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Invited review

Efficient detoxification of nerve agents by oxime-assisted reactivation of acetylcholinesterase mutants

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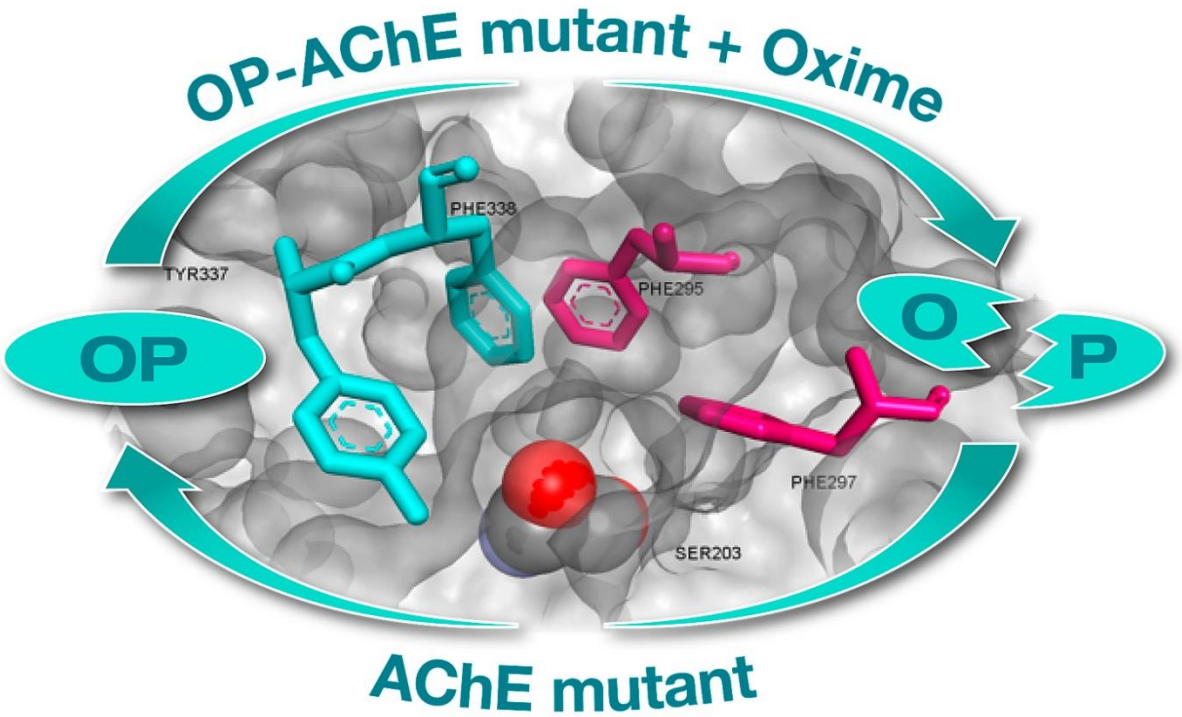

Highlights

- Detoxification of nerve agents by human AChE mutants is proved *ex vivo* and *in vivo.*
- Y337A/F338A mutant combined with HI-6 improves therapy in VX and soman exposed mice.
- AChE mutant reactivation and re-inhibition cycles attenuate symptoms of poisoning.

Abstract

The recent advancements in crystallography and kinetics studies involving reactivation mechanism of acetylcholinesterase (AChE) inhibited by nerve agents have enabled a new paradigm in the search for potent medical countermeasures in case of nerve agents exposure. Poisonings by organophosphorus compounds (OP) that lead to life-threatening toxic manifestations require immediate treatment that combines administration of anticholinergic drugs and an aldoxime as a reactivator of AChE. An alternative approach to reduce the *in vivo* toxicity of OP centers on the use of bioscavengers against the parent organophosphate. Our recent research showed that site-directed mutagenesis of AChE can enable aldoximes to substantially accelerate the reactivation of OP-enzyme conjugates while dramatically slowing down rates of OP-conjugate dealkylation (aging). Therefore, this review focuses on oxime-assisted catalysis by AChE mutants that provides a potential means for degradation of organophosphates in the plasma before reaching the cellular target site.

Keywords

antidotes; cholinesterase; organophosphates; oximes; phosphorylation; 2-PAM

1. Introduction

Organophosphorus compounds (OP) used as pesticides account for more than 3,000,000 accidental or deliberate cases of poisoning registered per year worldwide (Eddleston, 2000; Gunnell et al., 2007). Furthermore, OPs developed as nerve agents (soman, sarin, tabun, VX) still present a threat in terrorist attacks and conflicts, such as in the recent cases in Syria, Malaysia and UK (Dolgin, 2013; Stone, 2018). The main target of OP compounds is acetylcholinesterase (AChE, EC 3.1.1.7). Upon exposure, OP compounds covalently bind to the catalytic active site serine after which the enzyme's physiological activity is permanently lost. As AChE hydrolyses the neurotransmitter acetylcholine (ACh) in the cholinergic synapses of the central and peripheral nervous system, and thus sustains organism homeostasis, the loss of its activity has severe consequences for an organism. These consequences are linked to the pathologic over-stimulation of cholinergic receptors, which impacts numerous body functions and ultimately results in respiratory arrest and death (Clement et al., 1987; Marrs, 2007; Taylor, 2011).

There are two possibilities to counteract OP action; the first involves lowering the accumulated ACh concentrations in the synapse, while the second focuses on limiting accumulated ACh stimulation of nicotinic and muscarinic membrane receptors (Dawson, 1994; Grey, 1984). Therefore, therapy needs to combine both of these aspects in order to be efficient (Masson, 2011; Timperley et al., 2019a, 2019b). While overstimulation of receptors has been approached by ACh antagonists such as atropine, the greatest challenge is in overcoming the high concentration of ACh itself. Since the rate of ACh hydrolysis by AChE is one of the fastest known enzyme reactions (Tormos et al., 2010), the main focus was placed on restoring inhibited AChE catalytic activity (Franjesevic et al., 2019).

In the 1950s and 1960s, pyridinium-based compounds carrying an oxime group (C=N-OH) were developed as reactivators of OP-inhibited AChE (Wilson and Ginsburg, 1955). Reactivators act through the nucleophilic displacement of phosphoryl moiety from the AChE active site catalytic serine, in which a phosphorylated oxime and a free enzyme are formed (Mercey et al., 2012; Worek and Thiermann, 2013). However, the standard oximes in medical use, 2-PAM, HI-6 and obidoxime (**Fig. 1**), have limitations and are not as efficient in OP-inhibited AChE as one would expect (Dawson, 1994; de Yong and Worling, 1984; Stojiljković and Jokanović, 2006, Worek et al., 1998). The problem lies in the fact that the oxime-assisted reactivation of OP-inhibited AChE implies intertwined processes that depend not only on oxime structure, but also on the structure and characteristics of the OP compound and of the OP-AChE conjugate (Franjesevic et al., 2019; Katalinić and Kovarik, 2012; Katalinić et al, 2018; Kovarik et al., 2003, 2004, 2006, 2007a, 2009, 2019a; Mercay et al., 2012;

Radić et al., 2013a; Worek et al., 2004, 2012; Worek and Thiermann, 2013; Winter et al., 2016; Zorbaz et al., 2018a,b, 2019).

