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Oximes in organophosphate poisoning: 60 years of hope and despair

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ABSTRACT

The high number of annual fatalities following suicidal poisoning by organophosphorus (OP) pesticides and the recent homicidal use of the chemical warfare nerve agent sarin against civilian population in Syria underlines the continuous threat by these highly toxic agents. The need for an effective treatment of OP poisoning resulted in the implementation of a combination therapy with the muscarinic receptor antagonist atropine and an oxime for the reactivation of OP-inhibited acetylcholinesterase (AChE). Since the invention of the first clinically used oxime pralidoxime (2-PAM) in the 1950s ongoing research attempted to identify more effective oximes. In fact, several thousand oximes were synthesized in the past six decades. These include charged and non-charged compounds, mono- and bispyridinium oximes, asymmetric oximes, oximes with different substitutes and more recently non-oxime reactivators. Multiple in vitro and in vivo studies investigated the potential of oximes to reactivate OP-inhibited AChE and to reverse OP-induced cholinergic signs. Depending on the experimental model, the investigated species and the tested OP largely variable results were obtained by different laboratories. These findings and the inconsistent effectiveness of oximes in the treatment of OP-pesticide poisoned patients led to a continuous discussion on the value of oximes. In order to provide a forward-looking evaluation of the significance of oximes in OP poisoning multiple aspects, including intrinsic toxicity, in vitro reactivation potency, efficacy and pharmacokinetics, as well as the impact of the causative OP have to be considered. The different influencing factors in order to define the benefit and limitations of oximes in OP poisoning will be discussed.

1. Introduction

The high number of annual fatalities following suicidal poisoning by organophosphorus (OP) pesticides and the recent homicidal use of the OP nerve agent sarin in Syria underline the urgent need for drugs and concepts to treat OP poisoning effectively [1–3]. Since decades standard treatment of OP poisoning includes the muscarinic antagonist atropine and an oxime to reactivate acetylcholinesterase (AChE) inhibited by OPs [4–6]. Yet, a large number of in vitro and in vivo studies as well as clinical reports showed a limited effectiveness of standard therapy in different scenarios of OP pesticide and nerve agent poisoning [7–10].

The unsatisfactory situation led to intensive research efforts to identify potential new antidotes with superior effectiveness or as an adjunct to standard therapy. Focus was put on stoichiometric and catalytic bio- and small molecule scavengers for prophylaxis or therapy, advanced neuroprotectants and anticonvulsants, and on antinicotinics to restore neuromuscular transmission of OP-blocked respiratory muscles [11–19].

In addition, thousands of oxime structures were published since the invention of pralidoxime in 1955 in order to identify compounds with superior reactivating potency and therapeutic effectiveness [7,20–26]. Despite great efforts of research groups in different countries to present more effective oximes, current standard therapy of OP poisoning is still based on a small number of compounds firstly synthesized in the 1950s and 1960s, i.e. pralidoxime (2-PAM), trimedoxime (TMB-4), obidoxime and HI-6 [27–31]. Hence, there is the peculiar situation of using old oximes with numerous limitations while a huge number of new oximes are available. Moreover, there is an ongoing debate on the value of oximes in OP pesticide poisoning. Therefore, the present paper shall give insight into the potential and limitations of oxime therapy as well as an outlook on the requirements for superior future oximes.

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2. Value of oximes in OP poisoning

A countless number of studies on oximes were published in the open literature since the invention of pralidoxime in the mid 1950s [20,21]. These included in vitro reactivation studies using different AChE sources, OP pesticides and nerve agents, pharmacological studies on isolated organs and in vivo studies with a broad range of experimental protocols, animal species, antidote combinations and doses. In general, these studies provided convincing evidence that addition of an oxime to basal atropine treatment provides a therapeutic benefit [10]. Hereby, the therapeutic effectiveness is strongly dependent on the administered OP and on the experimental setup. Nevertheless, the use of atropine-oxime combinations is an accepted concept for treatment of nerve agent poisoning and is implemented for self and buddy aid by auto-injectors as well as for clinical therapy in military and civilian treatment facilities.

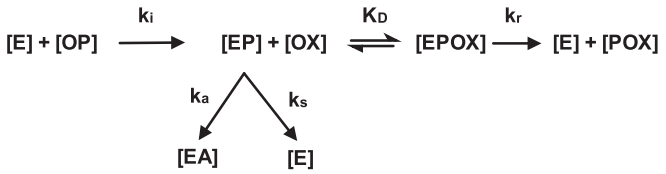
In contrast, there is an ongoing and fierce debate on the value of oximes in human OP pesticide poisoning and rather contradictory views were published in the last six decades [32–36]. These range from a very enthusiastic conclusion in the early paper by Namba and Hiraki stating "Hitherto, alkylphosphate poisoning has been treated mainly by atropine, but now atropine is replaced by PAM [pralidoxime]" to a complete denial of any oxime benefit by Peter et al. saying "Based on the current available data on human organophosphate poisoning, oxime was associated with either a null effect or possible harm" [32,35].

These inconsistent data on oxime effectiveness require a closer look on the mechanism, potential and limitations of oximes in OP poisoning.

