Treatment of Organophosphate Intoxication Using Cholinesterase Reactivators: Facts and Fiction

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Abstract: Basic part of the current standard treatment of organophosphate (OP) agent poisoning is administration of cholinesterase reactivators. It includes different types of oximes with a similar basic structure differing by the number of pyridinium rings and by the position of the oxime group in the pyridinium ring. Oximes hydrolytically cleave the organophosphates from acetylcholinesterase (AChE), restoring enzymatic function. This reactivation of AChE is dependent on the type of the agent and, on the reactivator used. From the common oximes, mono- and bisquaternary pyridinium oximes are more or less frequently used in clinical practice such as pralidoxime, obidoxime, trimedoxime, and HI-6. Though there are data on a good therapeutic effects of reactivators, some attempts to undermine the role of reactivators as effective antidotes against OP poisoning have been made. Some arguments on the necessity of their administration following OP poisoning are discussed with the aim to resolve the question on their effective use, possible repeated administration in the treatment of OP poisonig, their peripheral and central effects including questions on their penetration through the blood brain barrier as well as a possibility to achieve their effective concentration for AChE reactivation in the brain. Reactivation of cholinesterases in the peripheral and central nervous system is described and it is underlined its importance for the survival or death of the organism poisoned with OP. An universality of oximes able to reactivate AChE inhibited by all OP is questioned and trends (molecular modelling using neural network, structure-activity relationship, combination of reactivation and anticholinergic properties in one molecule) for future research are characterized.

Key Words: Acetylcholinesterase, nerve agent, organophosphate, cholinesterase reactivators.

INTRODUCTORY

Organophosphates (OP) including their most toxic representatives - nerve agents (NA) - can be used as chemical warfare agents and, they can be (and were) misused by terrorists. Moreover, organophosphorus pesticide (of less toxicity than NA) (Fig. **1**) poisonings are commonly reported internationally [1-12]. Therefore, the treatment of OP intoxication is a very hot topic at present [1-8].

The mechanism of action of nerve agents is based on irreversible acetylcholinesterase (AChE, EC 3.1.1.7) inhibition at the cholinergic synapses. The resulting accumulation of acetylcholine at the synaptic junctions overstimulates the cholinergic pathways and subsequently desensitizes the cholinergic receptor sites [1-3].

Based on our knowledge of the mechanism of action, two therapeutic principles for antidotal treatment are used. The main drugs are anticholinergics that antagonize the effects of accumulated acetylcholine at the cholinergic synapses and cholinesterase reactivators (oximes) reactivating inhibited AChE. Their effects are synergistic. Sedative-hypnotics such as benzodiazepines are also used to treat convulsions (anticonvulsants) [1-3].

TREATMENT WITH REACTIVATORS

The current standard treatment with reactivators includes different types of oximes with a similar basic structure differing by the number of pyridinium rings and by the position of the oxime group on the pyridinium ring. Oximes cleave the OP/NA from AChE, restoring enzymatic function. The reactivation of AChE is dependent on the type of the agent and, on the reactivator used. From the common oximes, mono- and bisquaternary pyridinium oximes are more or less frequently used in clinical practice such as pralidoxime, obidoxime, trimedoxime, and HI-6 (Fig. **1**) [1-3,9-14]. However, a lot of other reactivators have been synthesized and many of them were tested *in vitro* or *in vivo* [1,9,10] (Fig. **2**) though some attempts to undermine the role of reactivators. As effective antidotes against OP poisoning have been made. Thus, there arise questions dealing with antidotal effects of reactivators.

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IS ADMINISTRATION OF REACTIVATORS NECESSARY?

Two important aspects can be considered to compare different results of reactivator administration: clinical and experimental approach. It is necessary to differentiate between clinical care containing all necessary countermeasures for life saving (metabolic monitoring and regulation, ventilation, etc.) i.e., an approach to achieve better therapeutic effects, and experimental approach focusing to survival of experimental animals without other interventions.

