IN VITRO REACTIVATION OF TABUN-INHIBITED ACETYLCHOLINESTERASE USING NEW OXIMES - K027, K005, K033 AND K048

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SUMMARY

Four new AChE oximes for reactivation of acetylcholinesterase inhibited with tabun - K027 [1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoyl-pyridinium) propane dibromide], K005 [1,3-bis(2-hydroxyiminomethylpyridinium) propane dibromide], K033 [1,4-bis(2-hydroxyiminomethylpyridinium) butane dibromide] and K048 [1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium) butane dibromide] were prepared. Their efficacies to reactivate tabun-inhibited acetylcholinesterase were studied and compared with the currently used acetylcholinesterase reactivators (pralidoxime, obidoxime and HI-6). Reactivator K048 seems to be promising reactivator of tabun-inhibited AChE. Its reactivation potency is significantly higher than the efficacy of HI-6 and pralidoxime, and comparable with the potency of the obidoxime at human relevant doses.

Key words: acetylcholinesterase, nerve agents, oximes, reactivation

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INTRODUCTION

Organophosphorus coumpounds (OPCs) are broadly used in the industry, in the veterinar and human medicine, in the agriculture or for military use (1). There are many intoxications due to their broad extension over the world. Nerve agents such as sarin, soman and tabun represent extremely toxic OPCs. These compounds inhibit enzyme acetylcholinesterase (AChE, EC 3.1.1.7) by its phosphorylation at the serine hydroxy group in the active site of the enzyme. Afterwards, the enzyme is not able to subserve its physiological function and intoxicated organism can die due to acute respiratory failure (2).

Anticholinergics as functional antidotes and AChE reactivators (called oximes due to their oxime functional group) as causal antidotes are used in the case of the intoxications with nerve agents. Pralidoxime, obidoxime and HI-6 are considered to be the most important AChE reactivators (3). The reactivators are characterised by the presence of several structural signs: functional oxime

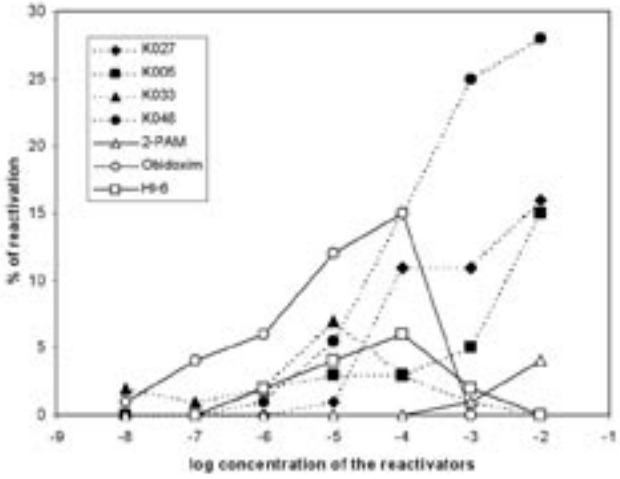

Fig. 1. *Concentration-reactivation relationship of the new oximes in comparison with currently used oximes to tabun-inhibited AChE*

group, quaternary nitrogen group, and different length of linking chain between two pyridinium rings in the case of bispyridinium reactivators (1).

No currently used AChE reactivator is able satisfactorily reactivate AChE inhibited by all types of nerve agents such as sarin, soman, tabun or VX (4).

At our department, four new reactivators of inhibited AChE were synthesized during last year (5, 6, 7). They are designated as K027 [1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium) propane dibromide], K005 [1,3-bis(2-hydroxyiminomethylpyridinium) propane dibromide], K033 [1,4-bis(2--hydroxyiminomethylpyridinium) butane dibromide] and K048 [1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium) butane dibromide]. Their chemical structures are derived from the structures of currently used AChE reactivators, especially trimedoxime and HI-6.

In this study, we have evaluated the reactivation potency of the new reactivators against by tabun-inhibited AChE and compared their potency to reactivate tabun-inhibited AChE with the currently used AChE reactivators (2-PAM, obidoxime and HI-6).

Tabun (O-ethyl-N,N-dimethylamidcyanophosphate) was chosen as a representative of nerve agents because of very low ability of the currently used oximes to reactivate tabun-inhibited AChE (8).

MATERIAL AND METHODS

All new oximes were synthesized at our department (5, 6, 7). Pralidoxime, obidoxime and HI-6 were purchased from Léčiva (Czech Republic), Merck (Germany) and Sevapharma (Czech Republic), respectively. Tabun of 89-95% purity was obtained from the Military Technical Institute (Brno, Czech Republic). All other chemicals of reagent grade were obtained from commercial sources.

In vitro testing of oximes involved a standard collection of experimental procedures (4, 9). The reactivating efficacy of oximes was evaluated in 10% rat brain homogenate that was incubated with tabun for 30 minutes and then the tested oxime was added for 10 minutes. The activity of brain AChE was measured by potentiostatic method with the help of automatic titrator RTS 822 (Radiometer, Denmark). The data of initial rate of enzyme reaction with substrate allowed possible the calculation of the dissociation constant of enzyme-reactivator complex (K_{DIS}), the dissociation constant of enzyme-inhibitor-reactivator complex (K_R) and the first-order rate constant (k_R). Bimolecular constants of reactivation (k_r) which represent overall reactivation ability were calculated from the equation ($k_r = k_R/K_R$). The ability of oxime to reactivate tabun-inhibited AChE was calculated as the percentage of increase in the activity of reactivated enzyme in the reaction mixture.