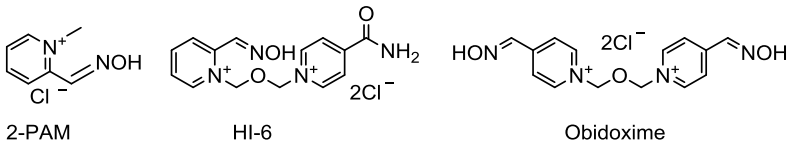

Fig. 1. Chemical structure of pyridinium oximes that present the mainstay of OP-treatment.

Nevertheless, new means of treatment such as bioscavengers have come under the spotlight of current research. Stoichiometric, catalytic or oxime-assisted catalytic bioscavengers are directed toward inactivating OP compounds before they react with the target AChE (Cerasoli et al., 2005; Doctor and Saxena, 2005, Lenz et al., 2007; Masson and Rochu, 2009; Nachon et al., 2013). So far, the administration of butyrylcholinesterase (BChE, EC 3.1.1.8) purified from human plasma has been indicated as the most promising prophylaxis (Raveh et al., 1997; Lenz et al., 2010; Saxena et al., 2011; Vučinić et al., 2013). However, high dose of the large protein of BChE (Lenz et al., 2007; Raveh et al., 1997) required for stoichiometric scavenging, may be minimized by mutants of human AChE assisted by oximes (Kovarik et al., 2007b; Mazor et al., 2008; Saxena et al., 1997; Taylor et al., 2007). Hence, an enzyme-reactivator pair that is catalytic for organophosphate hydrolysis, rather than stoichiometric for conjugation, would greatly reduce the dose requirements of the bioscavenger. Moreover, several mutants of human AChE were shown to have a slower aging rate than the wild type and increased oxime accessibility to the phosphylated catalytic serine (Cochran et al. 2011; Kovarik et al., 2015).

In this paper, we provide an overview of investigations that have shown that AChE mutants in combination with effective oximes could provide a reversal of OP toxicities, primarily in the case of poisoning with soman, tabun and VX. The bioscavenging potential and oxime-assisted degradation of OPs catalyzed by exogenous AChE mutants were proved *in vitro*, *ex vivo* in blood and *in vivo* in mice. Furthermore, we describe this unique bioscavenging system of a mixture of the aging-resistant human AChE mutant and an efficient reactivator that has significantly improved the treatment of soman exposure, which is the greatest challenge in nerve agent poisoning due to the high rate of aging that prevents the use of standard therapy and AChE reactivators.

2. The oxime-assisted degradation of OPs in combination with AChE

Continuous hydrolysis of nerve agents by AChE and oxime is comprised by cycles of inhibition and reactivation according to the following Scheme (**Fig. 2**). Both inhibition and reactivation mechanism involves a similar geometry of the transition state enabling the AChE mutants that enhance oxime reactivation (Kovarik et al., 2004) and react efficiently with the OPs (Kovarik et al., 2003). Therefore, a limit in scavenging capacity mostly depends on the efficiency of the oxime to regenerate the enzyme through continuous turnover. In other words, by enhancing reactivation rates, bioscavenging efficiency has a practical outcome (Kovarik et al., 2007b).

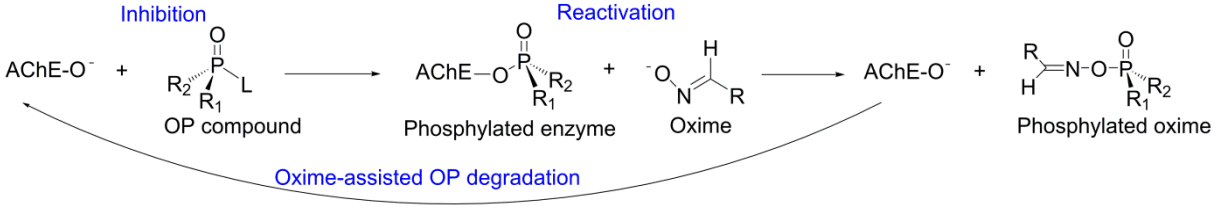

Fig. 2. A scheme of the degradation of OP compound by cycles of inhibition of AChE and oxime-assisted reactivation.

Many studies have shown that, in the reactivation of the phosphylated enzyme conjugate, a limiting step is the accommodation of a nucleophile, an oxime reactivator, within the OP-conjugated active centre (Kovarik et al., 2004, 2006, 2010, 2015). In other words, the flexibility/orientation of the oxime molecule is a key property for achieving stabilization of the oxime group directed towards the phosphylated active site serine (Kovarik et al. 2008a; Šinko et al., 2006). This property greatly defines the affinity of the enzyme for oxime compounds, which should be well-balanced with the nucleophilic substitution for efficient reactivators (Čalić et al., 2006, 2008; Kovarik et al. 2008a).

Oxime-assisted recovery of catalytic activity has been studied in far greater detail for AChE, owing to its greater physiological importance in neurotransmission compared to BChE, its structural analog. Consequently, most oxime reactivators are developed with the aim to recover AChE activity and are not efficient in recovering BChE activity (Lucić Vrdoljak et al., 2006; Kovarik et al., 2010). Certain progress has been made by pyridinium oximes K127 and K117 in tabun reactivation (Kovarik et al. 2010) and by non-pyridinium oximes in the reactivation of VX-, cyclosarin-, and paraoxon-BChE conjugates (Radić et al., 2013b). Moreover, we showed an improvement of BChE's endogenous scavenging capacity, mainly by trying to convert it into a pseudo-catalytic scavenger through adding a BChE-reactivation specific oxime (Radić et al., 2013b). This again implies that the geometry of oxime access to the phosphorus atom conjugated to the active serine is an important criterion for efficient reactivation, along with the chemical nature of the conjugated moiety: phosphorate, phosphonate, or phosphoramidate. Therefore, despite numerous oxime compounds being developed, after a rigorous testing of their reactivation efficacy, only several oximes have so far been selected as leads for bioscavenging and *in vivo* antidotal tests on mice (**Fig. 3**).

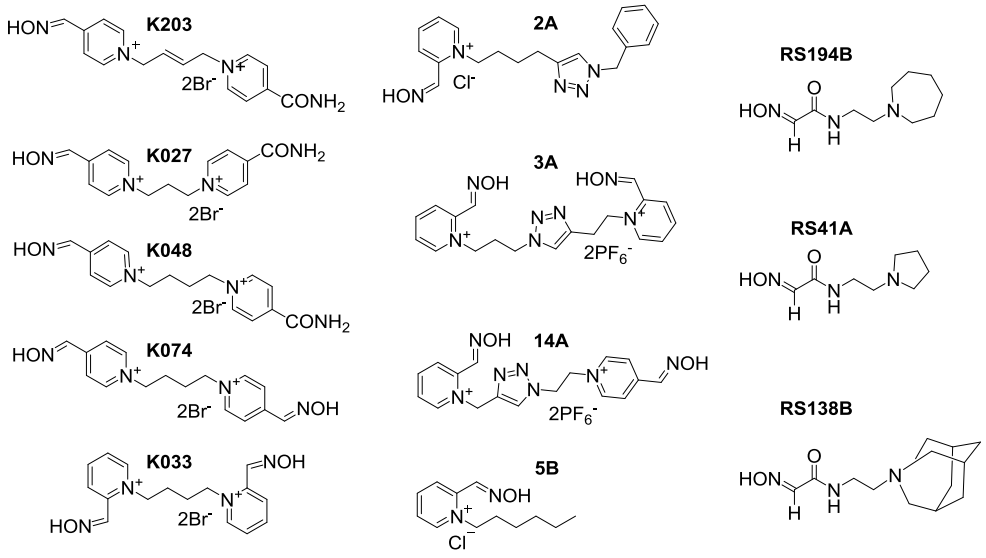

Fig. 3. Lead oximes tested for the oxime-assisted degradation of OPs in combination AChE.

