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REVIEW ARTICLE

Kinetic prerequisites of oximes as effective reactivators of organophosphate-inhibited acetylcholinesterase: a theoretical approach

Franz Worek[1], Nadine Aurbek[1], Timo Wille[1], Peter Eyer[2], and Horst Thiermann[1]

[1]*Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany, and* [2]*Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University, Munich, Germany*

Abstract

The standard treatment of poisoning by organophosphorus compounds (OP) includes the reversible muscarine receptor antagonist atropine and oximes for the reactivation of OP-inhibited acetylcholinesterase (AChE). There is an ongoing discussion on the benefit of oxime therapy in OP pesticide poisoning, and experimental data indicate a limited efficacy of oximes against various nerve agents. Oxime effectiveness can be quantified *in vitro* by determination of the reactivity (k_r) and affinity constants ($1/K_D$). These constants can be used to calculate reactivation velocities and oxime concentrations necessary for the reactivation of a desired fraction of inhibited AChE. Model calculations indicate that a $k_r > 0.1$ min^{-1} and $K_D < 100$ µM are minimal requirements for oxime effectiveness when reactivation is performed in the absence of free OP. In addition, the findings demonstrate that selective increase of either reactivity or affinity of an oxime would be insufficient. Hereby, it has to be taken into account that an increase of affinity to OP-inhibited AChE is generally accompanied by an increased affinity to native AChE and subsequent reduction in oxime tolerance. Hence, future developments of more effective oximes should consider kinetic demands by attempting to achieve a certain level of reactivity and affinity, preferentially towards OP-inhibited AChE.

Keywords: Oximes, organophosphorus compounds, acetylcholinesterase, reactivation kinetics, model calculations

Introduction

The clinical treatment of poisoning by organophosphorus compound (OP)-based acetylcholinesterase (AChE) inhibitors, i.e. pesticides and nerve agents, did not experience fundamental change since the first use of the oxime pralidoxime (2-PAM, Figure 1) in 1958[1]. Basically, the specific treatment of OP-poisoning is a combination of an anticholinergic drug, mostly atropine, and an oxime which is intended to reactivate OP-inhibited AChE[2]. The antidotal therapy is supplemented by benzodiazepines, catecholamines, antibiotics, and further drugs depending on the clinical signs and symptoms[3–6].

Apart from pralidoxime, only few oximes have been used in human OP-poisoning in the past 50 years[7]. Obidoxime (Figure 1) was first used in the early 1960s[8] followed by trimedoxime (TMB-4, Figure 1)[9] while HI-6 (Figure 1) was tested only in few cases of OP pesticide poisoning[10].

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Although only very few oximes are used clinically a huge number of oximes were synthesized in the past

Address for Correspondence: Franz Worek, Bundeswehr Institute of Pharmacology and Toxicology, Neuherbergstrasse 11, 80937 Munich, Germany. Tel: +49-89-3168-2930. Fax: +49-89-3168-2333. E-mail: franzworek@bundeswehr.org

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decades mainly in Germany, USA, Croatia, Israel, Czech Republic, and Korea[7,11]. The main purpose of these oxime development programs was to find oximes which are more effective than the clinically used compounds in nerve agent poisoning. The ultimate goal of this development was the search for highly effective oximes which can be used in autoinjectors with limited volume for self and buddy aid. Experimental oximes synthesized so far are in most cases derivatives of the *mono*-pyridinium oxime pralidoxime or the *bis*-pyridinium oxime obidoxime[7] and only few are bearing additional imidazolium or quinuclidinium groups[12].

There is convincing evidence that the main mechanism of action of oximes is the reactivation of OP-inhibited AChE by removal of the phosphoryl- or phosphonyl-moiety from the active site serine[7,13–15]. By restoring the function of the pivotal enzyme AChE OP-induced disruption of the cholinergic signalling chain may be counteracted and life-saving in severe cases of OP-poisoning. The ability of oximes to reactivate OP-inhibited AChE may be quantified *in vitro* by determination of the different reactivation rate constants (Figure 2). According to the scheme the ability of oximes to reactivate OP-inhibited AChE is determined by the affinity ($1/K_D$) and the reactivity (k_r). The efficiency of an oxime may be quantified by the second order rate constant k_{r2} determined by the ratio of k_r and K_D[16]. Based on these constants the reactivating ability of oximes may be estimated prior to *in vivo* evaluation of the oxime efficacy in animals or humans.