Clinical studies analyzing human poisonings with OP failed to show any beneficial affect of available oximes in such a setting: Peter *et al*. [15] demonstrated on the current available data on human poisoning (evaluated using meta-analytic techniques) that oxime was associated with either a null or possible harm. Rahimi *et al*. [16] concluded that oximes are not effective in management of OP-poisoned patients and, they can be dangereous and worsen the patient´s clinical situation. Current evidence is insufficient to indicate whether oximes are harmful or beneficial in the management of acute OP pesticide poisoning [17]. Eddleston *et al*. [18] published a systematic review of clinical trials dealing with oxime therapy in acute OP poisoning with generalized statements that oxime (especially pralidoxime) should not be used in OP poisoning. In these intoxications, intensive care with tight control of oxygenation, fluid and electrolyte balance, pH control and atropine titrated to effect are sufficient to treat patients oxposed to less toxic OP pesticides. This observation is in agreement with experimental results for pralidoxime. Its good reactivation or therapeutic effect was not demonstrated (for review, see e.g. [1,9]). On the other hand, it was demonstrated that unintentional injection of atropine and trimedoxime to children did not cause significant side effects [19]. In case of NA intoxication, it was formulated an anaesthesiological approach to NA victims: use of reactivators (pralidoxime or obidoxime) is vital [20].

Another approach is an experimental evaluation of oxime´s efficacy following NA poisoning. In these cases, the use of the reactivators is strongly supported [1,19-24] though some experiments showed that the effective treatment of NA intoxication without reactivator was possible [25-27]. Some questions dealing with reactivator´s effect in the treatment of NA intoxication arise as follows: Acute experimental intoxication with NA in doses higher

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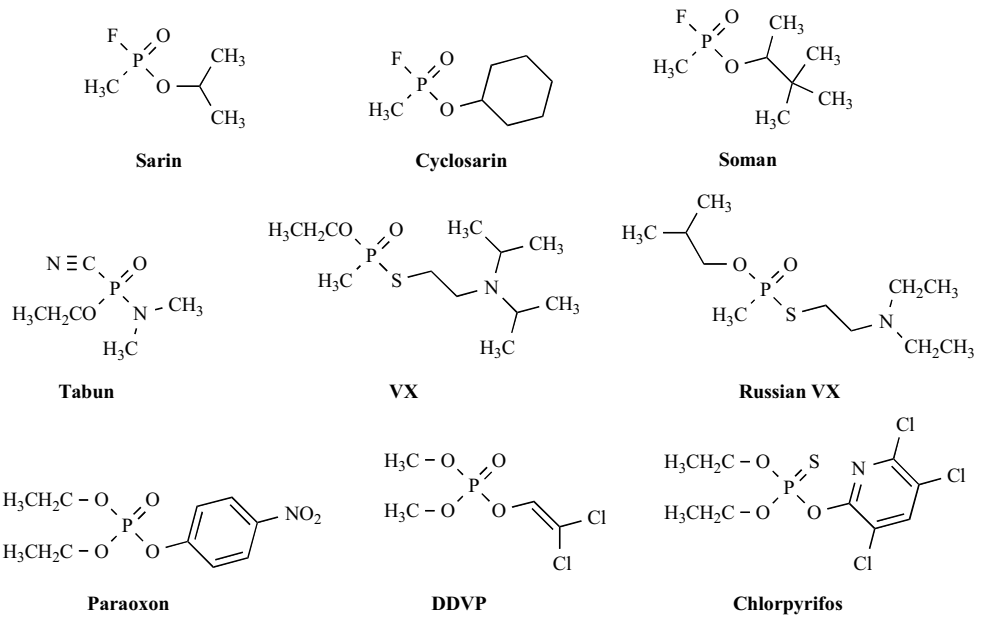