3. Counteracting OP inhibition by reactivation of ChE mutants

Kinetic and mechanistic studies of the site-directed mutants have added a new dimension in counteracting OP poisoning because structure/activity relationship of cholinesterases investigated by various compounds provides important information on the mechanisms of enzyme inhibition and protection as well as reactivation of inhibited enzymes. It became apparent that three domains govern reactivity of cholinesterases: the acyl pocket, the choline site, and the peripheral site (Kovarik, 1999; Radić et al., 1993, 1994; Taylor and Radić, 1994). Whereas aromatic side chains in the AChE acyl pocket sterically exclude ligands with particular size and dimension, the choline site contributes to the stabilization of a positively charged quaternary moiety of ligands thereby conferring selectivity to cationic ligands. The peripheral site at the rim of the gorge entrance dictates

specificity of bis-quaternary oximes and other compounds that cannot fit at the base of the AChE gorge (Čalić et., 2008; Katalinić and Kovarik, 2012, Katalinić et al. 2018; Radić et al., 1993, 1994; Taylor and Lappi, 1975).

Several mutants of human AChE have been created (**Fig. 4**) with the aim of slowing the aging rate, while providing increased oxime accessibility to the phosphylated catalytic serine (Cochran et al., 2011). Among nerve agents, soman is uniquely difficult to counteract because soman-inhibited AChE quickly becomes unresponsive to reactivation due to the rapid dealkylation (known as aging) of the soman-AChE conjugate with a half time of only 2 min (de Jong and Worling, 1984; Shafferman et al,. 1996). The anionic methylphosphonylated-AChE conjugate formed by aging is no longer susceptible to oxime reactivation, in part due to the charge repulsion between the anionic oximate and the conjugated methylphosphonic acid (Barak et al., 1997). Therefore, a novel approach to treat soman exposure is a necessity, and the most resistant to aging soman-inhibited human AChE mutant Y337A/F338A have opened new avenues in soman bioscavenging. An initial screening of a library of 840 oximes for reactivation of the soman-inhibited human AChE mutant Y337A/F338A (Cochran et al., 2011) and reiterative synthetic and efficacy oriented search for more effective reactivators by an analysis of a library of HI-6 analogs (Kovarik et al., 2015) identified the standard bispyridinium oxime HI-6 as the most potent reactivator of the soman-inhibited mutant. Although in the case of reactivation of the soman-AChE conjugate the primary problem is the rapid aging of that conjugate, these structure-activity investigations show a complexity of the reactivation and a steric interference by a substituted branched pinacolyl group of soman that result in reactivation selectivity.

None of the currently used oximes are sufficiently effective against all nerve agents and pesticides. The lack of universality is a consequence of substantially different reactivation constants depending on the conjugated organophosphate (Franjesevic et al., 2019; Katalinić et al., 2018; Kovarik et al., 2019a; Maček Hrvat et al., 2020; Zorbaz et al. 2018a,b). In other words, conformational changes and steric hindrance in the gorge induced by the OP conjugation consequently affect the possibility of the oxime to adopt the most favorable position for dephosphylation of catalytic serine (Eto, 1976; Ekström et al., 2006a, 2006b; Katalinić et al., 2018; Maček Hrvat et al., 2020; Millard et al., 1999). This was particularly noted in the case of tabun inhibition where there was no reactivation of either AChE or BChE (Maček Hrvat et al., 2020; Maraković et al., 2016; Zorbaz et al., 2018a,b, 2019). Yet, in our recent papers we reported triazole-anulated oxime 3A and mono-pyridinium oxime 5B as potent reactivators of the tabun-inhibited AChE and Y337A mutant, respectively (Kovarik et al., 2019a,b). In comparison to wild type AChE, the Y337A mutation resulted with a 95-fold enhancement of the maximal reactivation rate of 5B in case of tabun. Interestingly, the three most efficient reactivators of the single AChE mutant were 2-PAM analogues with extended alkyl chains butyl, pentyl and hexyl, while N-propyl, N-ethyl, and 2-PAM itself, the N-methyl analogue, were poor reactivators of the tabun-Y337A conjugate (Kovarik et al., 2019b). Although hydrophobicity increases with 5B, removal of the aromatic portion of the tyrosine in the mutant enzyme allows the compound to be accommodated in the active center presumably with a distinctive binding pose. A similar positive effect of the single mutation was observed in previous studies with other OPs where the Y337A mutation enhanced the dephosphylation rate and/or overall reactivation rate relative to wild type AChE (Kovarik et al. 2004, 2006, 2007b; Katalinić and Kovarik 2012, Katalinić et al. 2018). Out of a congeneric library of triazole compounds, the bis-pyridinium aldoxime 14A displays a high potency to reactivate both phosphonate and phosphorate AChE conjugates more efficiently (especially cyclosarin-conjugate) than the standard reference oxime, 2-PAM, but still not as potently as HI-6 (Kovarik et al., 2019a; Zorbaz et al, 2018a,b).

In contrast to tabun, VX-inhibited AChE is easier to reactivate with standard oximes HI-6 and 2-PAM (Dawson, 1994; Kuca et al., 2006; Maček Hrvat et al., 2016; Puu et al., 1986; Sidell and Grof, 1974; Sun et al., 1986; Worek et al., 1998) despite the fact that the crystal structure of the VX-AChE conjugate revealed that phosphonylation of the catalytic serine causes a conformational change of the imidazole ring of the catalytic histidine, similar to that of tabun, to accommodate the ethoxy

substituent of VX (Millard et al., 1999). Moreover, we have recently shown that mutagenesis could additionally improve the reactivation rate (Maček Hrvat et al., 2016). Two choline binding site mutations (Y337A/F338A) had a positive synergistic effect on the HI-6-assisted reactivation enhancing the rate of nucleophilic displacement of the phosphonyl-moiety from the active site serine about 5.5-fold when compared to the wild type AChE, while preserving its binding affinity toward the HI-6.

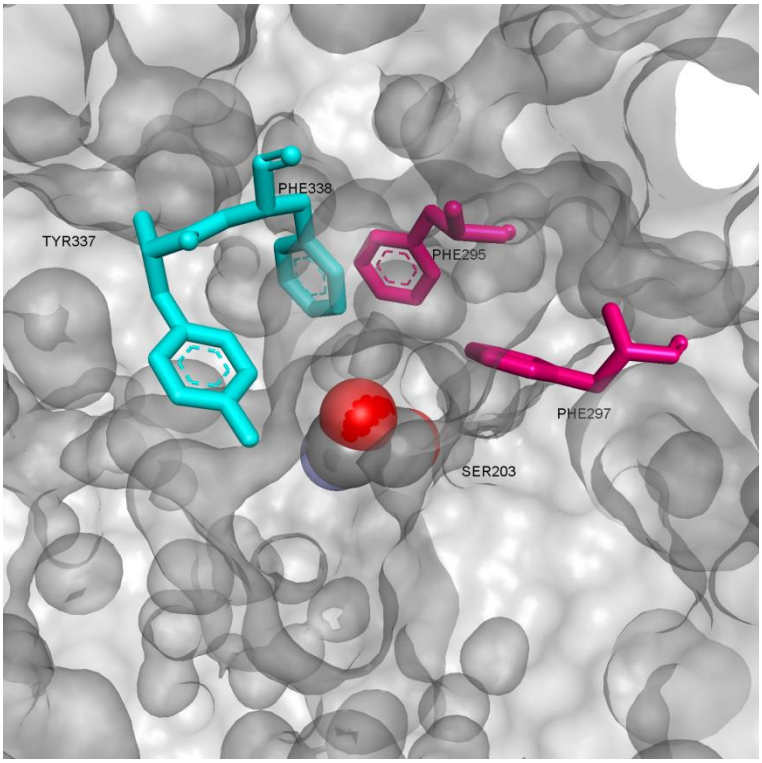

Fig. 4. Mutants of AChE created and tested as bioscavengers: Y337A, Y337A/F338A, F295L/Y337A, and F297I. Mutated residues in the active center gorge of hAChE (PDB code 3LII) are highlighted in blue (choline binding site) and in magenta (acyl pocket). The catalytic serine is shown as spheres with carbon atoms in grey, oxygen atoms in red and nitrogen atom in blue.