In the past decade we determined the reactivation rate constants for a variety of oximes with a large number of structurally different OP using AChE from different species[16–23]. Hereby it became apparent that the ability of oximes to reactivate OP-inhibited AChE is strongly dependent on the structure of the oxime, the OP-AChE conjugate and the AChE source. Due to, in part substantial, differences between human and animal AChE further considerations will focus only on human AChE. Moreover, only reactivation data from identical experimental set-up were considered for comparison, i.e. reaction conditions at pH 7.4, 37°C, in the absence of substrate and after removal of free OP.

The determination of the affinity and reactivity of oximes revealed enormous differences. The spectrum ranged from complete inability of oximes to reactivate inhibited AChE (e.g. HI-6 and tabun), hardly measurable k_r and K_D values to utmost high reactivity and affinity. Table 1 shows the extremes determined so far. Accordingly, the difference between the determined dissociation and reactivity rate constants is almost 2000 and 3000 fold, respectively.

Animal experiments and clinical data demonstrate that the therapeutic efficacy of oximes is also determined by additional factors[3,4,24–29]. Post-inhibitory reactions of the OP-inhibited AChE, i.e. spontaneous reactivation and aging (Figure 2), may enhance or impair the oxime efficacy. Further factors include pre-treatment, OP body load and competing acetylcholine concentration, time interval

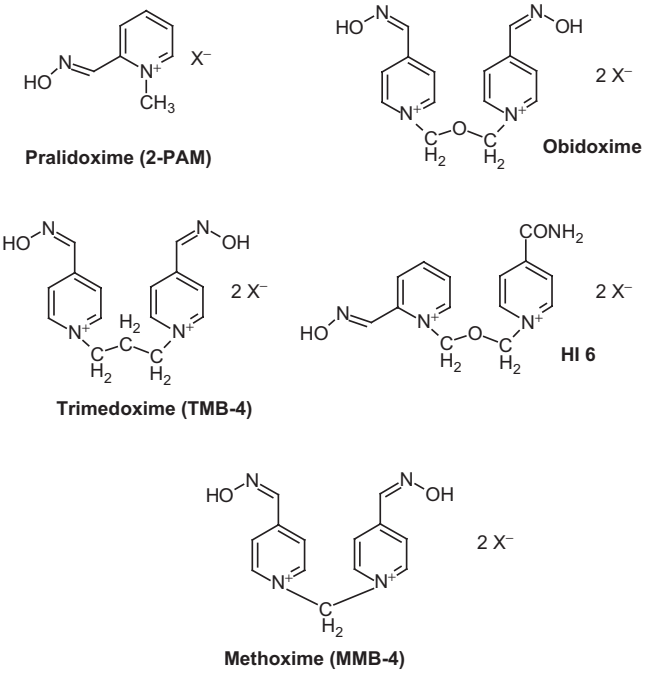

Figure 1. Chemical structures of important oximes.

Figure 2. Reaction scheme for the reactivation of organophosphate-inhibited acetylcholinesterase (AChE) by oximes. The respective concentrations are: [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_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 is the rate constant for the displacement of the phosphyl residue from [EPOX], indicating the reactivity of the oxime.

Table 1. Highest and lowest reactivation rate constants of oximes with OP-inhibited human AChE.

Oxime	OP	k_r (min^{-1})	K_D (μM)
Pralidoxime	MFPßCh[#]	0.002[a]	
Methoxime	*n*-Butylsarin	5.91[b]	
Pralidoxime	Methamidophos		2.1[c]
Pralidoxime	MFPhCh[§]		3837[d]

AChE, acetylcholinesterase; affinity (K_D) and reactivity (k_r) constants; OP, organophosphorus compounds.
[a,d]from[18], [b]from[19], [c]from[16].
[#]Methylfluoro-ß-phosphonylcholine.
[§]Methylfluorophosphonylhomocholine.

Table 2. Reactivation rate constants of selected oximes and OP.

Oxime	OP	k_r (min^{-1})	K_D (μM)
Obidoxime	Tabun	0.04	97.3
Obidoxime	Cyclosarin	0.395	945.6
Pralidoxime	Cyclosarin	0.182	3159
HI-6	Cyclosarin	1.3	47.2

Affinity (K_D) and reactivity (k_r) constants; OP, organophosphorus compounds.
Data are from[18].

between poisoning and onset of treatment, oxime dosing and duration of treatment. In view of multiple factors influencing the efficacy of oximes in OP-poisoning there is an ongoing debate on the benefit of oximes and on the necessity to develop more effective oximes as antidotes against pesticide and nerve agent poisoning[2–4,11,28–31].