Fig. (1). Structures of some nerve agents and organophosphorus pesticides. Toxicities of these compounds expressed as LD_{50} for rats (intramuscular administration) varied from μg/kg (10-20μg/kg for VX and Russian VX through 70-220 μg/kg for sarin, cyclosarin soman, and tabun) to mg/kg (0.3-0.5 mg/kg for Paraoxon through 10-20 mg/kg for DDVP, and 130-170 mg/kg for Chlorpyrifos), respectively.

than 2xLD50 is not possible to treat effectively without reactivators. It is indisputable question and, in intoxication with NA (e.g. sarin, soman, cyclosarin, VX, Russian VX, and tabun) treated with anticholinergics and anticonvulsants only (without reactivators) better therapeutic effect (i.e. therapeutic index calculated as a ratio LD_{50} treated/LD_{50} untreated animals to be higher than 2.0) was not achieved [28-32]. However, in case of poisoning with doses not exceeding 2xLD_{50} and lower, administration of reactivators is not necessary and survival of intoxicated animals was clearly demonstrated. This treatment consisted of administration of anticholinergics (atropine mostly) and different therapeutic interventions such as artificial ventilation or administration of sodium bicarbonate [25-27]. Artificial ventilation without any other treatment has led to survival of rats poisoned with 1xLD_{50} of sarin [33]. The results of Stefanovic *et al*. indicate that addition of sodium bicarbonate to standard antidotes significantly improved protective effects of atropine, obidoxime and trimedoxime against dichlorvos (1.3xLD_{50}) intoxication. Correlation between protection and biochemical outcome was clearly evident when bicarbonate is being added to atropine [27]. For the treatment of human poisoning, three reactivators – pralidoxime, obidoxime, and HI-6 - were the only antidotes clinically used [1,9]. In the past, trimedoxime was also clinically available. For military purposes (limited for military use only), methoxime was introduced into one army [1,34]. However, clinical experience with oximes (especially pralidoxime) is disappointing and its use was questioned. Though these objections, therapeutic effect of oximes is indisputable. Generally, the conventional oximes (pralidoxime or obidoxime) have been considered to be sufficiently effective against VX, sarin and cyclosarin, and rather ineffective against soman [1,9,10]. At present, HI-6 is only one oxime in the clinical practice able to treat soman poisoning (if administered short period after the intoxication) but without marked effects against OP insecticides. In this case, obidoxime is of importance (but rather ineffective against NA). However, following intoxication with lower doses of OP or with less toxic OP, administration of reactivators is not necessary and parasympatholytics and treatment of metabolic dysbalance is sufficient enough.

IF REACTIVATORS ARE EFFECTIVE ANTIDOTES, IS THEIR REPEATED ADMINISTRATION IN THE COURSE OF OP POISONING INDICATED?

The antidotal effectiveness of reactivators is dependent on the reactivatability of inhibited AChE [1,10,12,24,35]. The efficacy of AChE reactivation by oximes is dependent on both oxime and the conjugated phosphonate structure [11,36] and on the source of the enzyme. Simultaneously, the microenvironment of the gorge of active AChE surface plays a significant role in determining the selectivity of the substrate and inhibitors for cholinesterases [1,37]. Depending on the structure of the inhibitor, inhibited AChE is dealkylated (aged) and the complex formed is resistant to the reactivation effect (aging process). The molecular mechanism is explained by the splitting of the complex forming the alcohol and unreactivatable enzyme (Fig. **3**) [1,11,38].

This reaction, (aging or dealkylation) is very fast for soman-inhibited AChE (the half-life is about 10 min) and it is less expressed for sarin (the half-life is about 10 hours). For VX-inhibited AChE, this reaction was not observed within 24 hours [1,9,39,40]. This is one of the reasons for difficult therapeutic interventions in soman intoxication [1,10,29]. The importance of the orientation not only of the OP molecule but also the reactivator has been described by Luo *et al*. [36].

It appears from these results that the differences in the oxime efficacy against various NA are mainly due to the various aging rates [9.14,35]. As mentioned previously, the reactivation of VX, sarin, or GF-inhibited AChE is still possible hours after the intoxication while soman-inhibited AChE becomes unreactivatable within minutes and, therefore, renders the treatment of soman poisoning much more difficult [1,11,38,40].