RESULTS

Kinetics parameters (dissociation constants: K_{DIS} and K_R; rate constants: k_R and k_r) shown in Table 1 characterize the ability of the new oximes to reactivate tabun-inhibited AChE *in vitro*. Oxime K027 has the lowest affinity to the intact enzyme among all tested quaternary oximes. The values of the constant K_R characterizing the affinity of oximes to tabun-inhibited AChE indicate that the affinity of the compound K033 to the enzyme-inhibitor complex is the highest among all new oximes tested.

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

Oxime K033 has the highest bimolecular constant of reactivation (k_r) representing overall reactivation ability, followed by K048 [7.5-times], K027 [9.5-times] and K005 [323-times], respectively.

The potency of tested oximes to reactivate tabun-inhibited AChE is demonstrated in Fig. 1. In the case of high oxime concentration [10^{-3}M], 28% reactivation of tabun-inhibited AChE was obtained for K048, 16% for K027 and 15% for K005, respectively. Oxime K033 was not able at this concentration to reactivate sufficiently tabun-inhibited AChE. Unfortunately, this concentration is not suitable for humans. In the case of human relevant concentration [10^{-5}M], the percentage of reactivation of tabun-inhibited AChE does not reach 10% 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 K048, however, in the high concentrations only.

We have also measured reactivation potency of the currently used AChE reactivators (2-PAM, obidoxime and HI-6) for the comparison with our new oximes. Kinetic parameters of the reactivation for the currently used oximes are summarized in Table 1, too.

The oxime K048 has comparable value of K_{DIS} with 2-PAM and obidoxime. Its K_R is higher than that for obidoxime (29-times) and HI-6 (15-times). However, its first order (k_R) rate constant is 1.6 times higher in comparison with obidoxime and 4.6 times higher compared to HI-6. Bimolecular constant of reactivation (k_r) is the highest for obidoxime, followed by HI-6 and then by K048.

The potency of all tested oximes to reactivate tabun-inhibited AChE is shown in Figure 1. 10^{-2} M concentration of the oxime K048 is necessary to reach 28% reactivation of tabun-inhibited AChE. Nevertheless, this concentration is not available for the

Table 1. *Kinetics parameters of the reactivation of the new oximes*

Reactivator	K_{DIS} [μM]	K_R [μM]	k_R [min^{-1}]	k_r [min^{-1} M^{-1}]
K027	5,888	54	0.0148	273
K005	53	2510	0.0198	8
K033	65	5	0.0121	2591
K048	228	93	0.0324	348
2-PAM	210	-*	-*	-*
Obidoxime	280	3.2	0.020	6250
HI-6	24	6.3	0.007	1111

*We were not able to measure values of the kinetics constants because of very low ability of this oxime to reactivate tabun-inhibited AChE

use in vivo. The reactivation potency of K048 at the concentration 10^{-4} M (probably acceptable for human use) is similar to the potency obtained for obidoxime.

DISCUSSION

Reactivation potency of AChE reactivators depends on their reactivity and affinity to intact and inhibited AChE (4). The reactivity is characterized by k_R. This constant represents nucleophilic activity of the oximate anion which is incorporated into the structure of the AChE reactivators. Oxime K048 has the highest value of this constant. The reason for this result is the difference of its structure with regard to other oximes tested. Oxime K027 differs from K048 in the length of the connecting chain (three membered versus four membered connecting chains), only. However, its reactivation potency is lower. So that, the lenght of the connecting chain between both pyridinium rings of the bisquaternary oxime can affect the reactivation potency. The same results were obtained with compounds K005 and K033 and confirmed our former results dealing with the reactivation AChE inhibited by VX agent (7).

Steric compatibility, electrostatic effects and hydrophobic interactions affect the affinity constants K_{DIS} and K_R. The values of the constant K_{DIS} represent affinity of the reactivators to the intact enzyme. Our results show that the differences between the values of K_{DIS} for K027 and K048 were found, too. On the other hand, there are no differences in the affinity to the intact enzyme between K005 and K033. There are big differences in K_R values for K005 and K033 and small differences for K027 and K048. We can conclude that the negligible change in the structure of the AChE reactivator can greatly affects its affinity to the intact or inhibited enzyme. These data confirm our previous published data (10).

We have also tested our new oximes in comparison with the currently used AChE reactivators. No currently used AChE reactivator can sufficiently reactivate tabun-inhibited AChE (8). According to our results, oxime K048 is better reactivator of tabun-inhibited AChE than all other oximes tested at the concentrations 10^{-3} M and higher. However, the concentration 10^{-3} M seems to be to high for human use and can be toxic for humans (4). Reactivation potency of the oxime K048 at the concentration 10^{-4} M is practically the same as reactivation potency of obidoxime. This concentration of the AChE reactivators could be safe for human use. Nevertheless, this hypothesis needs to be verified by the further experiments in vivo.

In conclusion, we have prepared four new AChE reactivators (K027, K005, K033 and K048). Their reactivation potency was studied using tabun-inhibited AChE and compared with the currently used AChE reactivators (pralidoxime, obidoxime and HI-6). K048 seems to be promising reactivator of tabun-inhibited AChE. Its reactivation potency is significantly higher than the efficacy of HI-6 and pralidoxime and comparable with the potency of the obidoxime.

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