These controversies prompted us to use theoretical models, based on kinetic data obtained under identical experimental conditions, to characterize the requirements of an oxime as an effective antidote in OP-poisoning.

Which level OF AChE activity is necessary?

Impairment of neuromuscular transmission at respiratory muscles is one of the life threatening effects of OP which cannot be counteracted by anticholinergics[32]. Hence, the reactivation of AChE at neuromuscular synapses is a key parameter for the assessment of oxime efficacy *in vivo*. Unfortunately, synaptic AChE is hardly accessible to direct monitoring and the evaluation of oxime effects has to be based on surrogate parameters. Repetitive measurement of erythrocyte AChE activity can be used to follow changes in AChE activity as a result of oxime treatment provided that erythrocyte AChE reflects synaptic AChE.

Recently, *in vitro* studies using a dynamic model with real-time determination of membrane-bound AChE activity demonstrated almost identical kinetics of human erythrocyte and intercostal muscle AChE following inhibition by OP and reactivation by oximes[33,34]. A thorough analysis of data from OP pesticide poisoned patients revealed that erythrocyte AChE levels higher than 40% of normal were accompanied by normal neuromuscular transmission[26,35].

Consequently, an effective oxime does probably not require a complete reactivation of inhibited AChE and a 40% reactivation will be considered as lower edge for further considerations. Now, it was tempting to determine whether presently available oximes could meet this goal. Initial calculations were performed using determined reactivation rate constants[16] and excluding excess inhibitor and premature aging. Figure 3 shows the marked differences between the selected OP. None of the selected oximes would be able to reactivate tabun-inhibited AChE at clinically used oxime concentrations within a reasonable time, primarily due to low reactivity (Figure 3A). With cyclosarin-inhibited AChE HI-6 (high reactivity and affinity) and MMB-4 (high reactivity) should result in a rapid and complete restoration of enzyme activity within a few minutes (Figure 3B), obidoxime and 2-PAM would fail due to low affinity. With paraoxon- and methamidophos the oximes should be able to reactivate the enzyme although HI-6 would result in a rather slow increase of paraoxon-inhibited AChE activity (Figure 3C).

Reactivation of OP-inhibited AChE by oximes: model calculations

Initial model calculations were performed at following conditions:

- Complete AChE inhibition without premature aging,
- Use of fixed oxime concentrations at a clinical relevant level of established oximes, i.e. 10 and 100 μM[3,26], and

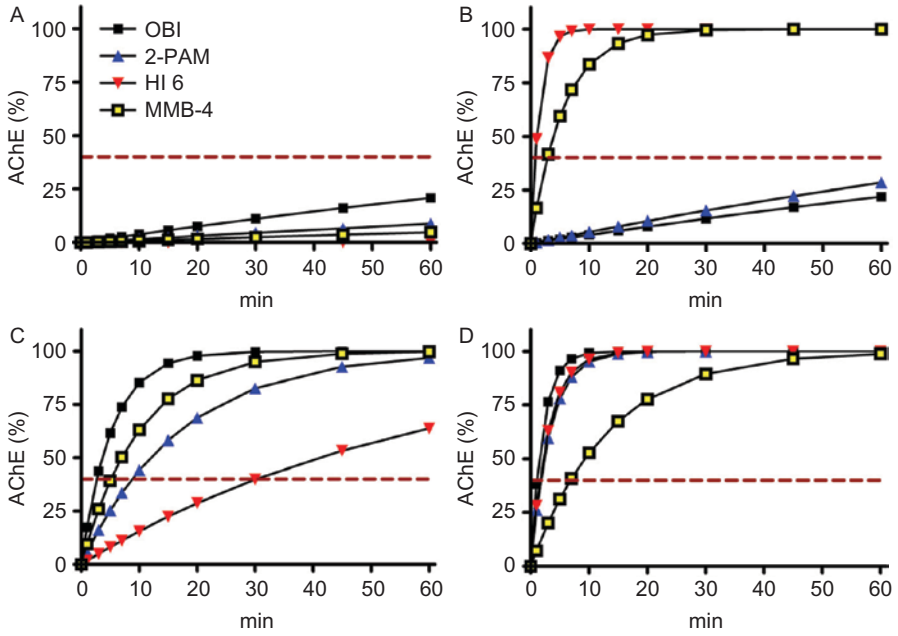

Figure 3. Calculated acetylcholinesterase (AChE) activities of tabun- (A), cyclosarin- (B), paraoxon-ethyl- (C), and methamidophos-inhibited AChE (D) after reactivation by obidoxime (OBI, 10 μM), 2-PAM (100 μM), HI-6 (50 μM), and MMB-4 (100 μM). Based on experimental reactivation rate constants[16], the AChE activities were calculated using the equation $AChE_{t\%} = 100 \times (1\text{-}exp^{-kobs \times t})$.