This fact led to the synthesis of a series of bisquaternary oximes, designated as "H-oximes", that in combination with anticholinergic drugs have been relatively successful in antagonizing soman intoxication [1,9,10]. Among the H-series oximes, HI-6 has been the best studied and, therefore, seems to be the most promising oxime against soman poisoning [1,9,29]. Worek *et al*. [24], based

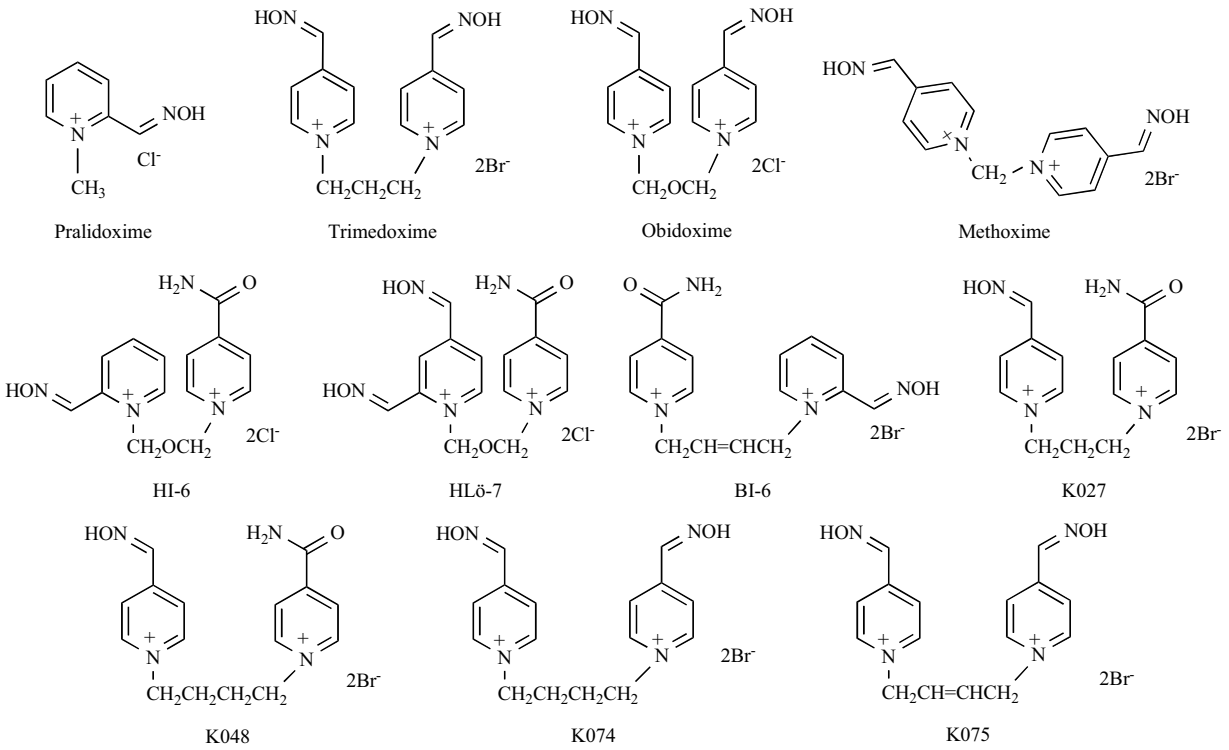

Fig. (2). Structures of currently available acetylcholinesterase reactivators. Pralidoxime, trimedoxime, obidoxime, methoxime, and HI-6 are available for clinical purposes (in some cases limited for military use only), other compounds are under research and/or development.

on experimental testing of the reactivation potency of obidoxime, pralidoxime, HI-6 and HLő-7 in human erythrocyte AChE inhibited by NA, suggested that HLő 7 may serve as a reactivator in NA poisoning at doses therapeutically relevant in humans.

