

REACTIVATION POTENCY OF THE ACETYLCHOLINESTERASE REACTIVATOR OBIDOXIME IS LIMITED

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Background: Obidoxime is the only one reactivator of acetylcholinesterase (AChE) approved in Czech Republic for the treatment of nerve agent and pesticide poisonings for civilian sector. Due to the fact that misuse of nerve agents by terrorists or by an accidental poisoning by farmers is possible, re-evaluation of its universality is needed. It is also needed by the fact that clinical findings considering this oxime are controversial.

Aim: In this study, we wanted to summarize if obidoxime is a universal reactivator or if its reactivation potency in case of some organophosphorus inhibitors is limited.

Method: Using our in vitro method, rat brain AChE was inhibited by eleven organophosphorus AChE inhibitors and then reactivated by obidoxime.

Results and Conclusion: It was found that obidoxime could not be termed as universal antidote. Due to this, development of new promising candidates as replacement of obidoxime is recommended.

INTRODUCTION

Nerve agents (sarin, tabun, agent VX, etc.) and pesticides (paraoxon, chlorpyrifos, DDVP, etc.) pose relatively high threat to mammals. They are extremely toxic compounds, especially nerve agents, influencing nerve system. They inhibit enzyme acetylcholinesterase (AChE; EC 3.1.1.7) by the covalent binding to the hydroxyl group of serine in enzyme's active site. After the inhibition, enzyme is not able to split the neuromediator – acetylcholine (ACh). ACh cumulates at the synaptic clefts, and due to this, subsequent cholinergic crisis occurs^{1–4}.

At present time, only five AChE reactivators (pralidoxime, obidoxime, trimedoxime, methoxime and HI-6) together with atropine (cholinergic drug) and diazepam (anticonvulsive) are clinically used worldwide. In Czech Republic, three reactivators of them are available. However, only obidoxime can be used in civilian sector^{5–6}.

Because of the fact, that we are living at the present time in the global war against terrorism, there is still a very high probability of the misuse of these compounds. Due to this, preparedness for the possible misuse of these agents is of high priority. As mentioned above, obidoxime is the only oxime introduced in the Czech civilian sector (Fig. 1). However in the current literature, the data discussing obidoxime antidotal potency are controversial^{7–11}.

Owing to the above mentioned factors, summarization of the reactivation activities of obidoxime are shown in this article. For this purpose, eleven AChE inhibitors were selected. Tabun, sarin, cyclosarin, soman, VX

agent, Russian VX were chosen as members of nerve agent group. Chlorpyrifos, paraoxon, methyl-chlorpyrifos, DDVP and DFP were chosen as members of the pesticide family. In vitro potentiometric method was used for the evaluation of obidoxime reactivation.

MATERIAL AND METHODS

Obidoxime (1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxapropane dichloride) was prepared at our department using the modified approach published earlier¹². Its purity was determined prior its use by using TLC and HPLC^{13–14}. Nerve agents were obtained from the Military Facility Brno (Czech Republic). Pesticides and all other chemicals used were obtained from Sigma-Aldrich (Czech Republic).

The whole in vitro method is described in the work of Musilek et al.¹⁵. Shortly: Lightly narcotized rats were killed (narcosis does not influence cholinesterase activity)¹⁶. Their brains were removed and then homogenized with water to obtain 10% rat brain homogenate as the enzyme source. The brain homogenate (0.5 ml) was mixed with isopropanol solution of AChE inhibitor and then incubated at 25°C for 30 minutes to achieve 95% inhibition of AChE. 2.5 ml of solution of sodium chloride (3 M) were added to the mixture and filled to the volume 23 ml with distilled water. Finally, 2 ml of acetylcholine iodide solution (0.02 M) were added. The enzyme activity was measured at pH 8.0 and temperature 25°C on

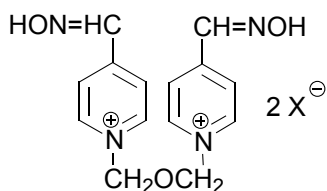


Fig. 1. Chemical structure of obidoxime.