4. *Ex vivo* reactivation of AChE inhibited by OPs

Potentially active oxime-mutant enzyme pairs capable of degrading nerve agents in cycles of inhibition and reactivation (**Fig. 2**) were identified in a comprehensive analysis of both reactivation and inhibition of the human AChE mutants. Since mutations can disturb the general mechanism of enzyme reactivity, and consequently a bioscavenger potential, it was important to verify that mutations did not affect the rate of phosphylation. In case of tabun, the selected mutations did not reduce the rate of inhibition significantly in comparison to AChE w.t. (Katalinić et al., 2018). Moreover, the phosphorylation rate of the Y337A/F338A mutant was 4-fold faster than that of the AChE w.t. probably because of the more direct access of the bulky tabun to the catalytic serine due to lower steric hindrance as a consequence of the two choline binding site mutations. The rate of AChE mutants inhibition by VX and soman also ensured that the phosphylation step in *ex vivo* decomposition of OPs would not compromise the oxime-assisted catalytic bioscavenging of VX or soman (Kovarik et al., 2015, 2019b; Maček Hrvat et al., 2016).

The *ex vivo* bioscavenger potential was tested in human whole blood (hWB) supplemented with the AChE mutants and inhibited by a 10- and 50-fold excess of OPs. OP detoxification set in quickly after adding oxime HI-6 in case of soman (Kovarik et al., 2015), and VX (Maček Hrvat et al., 2016) and oxime 5B in case of tabun (Kovarik et al., 2019b). A high recovery of total cholinesterase activity with respect to OP excess, while no recovery of activity when hWB was supplemented with only mutant

enzymes, prove that OP was degraded by cycles of re-inhibition and reactivation of the AChE mutant. Rates of decomposition and the overall bioscavenging potential were directly related to inhibition and reactivation rates. In other words, in case of efficient OP detoxification by several mutants, it seems that the rate-limiting step for bioscavenging was the rate of phosphylation as in case of bioscavenging of S_P-cycloheptyl methylphosphonyl thiocholine, a structural homolog of cyclosarin, with a combination of the acyl pocket AChE mutants and HI-6 (Kovarik et al., 2007). Nevertheless, our results confirmed that the rate-limiting step for bioscavenging may be either the rate of phosphylation or subsequent reactivation of AChE as the enzyme cycles through its phosphonylated and unconjugated species decompose OPs (Fig. 2).

In case of soman, the most rapid soman decomposition was observed within the initial twenty minutes of oxime-assisted catalysis, when hWB was supplemented with 0.5 μM Y337A/F338A and 1.0 mM HI-6 (Kovarik et al., 2015), while with 10 μM soman the initial recovery of activity had a lag phase preceding the recovery. Although the catalytic activity of the mutant increased to its maximum as a result of total soman decomposition, the observed multiphasic kinetics could also be due, in part, to differences in the inhibition and reactivation kinetics of the four soman diastereoisomers leading to different rates of their catalytic hydrolysis (Benshop and de Jong, 1988; Bucht and Puu, 1984).

5. Oxime-assisted catalytic detoxification of nerve agents in mice

Oxime-assisted catalytic detoxification of nerve agents in combination with the AChE mutants was assayed *in vivo* on mice in terms of symptoms of poisoning, protective index (PI), and maximal dose (MD) of OP that was fully counteracted by the treatment. The experimental paradigm uses mice pretreated intravenously with oxime and AChE mutant 5 or 15 min prior to nerve agent exposure and then treated by HI-6 and atropine. In case of soman, HI-6 in combination with the double mutant Y337A/F338A increased the protective index by 60% compared to the protection observed with HI-6 therapy only (Kovarik et al., 2015). Although the MD of soman was not affected (5.0 x LD_{50} of soman), the symptoms of toxicity in mice pretreated with a combination of mutant and HI-6 were significantly less intense; e.g. tremor, convulsions, breathing, and locomotion disturbances. Moreover, even at the highest administered dose of soman (10 x LD_{50}), a delay in the time of lethality was noticeable if mice were pretreated with the mutant plus HI-6 combination (Kovarik et al., 2015). Nevertheless, the investigation was guided by the hypothesis that the administration of a mixture of the aging-resistant human AChE mutant Y337A/F338A and HI-6, its efficient reactivator, could provide considerable improvement in soman exposure treatment creating a unique oxime-assisted catalytic bioscavenger system *in vivo* in soman-exposed mice. A varied administration regimen of HI-6 and mutant AChE (pretreatment/post-exposure therapy) was the first to establish an *in vivo* example of effective oxime-assisted catalytic soman bioscavenging based on a combined administration of sub-stoichiometric amounts of site-directed mutant AChE and an oxime reactivator. Similar results in the delay of symptoms of toxicity and time of death were observed in VX detoxification in mice by the same mutant-oxime combination of pretreatment/post exposure therapy (Maček Hrvat et al., 2016). These studies described a potential of the Y337A/F388A - HI-6 pair to act as a bioscavenger in situations of exposure to multiple organophosphates, where the oxime could assist scavenging in the blood as well serve as a conventional antidote in target tissues (Kovarik et al., 2015; Maček Hrvat et al., 2016). Further bioscavenging developments should consider not only the optimization of mutant/oxime doses applied but also improvements such as slowing oxime clearance in blood, and prolonging residence circulation time of the enzyme. A recent study by Pashirova et al. (2018) demonstrated that the encapsulation of an oxime in solid lipid nanoparticles increased its bioavailability and prolonged circulation time in the bloodstream by 8.5 times compared to free oxime form. On the other hand, it was shown that polyethylene glycol-modified enzyme retained all of the kinetic characteristics of its nonpegylated form, while having 20-fold greater mean residence time (Mazor et al., 2008). This extension in the circulatory lifetime of the mutant and oxime is important in terms of the practicality of administering such a scavenger in potential situations of

human exposure to a nerve agent. Moreover, the single administration of the enzyme would not raise concerns about the possible immunogenicity of the mutant AChE form.