Successful treatment with repeated administration of reactivators was observed. Its efficacy is very dependent on the type of OP used. Repeated administration of oximes can be recommended if the activity of peripheral AChE is reactivatable (easily monitored in blood erythrocyte AChE). Thus, AChE reactivation *in vitro* exists and it is dependent on the oxime concentration [35]. There exist also species differences and differences in AChE reactivation in homogenates of different organs and their parts (e.g. brain areas) [41-43]. If we compare the AChE reactivatability of different oximes and various NA, i.e. the dependence of the percentage of reactivation *vs*. concentration of the oxime [1], basically two different types of the curves can be obtained: the first depending on the oxime concentration shows an increase with a maximum followed by a decreased part of the curve. The second type is a sigmoid curve reaching to the maximum but the decrease cannot be demonstrated because of a too high concentration of the oxime (very probably it will be the same, i.e. containing a decreasing part): e.g. obidoxime and pralidoxime are effective against cyclosarin-and sarin-inhibited AChE at concentrations reaching to 10^{-3} – 10^{-2} M; reactivation 10-20% can be achieved at lower concentrations (10^{-5} - 10^{-6} M).

Some questions are focused to the presence of reactivators in the brain. It was generally accepted a view that oximes as the quaternary compounds are not able to penetrate the blood-brain barrier (BBB).

IS IT REALLY FACT?

There are direct and indirect evidences for ability of oximes to penetrate the BBB. Indirect evidence is based on AChE reactivation in the brain following intoxication with NA and administration of oximes to atropinized animals. Though in the whole brain homogenate reactivation was not observed [44,45], selective and but not very high AChE reactivation was demonstrated in different brain parts, in particular ponto-medullar area (PM) containing i.a. ncl. gigantoreticularis area [28,41-43,46].

Direct evidences for presence of oximes in the brain was demonstrated by Sakurada [47] using microdialysis detection of pralidoxime. Similar observation was described by Falb and Erdmann

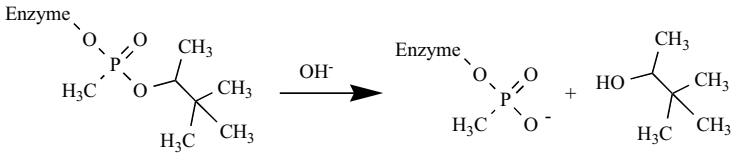

Fig. (3). Inhibition and dealkylation (aging) of acetylcholinesterase (AChE) inhibited by soman. AChE is represented as Enzyme; alcohol moiety (pinacolyl group) is released forming pinacolylalcohol and subsequently, dealkylated (aged) complex of AChE with the rest of the inhibitor molecule is unable to be reactivated.

for radiolabelled obidoxime [48] or Cassel using HPLC for demonstration of HI-6 presence in the brain [49]. Thus, reactivators are able to penetrate the BBB and the published data demonstrate their reactivation effect in the brain [28,41-43,46]. However, the reactivation effect is selective for different brain areas. This may be a reason for falls negative reactivation in the brain (the experiments were performed using the whole brain homogenate). This is not surprising: the effect of cholinesterase inhibitors is different in selected brain areas, varying from relatively resistant (striatum) to very sensitive structures (pontomedullar area, frontal cortex) [1,28,41-43]. Thus, oximes are able to penetrate the BBB depending on its dose administered *in vivo*. If we accepted that oximes penetrate through the BBB,

IS IT POSSIBLE TO ACHIEVE THEIR EFFECTIVE CONCENTRATION FOR ACHE REACTIVATION IN THE BRAIN?