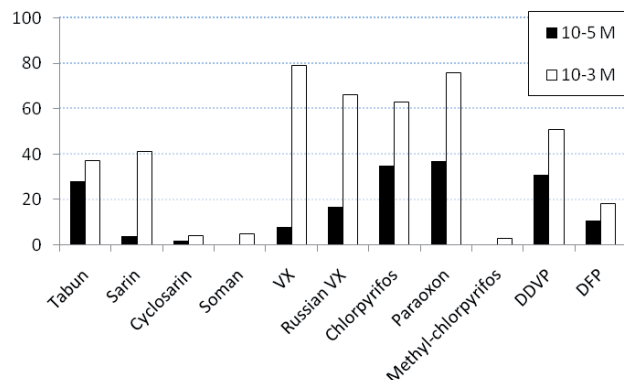


Fig. 2. Efficacy of oxime obidoxime in reactivation of different AChE inhibitors (% reactivation); concentration of the obidoxime 10⁻³ M and 10⁻⁵ M.

Table 1. Reactivation of nerve agents – and pesticide-inhibited AChE; concentration of the obidoxime 10⁻³ M and 10⁻⁵ M.

Nerve agent	Reactivation [%] (10 ⁻⁵ M)	Reactivation [%] (10 ⁻³ M)
Tabun	28	37
Sarin	4	41
Cyclosarin	2	4
Soman*	0	5
VX	8	79
Russian VX	17	66
Chlorpyrifos	35	63
Paraoxon	37	76
Methyl-chlorpyrifos	0	3
DDVP	31	51
DFP	11	18

* In case of soman, only 15 min inhibition was used because of aging.

autotitrator RTS 822 (Radiometer, Denmark). Activities of intact AChE (a₀) and inhibited AChE (a_i) were deducted from influence of consumption of NaOH solution (0.01 M) on time. After the incubation with nerve agent or pesticide-inhibited AChE (30 minutes), the reactivator was added to the solution and the mixture and incubated for 10 minutes. Activity of reactivated AChE (a_r) was calculated from the influence of consumption of NaOH solution on time.

The percentage of reactivation (%) was calculated from measured data according to formula:

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

RESULTS

All the obtained results are summarized in Table 1 and Fig. 2. As resulted, obidoxime was able to reactivate AChE inhibited by all tested nerve agents and pesticides. As it is known, the sufficient reactivation potency causing survival of the intoxicated organism should be over 10%¹⁷. From this point of view, cyclosarin-, soman – and methylchlorpyrifos-inhibited AChE was not sufficiently reactivated. At low for human relevant concentrations (10⁻⁵ M), obtained results are worse. At this concentration, only tabun, Russian VX, chlorpyrifos, paraoxon, DDVP and DFP (limiting activity) reached sufficient reactivation activity.

DISCUSSION

Probability of organophosphorus pesticide poisoning is in Czech Republic relatively rare. Number of people intoxicated by pesticides is increased mostly in developing countries^{18–20}. On the contrary, possibility of nerve agent misuse or misuse of pesticides as low-toxic and accessible nerve agents is more probable in advanced countries by terrorists who do not like this kind consumer society.

Owing to this, preparedness for this threat is of great interest of scientists working in this area. Obidoxime is the only accessible reactivator available for the civilian sector in the Czech Republic. Its reactivation potency is according to the literature not so good to think that it is a universal antidote^{21–24}. In our in vitro experiment, we confirmed this statement. If inhibitors such as cyclosarin, soman or methyl-chlorpyrifos would be misused, no antidote is available. Moreover, high doses of this oxime are needed to reactivate sufficiently – sarin and VX. However, high concentration of obidoxime could exert many side effects and in very high doses also death due to the cholinergic crisis²⁵.

Intoxication of those nerve agents, which were not reactivated by obidoxime, could be then treated by using atropine only, anticonvulsives and other supportive treatment. Second possibility is to use military antidotes, especially oxime HI-6, which is considered to be broad-

spectrum reactivator²⁶⁻²⁹. However, it is at present time not approved for civilian purposes.

Other possibility is to find quite new oxime which will be really universal antidote able to reactivate AChE inhibited by all nerve agents and pesticides. For this purpose, many laboratories throughout the world are working on this topic^{5, 12, 15, 30-35}.

Finally, combination of two oximes could solve this problem. This topic was already discussed in 1989 (ref.^{36, 37}). Nowadays, several scientists re-open this approach. However, there will be probably problem with licensing of such antidotal mixture consisting of two compounds³⁸.

CONCLUSION

In conclusion, AChE reactivator obidoxime is not a universal antidote applicable in all cases of organophosphorus AChE inhibitor poisonings. Due to this, it should be replaced by novel oximes such as HI-6 or novel candidates, which are just now only in laboratory testing phases.

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