- Use of four different affinities and reactivities of theoretical oximes in the limits of experimentally determined numbers (Table 1)[16].

One important determinant for the efficacy of oximes is the reactivation velocity (half-time $t_{1/2}$) which is determined by the reactivity (k_r) and affinity ($1/K_D$) as well as by the concentration of an oxime (Equation 1)[2].

$$t_{1/2} = \ln 2 \times \left(\frac{K_D + [OX]}{k_r \times [OX]} \right) \quad (1)$$

Figure 4 shows the reactivation half-time in relation to the reactivity rate constants at different K_D values. Hereby, a $t_{1/2}$ of 5 min was considered as upper limit since this was shown to be a reasonable value in OP pesticide poisoned patients[36]. This example demonstrates that a certain level of oxime reactivity and affinity is necessary to achieve the desired reactivation half-time. At clinically relevant oxime concentrations, i.e. between 10 and 100 µM, k_r of 0.1 min^{-1} at a K_D ~ 10 µM would be the lower limit. Consequently, markedly higher k_r values would be necessary at lower affinities of the oxime. Figure 4B shows additionally the corresponding reactivation half-times of obidoxime with tabun-inhibited AChE and of obidoxime, pralidoxime, and HI-6 with cyclosarin-inhibited AChE at an oxime concentration of 100 µM. This example demonstrates the relevance of adequate reactivities and affinities of oximes.

Apart from the reactivation velocity the oxime concentration required for the regeneration of a defined part of inhibited AChE is an important factor for defining properties of oximes[2].

$$[OX] = -\frac{K_D}{1 + \frac{t * k_r}{\ln\left(\frac{v_0 - v_t}{v_0 - v_i}\right)}} \quad (2)$$

Equation 2 may be used for calculating the oxime concentration necessary to obtain a certain fraction of reactivated enzyme within a given time in the absence of excess inhibitor[17]. At clinically relevant conditions, i.e. 40% reactivation within 10 min (i.e. $2 \times t_{1/2}$), the minimal requirements for an oxime would be a k_r of 0.1 min^{-1} and a K_D of 100 µM (Figure 5). Lower oxime reactivity would result in an enormous rise of the required oxime concentration far above the therapeutic range while higher K_D values could be compensated by a substantial increase in reactivity. At the anticipated conditions (40% reactivation within 10 min) and a $k_r < 0.052$ min^{-1} the denominator would become positive resulting in negative oxime concentrations. Therefore, Figure 5 shows only calculations with k_r values of 0.1, 1, and 4 min^{-1}. Again, the results of the model calculations are supported by

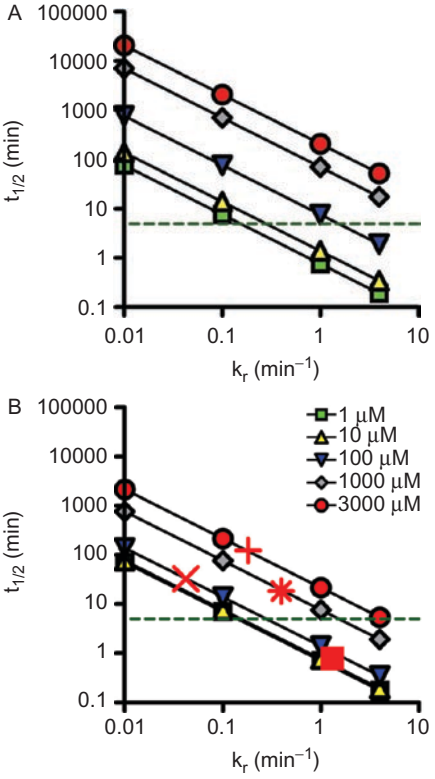

Figure 4. Double-logarithmic plot showing the relationship between reactivation half-time ($t_{1/2}$, min) and oxime reactivity (k_r in min^{-1}). The $t_{1/2}$ was calculated using equation (1) assuming five different dissociation constants (K_D), i.e. 1 µM, 10 µM, 100 µM, 1000 µM, and 3000 µM for two different oxime concentrations, i.e. 10 µM (A) and 100 µM (B). The dashed line indicates a reactivation $t_{1/2}$ of 5 min. In addition, reactivation half-times of selected oximes and organophosphorus compounds were included using measured reactivation rate constants (cf. Table 2): Tabun and obidoxime (x), cyclosarin and pralidoxime (+), obidoxime (*), and HI-6 (■).