This is connected with the oxime concentration in the target organ or in its defined areas and with the ability of such oxime to reactivate brain AChE. The effectivity of the oxime in a human can be influenced by the concentration in the target organs, i.e. when administered parenterally, in the dose range of 470-2280 μmol/kg, the concentration in the brain can be about 10^{-4} – 10^{-5} M [1,9]. These concentrations are able to reactivate sufficiently inhibited AChE in the brain especially in the ponto-medullar area (the increase by 10-20%): the minimal level of AChE activity in the pontomedullar area necessary for the survival of NA-intoxicated animals was assessed to be about 5-20 % [28,41-43]. This concentration *in vitro* varies with the type of oxime used. In general, concentration 10^{-4}-10^{-5} M can be considered as suitable for AChE reactivation *in vitro* [50-54] but lower concentrations of some oximes can also be considered as suitable for small but significant reactivation. It is connected with a question:

WHICH OXIME CONCENTRATION IS NECESSARY FOR THERAPEUTIC ACTION?

For this purpose, it is necessary to determine the oxime concentration able to reactivate satisfactorily AChE not in the whole brain but in selected area, functionally most important for central respiratory control. This concentration will be different for various oximes. It is dependent on the type of reactivation and its attainable concentration in the brain that is supposed to be maximally 10^{-4} M and less.

CAN BE THIS CONCENTRATION DISCUSSED AS ACCESSIBLE AND REAL?

The crucial question dealing with the reactivator's effect on the central nervous system was discussed in the past and present [18,44,55-58]. Because of their quaternary structure, at intact BBB, the penetration of the reactivators is slow. In order to reach an effective concentration of the reactivator in CNS, its extremely high plasma concentration is necessary. It is known from other results that the inhibition and reactivation of AChE in the brain is selective for different OP [1,59]. On the other hand, the central reactivation effect exists and following administration of the reactivators to nerve agent-intoxicated animals, reactivation of AChE in different brain parts was demonstrated [9,28,41-43,46]. It was also demonstrated [60,61] that real concentrations of some oximes (obidoxime, K27 and K48) in the rat brain are of about 1/10 of that in plasma. Therefore attainable concentration in the brain is about 10^{-5} M. The brain is well-organized and complex organ containing different levels of neuromediators and relevant enzymes in various structures of the brain. It is known that the AChE activity varied minimally 14 times between striatum and cerebellum [59]. In toxicodynamic studies, AChE determination in the whole brain homogenate was determined [31,32]. The percentage of AChE inhibition is reaching to 70 % (32 % of normal activity). On the other hand, administration of soman (the same dose, $1xLD_{50}$) decreased the normal activity to 29.6 μcat/kg, i.e., 10.1 % of control values (catals per kg can be converted as follows: Unit/kg=16.67ncat/kg). It was also demonstrated that after administration of other than OP inhibitors (Tacrine, its 7-methoxyderivative, and galanthamine) [62,63], the AChE inhibition and its influencing by L-carnitine [63] was different for various brain structures. The effect varied in range of 1:10-20. It can not be excluded that it would be similar in case of oxime administration. Thus, the effect of chemical substances is different for different brain parts.

WHERE IS THE ACHE REACTIVATION MORE IMPORTANT – ON THE PERIPHERY OR IN THE BRAIN?

Some problems are dealing with AChE reactivatability in the brain. There are papers describing presence of reactivation in the peripheral targets but not in the central nervous system [41]. Peripheral AChE reactivation is of importance because of support of neuromuscular transmission important for right ventilation function. However, centrally acting OP such as sarin, soman, cyclosarin, and tabun have strong inhibitory effect on AChE in different brain structures [28,64] and AChE activity here [28,41-43] can be of vital importance [64].

WHEN THE OXIMES ARE PRESENT IN THE BRAIN, CAN BE THEIR EFFECT BASED ON REACTIVATION OF ACHE HERE AND ARE THERE SOME OTHER EFFECTS OF OXIMES (NOT REACTIVATION)?

The activity determined in the whole brain homogenate can be considered as a "mean" of the AChE activities in different brain structures. In this connection, special importance can be focused to pontomedullar area where the respiration is regulated [65] controlled by cholinergic neurons [65,66]. The depression of central respiratory control centers in the pontomedullar area is considered as a primary event leading to death [65,67,68]. When the AChE reactivation is present in this area, good therapeutic effect was observed: survival of intoxicated animals correlated with the AChE activity in pontomedullar area [28,43].