We recently reported detoxification of tabun by assembling oxime-assisted catalytic scavenging *in vivo* using the AChE mutant Y337A and its efficient reactivator 5B, a 2-PAM analog (Kovarik et al., 2019c). Unfortunately, the determined acute toxicity of 5B classified it as a relatively toxic compound especially for intravenous application. Therefore, the oxime was administrated intramuscularly as post-exposure treatment, while the mutant enzyme intravenously as pre-exposure treatment. The antidotal efficacy of the tested scavenging system in terms of PI and MD was poor and did not improve lethality outcomes. It seems that the failure to prove the scavenging capacity to detoxify tabun in mice could be a consequence of many factors such as: oxime toxicity, low application dose of oxime, two routes of therapy application, etc. It should also be pointed out that oxime 5B showed poor potency for reactivation of phosphorylated endogenous AChE and BChE as reported previously (Kovarik et al., 2019b). Therefore, it seems that for an efficient treatment, an oxime alone should not only reactivate an exogenous enzyme but also endogenous erythrocyte AChE and plasma BChE. This is in accordance with our previous studies with potent reactivators of tabun-inhibited AChE (oximes K203, K048 and 3A), where we reported that the therapeutic efficacy *in vivo* corresponded to the reactivating efficacy *in vitro*, meaning that the pharmacological effect of these oximes was indeed primarily related to the reactivation of tabun-inhibited native AChE (Berend et al., 2010; Čalić et al., 2006, 2008; Kovarik et al., 2008b, 2009). Moreover, this was confirmed with the best triazole oxime reactivators of the AChE wild type, where the protection index was 8.9, and all of the mice survived a dose of 7.9 LD_{50} of tabun (Kovarik et al., 2019a) despite the relatively high toxicity and low dose (2.2 mg/kg) of oxime. Furthermore, the translation of data obtained *in vitro* to *in vivo* application could hinder efficient therapy, i.e. the oxime circulation, cytotoxicity and tissue-specific distribution (Katalinić et al., 2015; Sit et al., 2018), and therefore the applied dose became important. When we administered the non-toxic oxime HI-6 intravenously together with the mutant to poisoned mice, soman and VX were efficiently detoxified (Kovarik et al., 2015; Maček Hrvat et al., 2016). Therefore, bioscavenger development should consider optimization of mutant/oxime dose applied as well as adjunct therapy to slow down oxime clearance from blood which would extent scavenging in plasma before OP distributes in the body and crosses the blood-brain-barrier. Indeed, as shown in a recent study on zwitterionic reactivators of AChE oximes (RS41A, RS194B and RS138B), efficacy relates to achieving pharmacokinetics and tissue distribution (Sit, et al., 2011, 2018; Radić et al., 2012, 2013a). The therapy was most effective in the case of VX and sarin exposure. Moreover, when RS194B was administered as pretreatment, 15 min prior to VX exposure and again as therapy post VX exposure, a particularly high protective index of 45 was produced and all mice survived 31.1 multiple doses of VX LD_{50} (Radić et al., 2012, 2013a). Since OP driven respiratory and cardiovascular defect arises from both central and peripheral AChE inhibition, an antidote with the potential to cross the blood-brain barrier rapidly would not only reactivate the immediately exposed and inhibited OP-sensitive sites in the CNS, but should also prevent secondary inhibition (Sit et al., 2018). Hence, there may be a justification for the longer term reactivation potential of an oxime antidote that is retained in the brain. Multiple ionization states, a capacity to be retained in tissue, a larger distribution volume and prolonged oral absorption may favor less frequent repeated dosing of the antidote (Sit et al., 2018; Taylor et al., 2019).

6. Conclusion

Variations in oxime structure and further refinements of AChE mutations should improve the catalytic potential of these scavenging pairs. Efficiency of scavenging is also a pharmacokinetic consideration since the introduced organophosphate should be scavenged in the plasma before it distributes into extracellular space and/or crosses the blood-brain barrier. Further bioscavenging developments should consider not only the optimization of mutant AChE/oxime doses applied but also adjunct therapy to slow oxime clearance in blood. The latter would extend pretreatment times

and increase efficiency of scavenging in the plasma before an organophosphate distributes and/or crosses the blood-brain barrier. One advantage of the cholinesterases as bioscavengers is that stereoselective preference for the organophosphorus enantiomer for inactivation likely matches the stereo preference for reactivation. Hence the more toxic enantiomer formed with excess organophosphate is also most susceptible to reactivation and detoxification.

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References

Barak, D., Ordentlich, A., Segall, Y., Velan, B., Benschop, H.P., De Jong, L.P.A., Shafferman, A., 1997. Carbocation-mediated processes in biocatalysts: contribution of aromatic moieties. J. Am. Chem. Soc. 119, 3157–3158.

Benschop, H.P., De Jong, L.P.A., 1988. Nerve agent stereoisomers: analysis isolation and toxicology. Acc. Chem. Res. 21, 368–374.

Berend, S., Katalinić, M., Lucić Vrdoljak, A., Kovarik, Z., Kuča, K., Radić, B., 2010. In vivo experimental approach to treatment against tabun poisoning. J. Enz. Inhib. Med. Chem. 25, 531–536.

Bucht, G., Puu, G., 1984. Aging and reactivatability of plaice cholinesterase inhibited by soman and its stereoisomers. Biochem. Pharmacol. 33, 3573–3577.

Cerasoli, D.M., Griffiths, E.M., Doctor, B.P., Saxena, A., Fedorko, J.M., Greig, N.H., Yu, Q.S., Huang, Y., Wilgus, H., Karatzas, C.N., Koplovitz, I., Lenz, D.E., 2005. In vitro and in vivo characterization of recombinant human butyrylcholinesterase (Protexia) as a potential nerve agent bioscavenger. Chem. Biol. Interact. 157-158, 363–365.

Clement, J.G., Shiloff, J.D., Gennings, C., 1987. Efficacy of a combination of acetylcholinesterase reactivators, HI-6 and obidoxime against tabun and soman poisoning of mice. Arch. Toxicol. 61, 70–75.

Cochran, R., Kalisiak, J., Küçükkılınç, T., Radić, Z., Garcia, E., Zhang, L., Ho, Y. K., Amitai, G., Kovarik, Z., Fokin, V.V., Sharpless, K.B., Taylor, P., 2011. Oxime-assisted acetylcholinesterase catalytic scavengers of organophosphates that resist aging. J. Biol. Chem. 286, 29718–29724.

Čalić, M., Lucić Vrdoljak, A., Radić, B., Jelić, D., Jun, D., Kuča, K., Kovarik, Z. 2006. In vitro and in vivo evaluation of pyridinium oximes: Mode of interaction with acetylcholinesterase, effect on tabun- and soman-poisoned mice and their cytotoxicity. Toxicology 219 (1-3), 85–96. https://doi.org/ 10.1016/j.tox.2005.11.003

Čalić, M., Bosak, A., Kuča, K., Kovarik, Z., 2008. Interactions of butane, but-2-ene or xylene-like linked bispyridinium para-aldoximes with native and tabun-inhibited human cholinesterases. Chem. Biol. Interact. 175(1– 3), 305–308. https://doi.org/ 10.1016/j.cbi.2008.04.010

Dawson, R.M., 1994. Review of oximes available for the treatment of nerve agent poisoning. J. Appl. Toxicol. 14, 317–331.

de Jong, L.P.A., Wolring, G.Z., 1984. Stereospecific reactivation by some Hagedornoximes of acetylcholinesterases from various species including man, inhibited by soman. Biochem. Pharmacol. 33, 1119–1125.

Doctor, B.P., Saxena, A., 2005. Bioscavengers for the protection of humans against organophosphate toxicity. Chem. Biol. Interact. 157-158, 167–171. http://dx.doi.org/10. 1016/j.cbi.2005.10.024.

Dolgin, E., 2013. Syrian gas attack reinforces need for better anti-sarin drugs. Nat. Med. 19, 1194–1195.