3. Factors limiting oxime effectiveness

The main mechanism of action of oximes is reactivation of OP-inhibited AChE in order to restore the physiological function of this pivotal enzyme [21]. Successful reactivation can terminate the toxic OP effects at muscarinic and nicotinic receptors. The velocity and extent of reactivation is determined by various factors (Scheme 1):

- The reactivating potency of an oximes which can be quantified by in vitro determination of the reactivation constants k_r (reactivity constant for the displacement of the phosphyl moiety from AChE) and K_D (apparent dissociation constant which is inversely proportional to the affinity of an oxime to the phosphylated AChE),
- The susceptibility of phosphylated AChE towards nucleophilic attack by an oxime,



Scheme 1. Reaction scheme for the inhibition of AChE by OP, the spontaneous and oxime-induced reactivation and dealkylation of OP-inhibited AChE. The respective concentrations are denoted [EP] the phosphylated AChE, [OX] the reactivator, [EPOX] the Michaelis-type phosphyl-AChE-oxime-complex, [E] the active enzyme and [POX] the phosphylated oxime. k_i is the second order inhibition rate constant, k_s and k_a the first order rate constants for spontaneous reactivation and dealkylation, respectively. K_D is equal to the ratio [EP]*[OX]/[EPOX]) and describes the dissociation constant which is inversely proportional to the affinity of the oxime to [EP], and k_r denotes the rate constant for the displacement of the phosphyl residue from [EPOX], indicating the reactivity of the oxime.

- The velocity of spontaneous dealkylation of phosphylated AChE resulting in a reactivation-resistant enzyme species ("aging") and
- The intrinsic inhibitory activity of an oxime towards native AChE which may be a limiting factor for the maximum therapeutic concentration.

In general, the majority of the classical nerve agents (sarin, cyclosarin, VX, VR) and the most important pesticides bearing a dimethoxy or diethoxy group at the phosphorus form phosphylated AChE which can be easily reactivated [20]. AChE inhibited by selected OP, e.g. soman or profenophos, undergoes extremely rapid aging thus preventing a sufficient reactivation by oximes [37,38] and inhibition of AChE by various phosphoramidates, i.e. tabun and fenamiphos, results in OP-AChE conjugates which are difficult to reactivate by any oxime [39].

There is still uncertainty on potential additional pharmacological effects of oximes. A therapeutic effect of oximes not related to reactivation of AChE was shown in rodent models but could not be verified with human tissue (intercostal muscles) and at present there is no evidence for a direct, pharmacological effect of oximes in humans in vivo [40,41].

The investigation of interactions between AChE, OP and oximes in vitro can provide the basic information on the possibility of reactivation of an OP-AChE conjugate by a selected oxime. However, such data cannot be translated directly to the in vivo situation. Here, additional factors are affecting the therapeutic effectiveness of oximes:

- Initiation of therapy, dosing, duration and pharmacokinetics of oximes as well as the ability of an oxime to enter the central nervous system, an issue which is still a limitation of established pyridinium oximes [42,43] and
- The inhibitory potency, blood and tissue concentration and persistence of an OP.

The persistence of toxic OP concentrations is considered as a major factor affecting the effectiveness of oxime therapy and is a determining issue in suicidal OP pesticide and percutaneous VX poisoning [44,45]. In fact, by utilizing a dynamic in vitro model for

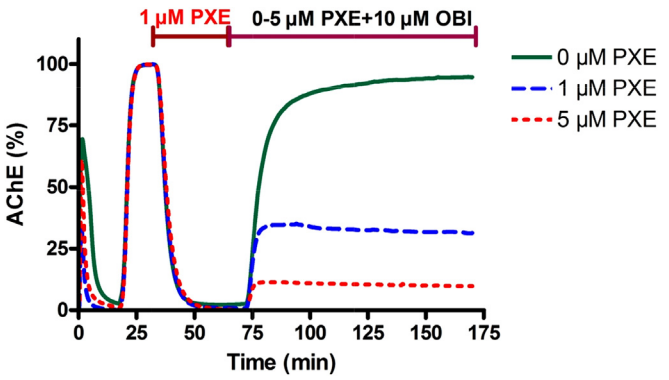


Fig. 1. Human erythrocyte AChE activity in the presence of paraoxon (PXE) and obidoxime (OBI) in a dynamic model [57,58]. Human erythrocytes serving as AChE source were loaded on a 0.22 µm syringe filter and continuously perfused with phosphate buffer (0.1 M, pH 7.4), Ellman's reagent (DTNB; 0.3 mM) and the substrate acetylthiocholine (0.45 mM) at a flow rate of 0.5 ml/min and 37 °C. Absorbance changes (470 nm) were continuously recorded by an UV/VIS detector and are given as % of the individual maximum AChE activity determined at 30 min. Enzyme reactors were perfused with 1 µM PXE from 30 to 60 min to induce complete inhibition of immobilized AChE followed by perfusion with 0, 1 or 5 µM PXE until 170 min. Perfusion with 10 µM OBI was started at 70 min and continued until the end of the experiment at 170 min. The figure presents original recordings of single experiments.