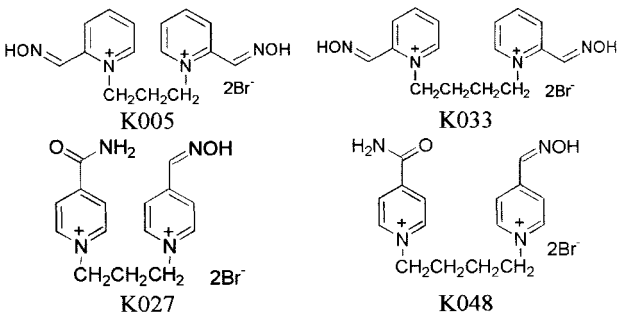

Fig. 1: Structures of the tested oximes.

Material and methods

All new oximes were synthesized previously at our department (10,11,13). Cyclosarin [GF; O-cyclohexylmethylfluorophosphate] of 95% purity was obtained from the Military Technical Institute (Zemianské Kostolany, Slovak Republic). All other chemicals of a reagent grade were obtained from commercial sources.

Standard in vitro method was used for the evaluation of reactivation potencies of these new drugs (14,15). General conditions were taken as follows. Rat brain homogenate diluted in distilled water (10%, w/v) was used as a source of AChE. Measurement was taken at 25 °C, pH 8, and concentration of the AChE reactivators in the scale from 10^{-8} to 10^{-2} M. The activity of AChE was determined by pH static titration of acetic acid released from acetylcholine iodide using the autotitrator (Copenhagen, Danmark).

Results

Affinity to the acetylcholinesterase

Values of the constant K_{DIS} characterizing the affinity of newly synthesized oximes to the intact AChE are shown in Table 1. As lower its value is as higher the affinity of tested oximes towards the AChE is. As indicated in the Table 1, oxime K005 has the lowest value of the constant K_{DIS}. Due to this fact, its affinity towards the enzyme is the highest among all the tested oximes. Value of the affinity of the oxime K033 is comparable with that of oxime K005.

Tab. 1: Affinity of the tested oximes towards acetylcholinesterase.

Oxime	K_{DIS} [μM]
K005	53
K033	65
K027	5888
K048	228

Reactivation of the acetylcholinesterase

Kinetics parameters (dissociation constant: K_R and rate constants: k_R and k_r) shown in Table 2 characterize the ability of the new oximes to reactivate cyclosarin-inhibited AChE in vitro. We were not able to measure rate constants of the compounds K027 and K048, due to the very low ability of these oximes reactivate cyclosarin-inhibited AChE.

The values of the constant K_R characterizing the affinity of oximes to cyclosarin-inhibited AChE indicate that the affinity of the compound K005 to the enzyme-inhibitor complex is the highest in the comparison to all new oximes tested.

Tab. 2: Kinetic constants of the reactivation potency of tested oximes.

Oxime	K_R [μM]	k_R [min.$^{-1}$]	k_r [M^{-1}.min.$^{-1}$]
K005	5	0,010	2196
K033	20	0,095	4872
K027	–	–	–
K048	–	–	–

(–) = not measurable values

The values of the constant k_r express the breakdown of the intermediate complex. The highest value of this constant was obtained for oxime K033. The values of this constant decrease as follows: K033 > K005 > K027 = K048.

Oxime K033 has the highest bimolecular constant of reactivation (k_r) representing overall reactivation ability.

The potency of tested oximes to reactivate cyclosarin-inhibited AChE is demonstrated in Figure 2.

In the case of oxime K033, 46% reactivation of cyclosarin-inhibited AChE was obtained at the concentration 10^{-4} M. Maximal reactivation potency for the oxime K005 (9 %) was obtained at the same concentration of the reactivators. Oxime K048 reached the maximum reactivation potency 11 % at the concentration 10^{-2} M. Unfortunately, this concentration is not suitable for human use. Oxime K027 was not able to reactivate AChE inhibited by cyclosarin. In the case of human relevant concentration [10^{-5}], the percentage of reactivation of cyclosarin-inhibited AChE does not reach 30% regardless of the oxime used.

According to our evaluation of all kinetic constants and concentration-reactivation relationship, we can confirm that the best reactivator from the new oximes seems to be oxime K033.