Eddleston, M., 2000. Patterns and problems of deliberate self-poisoning in the developing world. QJM 93, 715–731.

Ekström, F., Akfur, C., Tunemalm, A.-K., Lundberg, S., 2006a. Structural changes of phenylalanine 338 and histidine 447 revealed by the crystal structures of tabun-inhibited murine acetylcholinesterase. Biochemistry, 45 (1), 74–81. https://doi.org/10.1021/bi051286t

Ekström, F., Pang, Y.-P., Boman, M., Artursson, E., Akfur, C., Börjegren, S., 2006b. Crystal structures of acetylcholinesterase in complex with HI-6, Ortho-7 and obidoxime: Structural basis for differences in the ability to reactivate tabun conjugates. Biochem. Pharmacol. 72 (5), 597–607. https://doi.org/10.1016/j.bcp.2006.05.027

Eto, M., 1976. Organic and biological chemistry, in: Zweig, G. (Ed), The Organophosphorus Pesticides. CRC Press Inc., Cleveland, pp 142.

Franjesevic, A.J., Sillart, S.B., Beck, J.M., Vyas, S., Callam, C.S., Hadad, C.M., 2019. Resurrection and reactivation of acetylcholinesterase and butyrylcholinesterase. Chemistry 25 (21), 5337–5371.

Gray, A.P., 1984. Design and structure-activity relationships of antidote to organophosphorus anticholinesterase agents. Drug. Metab. Rev. 15, 557–589.

Gunnell, D., Eddleston, M., Phillips, M.R., Konradsen, F., 2007. The global distribution of fatal pesticide self-poisoning: systematic review. BMC Public Health. 7, 357.

Katalinić, M., Kovarik, Z., 2012. Reactivation of tabun-inhibited acetylcholinesterase investigated by two oximes and mutagenesis. Croat. Chem. Acta 85 (2), 209–212.

Katalinić, M., Maček Hrvat, N., Ždárová Karasová, J., Misik, J., Kovarik, Z., 2015. Translation of in vitro to in vivo pyridinium oxime potential in tabun poisoning. Arh. Hig. Rada Toksikol. 66, 291–298.

Katalinić, M., Šinko, G., Maček Hrvat, N., Zorbaz, T., Bosak, A., Kovarik, Z., 2018. Oxime-assisted reactivation of tabun inhibited acetylcholinesterase analysed by active site mutations. Toxicology 406–407, 104–113.

Kovarik, Z., 1999. Amino acid residues conferring specificity of cholinesterases. Period. Biol. 101 (1), 7–15.

Kovarik, Z., Radić, Z., Berman, H.A., Simeon-Rudolf, V., Reiner, E., Taylor, P., 2003. Acetylcholinesterase active centre and gorge conformations analyzed by combinatorial mutations and enantiomeric phosphonates. Biochem. J. 373 (1), 33–40.

Kovarik, Z., Radić, Z., Berman, H.A., Simeon-Rudolf, V., Reiner, E., Taylor, P., 2004. Mutant cholinesterases possessing enhanced capacity for reactivation of their phosphonylated conjugates. Biochemistry, 43 (11), 3222–3229. https://doi.org/ 10.1021/bi036191a

Kovarik, Z., Ciban, N., Radić, Z., Simeon-Rudolf, V., Taylor, P., 2006. Active site mutant acetylcholinesterase interactions with 2-PAM, HI-6, and DDVP. Biochem. Biophys. Res. Commun. 342 (3), 973–978. https://doi.org/10.1016/j.bbrc.2006.02.056

Kovarik, Z., Čalić, M., Šinko, G., Bosak, A., 2007a. Structure-activity approach in the reactivation of tabun-phosphorylated human acetylcholinesterase with bispyridinium para-aldoximes. Arh. Hig. Rada Toksikol. 58 (2), 201–209. https://doi.org/10.2478/v10004-007-0013-7

Kovarik, Z., Radić, Z., Berman, H.A., Taylor, P., 2007b. Mutation of acetylcholinesterase to enhance oxime assisted catalytic turnover of methylphosphonate. Toxicology 233, 79–84.

Kovarik, Z., Čalić, M., Bosak, A., Šinko, G., Jelić D., 2008a. In vitro evaluation of aldoxime interactions with human acetylcholinesterase. Croat Chem Acta, 81, 47–57.

Kovarik, Z., Čalić, M., Šinko, G., Bosak, A., Berend, S., Lucić Vrdoljak, A., Radić, B., 2008b. Oximes: Reactivators of phosphorylated acetylcholinesterase and antidotes in therapy against tabun poisoning. Chem. Biol. Interact. 175 (1-3), 173–179. https://doi.org/10.1016/j.cbi.2008.04.011

Kovarik, Z., Lucić Vrdoljak, A., Berend, S., Katalinić, M., Kuča, K., Musilek, K., Radić, B., 2009. Evaluation of oxime K203 as antidote in tabun poisoning. Arh. Hig. Rada Toksikol. 60(1), 19–26.

Kovarik, Z., Katalinić, M., Šinko, G., Binder, J., Holas, O., Jung, Y.-S., Musilova, L., Jun, D., Kuča, K., 2010. Pseudo-catalytic scavenging: Searching for a suitable reactivator of phosphorylated butyrylcholinesterase. Chem. Biol. Interact. 187 (1-3), 167–171. https://doi.org/10.1016/j.cbi.2010.02.023

Kovarik, Z., Maček Hrvat, N., Katalinić, M., Sit, R.K., Paradyse, A., Žunec, S., Musilek, K., Fokin, V.V., Taylor, P., Radić, Z., 2015. Catalytic soman scavenging by the Y337A/F338A acetylcholinesterase mutant assisted with novel site-directed aldoximes. Chem. Res. Toxicol. 28 (5), 1036–1044. https://doi.org/10.1021/acs.chemrestox.5b00060

Kovarik, Z., Kalisiak, J., Maček Hrvat, N., Katalinić, M., Zorbaz, T., Žunec, S., Green, C., Radić, Z., Fokin, V.V., Sharpless, K.B., Taylor, P., 2019a. Reversal of tabun toxicity enabled by a triazole annulated oxime library-reactivators of acetylcholinesterase. Chem. Eur. J. 25(16), 4100–4114.

Kovarik, Z., Maček Hrvat, N., Kalisiak, J., Katalinić, M., Sit, R.K., Zorbaz, T., Radić, Z., Fokin, V.V., Sharpless, K.B., Taylor, P., 2019b. Counteracting tabun inhibition by reactivation by pyridinium aldoximes that interact with active center gorge mutants of acetylcholinesterase. Toxicol. Appl. Pharmacol. 372, 40–46.

Kovarik, Z., Maček Hrvat, N., Žunec, S., Katalinić, M., 2019c. Detoxification of tabun-exposed mice by an acetylcholinesterase mutant assisted with a novel pyridinium aldoxime. Biol. Serb. 41, 4–8. https://doi.org/10.5281/zenodo.3532038

Kuca, K., Jun, D., Cabal, J., Hrabinova, M., Bartosova, L., Opletalova, V., 2006. Russian VX: inhibition and reactivation of acetylcholinesterase compared with VX agent. Basic Clin. Pharmacol. Toxicol. 98, 389–394.

Lenz, D.E., Yeung, D., Smith, J.R., Sweeney, R.E., Lumley, L.A., Cerasoli, D.M., 2007. Stoichiometric and catalytic scavengers as protection against nerve agent toxicity: a mini review. Toxicology 233, 31–39.