Some other effects of reactivators were observed (parasympatholytic action). It can be supporting factor in the treatment, but it is not yet fully elucidated. Moreover, the behavioral changes are positively influenced by oximes [30,39]. It is undirect evidence that these drugs are effective on the central level but it is not yet clear if this effect is connected with AChE reactivation.

There are permanent attempts to improve the treatment of OP intoxication. In this context,

IS IT NECESSARY TO SEARCH FOR NEW OXIMES? IS THERE AN UNIVERSAL OXIME ABLE TO REACTIVATE ACHE INHIBITED BY ALL OP?

There were and are some attempts to synthesize new reactivators with the aim of making them universal or more effective especially against soman or tabun inhibited AChE either in the past (for a review, see [1, 9,11]) or presently [50-53,69-72]. A number of alternative oximes have been shown to be significantly more effective and have a broader spectrum of action than the pralidoxime and several of these may be as or more effective than HI-6 [59-63,69-72]. However, the results obtained up to now are not of interest for introducing them into medical practice. It can be concluded that currently available oximes (pralidoxime, methoxime, obidoxime) are sufficient for therapy of poisonings with OP [70,71] but they are not very effective against NA (especially soman) poisoning [1,9, 10,11,72-74]. The H-oximes (HI-6, Hlő-7, in some cases methoxime) appear to be very promising antidotes against NA including soman. However, there is no universal oxime suitable for antidotal treatment of poisoning with all OP/NA.

WHAT WILL BE BENEFIT IN THIS REGARD?

To use of new approaches – molecular modelling using neural network, structure-activity relationship, combination of reactivation

and anticholinergic properties in one molecule, as well as new strategies for the treatment (timing, new drugs), and antagonizing of the neuronal death needs to be further studied.

QUESTIONS ANSWERED

1. Oximes are good a not replaceable part of antidotal therapy in the treatment of OP/NA intoxication in high doses and at very beginning of the poisoning. However, in case of poisoning with sublethal doses its administration can not be necessary.
2. Repeated administration of oximes is indicated if the inhibited AChE can be reactivated according to results of laboratory examination or positive reaction on repeated administration of oximes.
3. Penetration of oximes into the brain has been demonstrated and AChE reactivation exists in the brain.
4. Effective concentration of oximes in the brain is attainable depending on the type of such oxime.
5. Necessary concentration of oxime in the brain depends on the reactivation ability of such oxime. It can be assessed to be 10^{-4} M in rare examples. More realistic assessment is a concentration 10^{-5} M and lower.
6. The concentration of oxime in the brain is accessible and real and it corresponds to AChE reactivation in the target site.
7. In some cases, peripheral AChE reactivation can be suitable for effective treatment. The importance of central reactivation effect is increased in poisoning with centrally acting OP such as sarin and soman.
8. The main effect of oxime is based on its ability to reactivate inhibited AChE. However, in some cases, other (parasympatholytic) effects are important.
9. Side effects and other than reactivation effects of oximes need to be carefully considered.

10.-11. Up to now, there is not a broad-spectrum oxime reactivating AChE inhibited by all OP/NA. Therefore, development of new oximes is still necessary. Further studies on mechanism of action of oximes (both therapeutic and toxic) are needed. Different AChE reactivation in various brain structures may have implications in the pattern of reactivation effects produced by different oximes. Therefore the AChE activity changes in brain areas are of high importance in toxicodynamic studies focused especially to differentiate among treatment with various oximes.

CONCLUSIONS

Action of reactivators as non replaceable part of antidotal therapy against OP/NA intoxication is based on AChE reactivation in the different structures of the nervous system including the brain. However, their effects need to be studied in more detailed way, and a new approach with the aim to improve the treatment of poisoning with these agents is necessary.

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