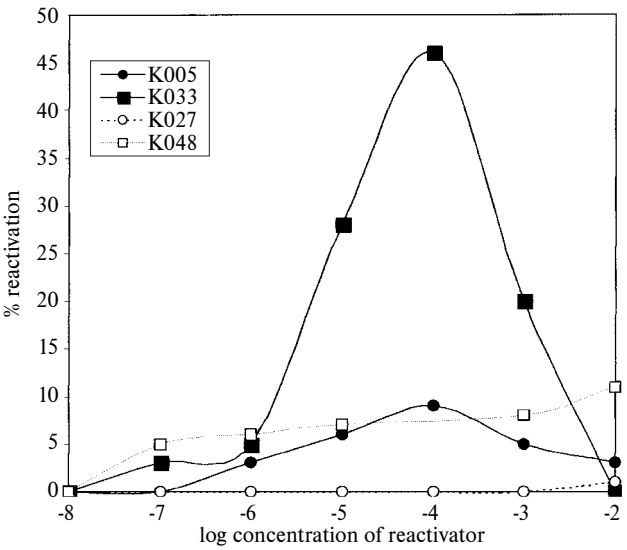

Fig. 2: Concentration versus reactivity relationship of the tested oximes.

Discussion

Thanks to the fact, that currently used AChE reactivators do not reactivate AChE inhibited by all kinds of nerve agents, searching for new AChE reactivator, which could reactivate AChE inhibited by more nerve agents, is needed. In our work, we have presented reactivation potency of new bisquaternary pyridinium AChE reactivators – K005, K033, K027 and K048 in cyclosarin intoxications. Although, this work presents just in vitro experiments our results are important because of the close relationship between in vitro and in vivo results (4,5).

It is very difficult to find an ideal broad-spectrum reactivator of nerve agents-inhibited AChE. It should have the value of K_R from 10 to 100 times lower than K_m of the native substrate (K_m for AChE + acetylcholine iodide is 200 μM) and the value of KDIS from 10 to 100 times higher than K_m (3,4). Thus, the affinity of the AChE reactivator to the inhibited enzyme should be higher than the affinity to the reactivated, respectively, intact enzyme. Both oximes K005 and K033 fulfil this rule.

In the case of the oximes K005 and K033, the rate constant k_R is not affected by pKa of these oximes because of their similar values of this constant (K005 – 7.8; K033 – 7.7). Their differencies (rate constant k_R) are caused just thanks to the length of the connecting chain between both pyridinium rings.

Our results presented in this work confirm the fact that the reactivation of inhibited AChE depends not only on the inhibitors but also on the chemical structure of the reactivators (10). As you can notice in Figure 1, chemical structures of the AChE reactivators K005 and K033 differ in the symmetry from the AChE reactivators K027 and K048. Bisquaternary symmetric pyridinium aldoximes K005 and K033 with functional aldoxime groups at both pyridinium rings are better reactivators of AChE inhibited by cyclosarin. On the other hand, asymmetric AChE reactivators K027 and K048 are not able to reactivate AChE inhibited by this nerve agent. As publicated earlier, these AChE reactivators (K027 and K048) are potent reactivators of AChE inhibited by tabun and sarin (11,15).

Our data confirm earlier mentioned results presented by Dohnal et al. that higher symmetry of the AChE reactivators increases reactivation ability of the oximes (2). These results are in good agreement with work presented by Kassa and Cabal (3). They have evaluated MMC-4 (1,1-bis(4-hydroxyiminomethylpyridinium) methane dibromide), symmetric bisquaternary pyridinium salt with aldoxime groups at the position four, as the best oxime for the reactivation of AChE inhibited by cyclosarin.

In conclusion, we have tested in vitro four new AChE reactivators (K005, K033, K027 and K048). Their reactivation potency was studied using cyclosarin-inhibited AChE. K033 seems to be promising reactivator of cyclosarin-inhibited AChE. Its reactivation potency is significantly higher than the efficacy of all other tested AChE reactivators. Thanks to the high reactivation potency of this AChE-reactivator, oxime K033 will be soon evaluated in vivo using standard reactivation test (19).

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