Lenz, D.E., Clarkson, E.D., Schulz, S.M., Cerasoli, D.M., 2010. Butyrylcholinesterase as a therapeutic drug for protection against percutaneous VX. Chem. Biol. Interact. 187, 249–252.

Lucić Vrdoljak, A., Čalić, M., Radić, B., Berend, S., Jun, D., Kuča, K., Kovarik, Z. 2006. Pretreatment with pyridinium oximes improves antidotal therapy against tabun poisoning. Toxicology, 228 (1), 41–50. https://doi.org/10.1016/j.tox.2006.08.012

Maček Hrvat, N., Žunec, S., Taylor, P., Radić, Z., Kovarik, Z., 2016. HI-6 assisted catalytic scavenging of VX by acetylcholinesterase choline binding site mutants. Chem. Biol. Interact. 259, 148–153.

Maček Hrvat, N., Kalisiak, J., Šinko, G., Radić, Z., Sharpless, K.B., Taylor, P., Kovarik, Z., 2020. Evaluation of high-affinity phenyltetrahydroisoquinoline aldoximes, linked through anti-triazoles, as reactivators of phosphylated cholinesterases. Toxicol. Lett. 321, 83–89.

Maraković, N., Knežević, A., Vinković, V., Kovarik, Z., Šinko, G., 2016. Design and synthesis of N-substituted-2-hydroxyiminoacetamides and interactions with cholinesterases. Chem. Biol. Interact. 259, 122–132.

Marrs, T.C., 2007. Toxicology of organophosphate nerve agents, in: Marrs, T.C., Maynard, R.L., Sidell, F.R. (Eds), Chemical Warfare Agents: toxicology and Treatment. John Wiley & Sons Ltd, West Sussex, pp 191–222.

Masson, P., Rochu, D., 2009. Catalytic bioscavengers against toxic esters, an alternative approach for prophylaxis and treatments of poisonings. Acta Naturae 1, 68–79.

Masson, P., 2011. Evolution of and perspectives on therapeutic approaches to nerve agent poisoning. Toxicol. Lett. 206, 5-13.

Mazor, O., Cohen, O., Kronman, C., Raveh, L., Stein, D., Ordentlich, A., Shafferman, A., 2008. Aging-resistant organophosphate bioscavenger based on polyethylene glycol-conjugated F338A human acetylcholinesterase. Mol. Pharmacol. 74, 755–763.

Mercey, G., Verdelet, T., Renou, J., Kliachyna, M., Baati, R., Nachon, F., Jean, L., Renard, P.Y., 2012. Reactivators of acetylcholinesterase inhibited by organophosphorus nerve agents. Acc. Chem. Res. 45, 756–766.

Millard, C.B., Koellner, G., Ordentlich, A., Shafferman, A., Silman, I., Sussman, J.L., 1999. Reaction products of acetylcholinesterase and VX reveal a mobile histidine in the catalytic triad. J. Am. Chem. Soc. 121, 9883–9884.

Nachon, F., Brazzolotto, X., Trovaslet, M., and Masson, P., 2013. Progress in the development of enzyme-based nerve agent bioscavengers. Chem. Biol. Interact. 206, 536–544.

Pashirova, T.N., Braïki, A., Zueva, I.V., Petrov, K.A., Babaev, V.M., Burilova, E.A., Samarkina, D.A., Rizvanov, I.K., Souto, E.B., Jean, L., Renard, P.Y., Masson, P., Zakharova, L.Y., Sinyashin, O.G., 2018. Combination delivery of two oxime-loaded lipid nanoparticles: time-dependent additive action for prolonged rat brain protection. J. Control Release. 290, 102-111.

Puu, G., Artursson, E., Bucht, G., 1986. Reactivation of nerve agent inhibited human acetylcholinesterases by HI-6 and obidoxime. Biochem. Pharmacol. 35, 1505–1510.

Radić, Z., Pickering, N.A., Vellom, D.C., Camp, S., Taylor, P., 1993. Three distinct domains in the cholinesterase molecule confer selectivity for acetylcholinesterase and butyrylcholinesterase inhibitors. Biochemistry, 32, 12074–12084.

Radić, Z., Duran, R., Vellom, D.C., Li, Y., Cervenansky, C., Taylor, P., 1994. Site of fasciculin interaction with acetylcholinesterase. J. Biol. Chem. 269, 11233–11239.

Radić, Z., Sit, R.K., Kovarik, Z., Berend, S., Garcia, E., Zhang, L., Amitai, G., Green, C., Radić, B., Fokin, V.V., Sharpless, K.B., Taylor, P., 2012. Refinement of structural leads for centrally acting oxime reactivators of phosphylated cholinesterases. J. Biol. Chem. 287 (15), 11798–11809. https://doi.org/10.1074/jbc.M111.333732

Radić, Z., Sit, R.K., Garcia, E., Zhang, L., Berend, S., Kovarik, Z., Amitai, G., Fokin, V.V., Sharpless, K.B., Taylor, P., 2013a. Mechanism of interaction of novel uncharged, centrally active reactivators with OP-hAChE conjugates. Chem. Biol. Interact. 203, 67–71.

Radić, Z., Dale, T., Kovarik, Z., Berend, S., Garcia, E., Zhang, L., Amitai, G., Green, C., Radić, B., Duggan, B.M., Ajami, D., Rebek Jr., J., Taylor, P., 2013b. Catalytic detoxification of nerve agent and pesticide organophosphates by butyrylcholinesterase assisted with nonpyridinium oximes. Biochem. J. 450 (1), 231–242.

Raveh, L., Grauer, E., Grunwald, J., Cohen, E., Ashani, Y., 1997. The stoichiometry of protection against soman and VX toxicity in monkeys pretreated with human butyrylcholinesterase. Toxicol. Appl. Pharmacol. 145, 43–53.

Saxena, A., Maxwell, D.M., Quinn, D.M., Radić, Z., Taylor, P., Doctor, B.P., 1997. Mutant acetylcholinesterases as potential detoxification agents for organophosphate poisoning. Biochem. Pharmacol. 54 (2), 269–274.

Saxena, A., Sun, W., Fedorko, J.M., Koplovitz, I., Doctor, B.P., 2011. Prophylaxis with human serum butyrylcholinesterase protects guinea pigs exposed to multiple lethal doses of soman or VX. Biochem. Pharmacol. 81, 164–169.

Shafferman, A., Ordentlich, A., Barak, D., Stein, D., Aiel, N., and Velan, B., 1996. Aging of phosphylated human acetylcholinesterase: catalytic processes mediated by aromatic and polar residues of the active centre. Biochem. J. 318, 833–840.

Sidell, F.R., Grof, W.A., 1974. The reactivatibility of cholinesterase inhibited by VX and sarin in man. Toxicol. Appl. Pharmacol. 27, 241–252.

Sit, R.K., Radić, Z., Gerardi, V., Zhang, L., Garcia, E., Katalinić, M., Amitai, G., Kovarik, Z., Fokin, V.V., Sharpless, K.B., Taylor, P., 2011. New structural scaffolds for centrally acting oxime reactivators of phosphylated cholinesterases. J. Biol. Chem. 286 (22), 19422–19430.

Sit, R.K., Kovarik, Z., Maček Hrvat, N., Žunec, S., Green, C., Fokin, V.V., Sharpless, K.B., Radić, Z., Taylor, P., 2018. Pharmacology, pharmacokinetics, and tissue disposition of zwitterionic hydroxyiminoacetamido alkylamines as reactivating antidotes for organophosphate exposure. J. Pharmacol. Exp. Ther. 367(2), 363–372.

Stojiljković, M.P., Jokanović, M., 2006. Pyridinium oximes: Rationale for their selection as causal antidotes against organophosphate poisonings and current solutions for auto-injectors. Arh. Hig. Rada Toksikol. 57 (4), 435–443.

Stone, R.K., 2018. Attack puts nerve agent in the spotlight: Researchers race to understand deadly compound developed by Soviet scientists. Science, 359 (6382), 1314–1315.

Sun, M.C., Li, F.Z., Chou, T.C., 1986. Reactivation of sarin- or soman phosphonylated human acetylcholinesterase by bis-pyridinium mono-oximes. Biochem. Pharmacol. 35, 337–339.

Šinko, G., Čalić, M., Kovarik, Z., 2006. Para- and ortho-pyridinium aldoximes in reaction with acetylcholinesterase. FEBS Letters, 580, 3167–3172

Taylor, P., Lappi, S., 1975. Interaction of fluorescence probes with acetylcholinesterase. The site and specificity of propidium binding. Biochemistry, 14, 1989–1997.

Taylor, P., Radić, Z., 1994. The cholinesterases: from genes to proteins. Annu. Rev. Pharmacol. Toxicol. 34, 281–320.

Taylor, P., Kovarik, Z., Reiner, E., Radić, Z., 2007. Acetylcholinesterase: Converting a vulnerable target to a template for antidotes and detection of inhibitor exposure. Toxicology, 233, 70–78.

Taylor, P., 2011. Anticholinesterase agents, in: Brunton, L.L., Chabner, B., Knollman, B. (Eds), Goodman & Gilman's The Pharmacological Basis of Therapeutics, 13th edn. McGraw-Hill, New York, pp 239–254.

Taylor, P., Yan-Jye, S., Momper, J., Hou, W., Camacho-Hernandez, G.A., Radić, Z., Rosenberg, Y., Kovarik, Z., Sit, R., Sharpless, K.B., 2019. Assessment of ionizable, zwitterionic oximes as reactivating antidotal agents for organophosphate exposure. Chem. Biol. Interact. 308, 194–197.

Timperley, C.M., Forman, J.E., Abdollahi, M., Al-Amri, A.S., Baulig, A., Benachour, D., Borrett, V., Cariño, F.A., Geist, M., Gonzalez, D., Kane, W., Kovarik, Z., Martínez-Álvarez, R., Mourão, N.M.F., Neffe, S., Raza, S.K., Rubaylo, V., Suárez, A.G., Takeuchi, K., Tang, C., Trifirò, F., van Straten, F.M., Vanninen, P.S., Vučinić, S., Zaitsev, V., Zafar-Uz-Zaman, M., Zina, M.S., Holen, S., 2019a. Advice on assistance and protection provided by the Scientific Advisory Board of the Organisation for the Prohibition of Chemical Weapons: Part 1. On medical care and treatment of injuries from nerve agents. Toxicology 415, 56–69.

Timperley, C.M., Abdollahi, M., Al-Amri, A.S., Baulig, A., Benachour, D., Borrett, V., Cariño, F.A., Geist, M., Gonzalez, D., Kane, W., Kovarik, Z., Martínez-Álvarez, R., Fusaro Mourão, N.M., Neffe, S., Raza, S.K., Rubaylo, V., Suárez, A.G., Takeuchi, K., Tang, C., Trifirò, F., van Straten, F.M., Vanninen, P.S., Vučinić, S., Zaitsev, V., Zafar-Uz-Zaman, M., Zina, M.S., Holen, S., Forman, J.E., Alwan, W.S., Suri, V., 2019b. Advice on assistance and protection from the Scientific Advisory Board of the Organisation for the Prohibition of Chemical Weapons: Part 2. On preventing and treating health effects from acute, prolonged, and repeated nerve agent exposure, and the identification of medical countermeasures able to reduce or eliminate the longer term health effects of nerve agents. Toxicology 413, 13–23.

Tormos, J.R., Wiley, K.L., Wang, Y., Fournier, D., Masson, P., Nachon, F., Quinn, D.M., 2010. Accumulation of tetrahedral intermediates in cholinesterase catalysis: a secondary isotope effect study. J. Am. Chem. Soc. 132, 17751–17759.

Vučinić, S., Zlatković, M., Antonijević, B., Čurčić, M., Bošković, B., 2013. Fresh frozen plasma as a successful antidotal supplement in acute organophosphate poisoning. Arh. Hig. Rada Toksikol. 64, 273–277.

Wilson, I.B., Ginsburg, S., 1955. Reactivation of acetylcholinesterase inhibited by organophosphates. Arch. Biochem. Biophys. 54, 569–571.

Winter, M., Wille, T., Musilek, K., Kuca, K., Thiermann, H., Worek, F., 2016. Investigation of the reactivation kinetics of a large series of bispyridinium oximes with organophosphate- inhibited human acetylcholinesterase. Toxicol. Lett. 244, 136–142.

Worek, F., Widmann, R., Knopff, O., Szinicz, L., 1998. Reactivating potency of obidoxime, pralidoxime, HI 6 and HLo 7 in human erythrocyte acetylcholinesterase inhibited by highly toxic organophosphorus compounds. Arch. Toxicol. 72 (4), 237–243.

Worek, F., Thiermann, H., Szinicz, L., Eyer, P., 2004. Kinetic analysis of interactions between human acetylcholinesterase, structurally different organophosphorus compounds and oximes. Biochem. Pharmacol. 68 (11), 2237–2248.

Worek, F., von der Wellen, J., Musilek, K., Kuca, K., Thiermann, H., 2012. Reactivation kinetics of a homologous series of bispyridinium bis-oximes with nerve agent-inhibited human acetylcholinesterase. Arch. Toxicol. 86 (9), 1379–1386.

Worek, F., Thiermann, H., 2013. The value of novel oximes for treatment of poisoning by organophosphorus compounds. Pharmacol Therapeut. 139 (2), 249–259.

Zorbaz, T., Braïki, A., Maraković, N., Renou, J., de la Mora, E., Maček Hrvat, N., Katalinić, M., Silman, I., Sussman, J.L., Mercey, G., Gomez, C., Mougeot, R., Pérez, B., Baati, R., Nachon, F., Weik, M., Jean, L., Kovarik, Z., Renard, P.-Y., 2018a. Potent 3-hydroxy-2-pyridine aldoxime reactivators of organophosphate-inhibited cholinesterases with predicted blood–brain barrier penetration. Chem. Eur. J. 24 (38), 9675–9691.

Zorbaz, T., Malinak, D., Maraković, N., Maček Hrvat, N., Zandona, A., Novotny, M., Skarka, A., Andrys, R., Benkova, M., Soukup, O., Katalinić, M., Kuca, K., Kovarik, Z., Musilek, K., 2018b. Pyridinium oximes with ortho-positioned chlorine moiety exhibit improved physicochemical properties and efficient reactivation of human acetylcholinesterase inhibited by several nerve agents. J. Med. Chem. 61 (23), 10753–10766.

Zorbaz, T., Malinak, D., Kuca, K., Musilek, K., Kovarik, Z., 2019. Butyrylcholinesterase inhibited by nerve agents is efficiently reactivated with chlorinated pyridinium oximes. Chem. Biol. Interact. 307, 16–20.