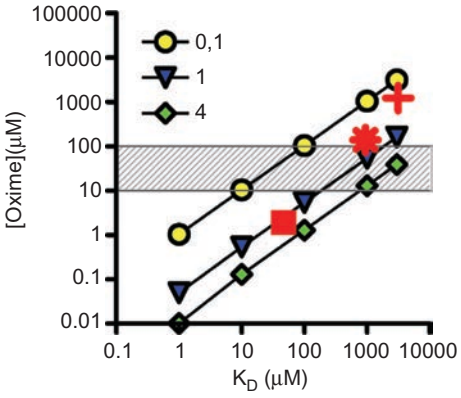

Figure 5. Double-logarithmic plot showing the relationship between oxime concentration (µM) and dissociation constant (K_D in µM). Oxime concentrations necessary to obtain 40% reactivation of inhibited acetylcholinesterase within 10 min were calculated according to[17] for three different oxime reactivity constants (k_r) of 0.1, 1 and 4 min^{-1}. The hatched area resembles the range of clinically used oxime concentrations. In addition, necessary oxime concentrations of selected oximes and organophosphorus compounds were included using measured reactivation rate constants (cf. Table 2): Cyclosarin and pralidoxime (+), obidoxime (*), and HI-6 (■).

calculating the necessary oxime concentrations for obidoxime, pralidoxime, and HI-6. It becomes evident that for the adequate reactivation of cyclosarin-inhibited AChE by pralidoxime extraordinary high and toxic oxime concentrations would be required to achieve the desired reactivation. On the other hand, the high reactivity and affinity of HI-6 would result in adequate reactivation with very low oxime concentrations.

Affinity of oximes towards native AChE and therapeutic concentration

Oximes inhibit reversibly AChE[7] and the dissociation constant K_i of the oxime from the substrate-free human AChE was determined to be in the range of 150–500 μM for obidoxime, pralidoxime, HI-6, TMB-4, and MMB-4[2,37–40]. The consequence of the intrinsic inhibitory potency of oximes, which may contribute to the observed oxime toxicity at high doses[41,42], is a limitation of the therapeutic oxime dose and the resulting *in vivo* oxime concentration. Consequently, recommended therapeutic obidoxime and pralidoxime concentrations are in the range of 10–20 and 100 μM, respectively[3,36].

The relationship between the affinity towards native and OP-inhibited human AChE was investigated by Pang and co-workers with a homologous series of alkylene-linked *bis*-pyridinium aldoximes, i.e. Ortho-3 to Ortho-9[43]. It could be shown that the increase of affinity of the oximes towards diethyl-phosphoryl-inhibited AChE, i.e. K_D from 21 (Ortho-3) to 0.6 μM (Ortho-9), was accompanied by an increasing inhibitory potency of the compounds, i.e. K_i from 65 (Ortho-3) to 0.3 μM (Ortho-9). Consequently, the LD_{50} of Ortho-7 (K_i 1.3 μM) was less than 5 μmol/kg in rats[44] while the LD_{50} of pralidoxime in rats is approximately 1 mmol/kg[42].

These data demonstrate that a simultaneous increase of oxime affinity towards native and OP-inhibited AChE in the absence of a substantial increase in reactivity does not necessarily result in an improved therapeutic benefit due to the reduced side effect-free oxime concentration. Desirable would be a selective affinity of an oxime towards OP-inhibited AChE, a requirement that seems difficult to be accomplished in view of the huge number of structurally different OP.

Conclusions

The theoretical considerations based on the kinetic properties of oximes indicate that oximes presently used clinically (obidoxime, pralidoxime) or under development for human use (HI-6, MMB-4) should be adequate reactivators for a larger number of OP pesticides and nerve agents. Established and experimental oximes are inadequate against particular OP, e.g. tabun, fenamiphos, and methylfluorophosphonylcholines, due to low affinity and reactivity. Future developments of more effective oximes should consider kinetic demands by attempting to achieve an at least moderate reactivity and affinity, preferentially towards OP-inhibited AChE. A minimal k_r of 0.1 min^{-1} and a K_D lower than 100 μM would be the minimal requirements for a rapid and sufficient reactivation of inhibited AChE.

The availability of effective reactivators does not necessarily imply that such oximes are effective antidotes *in vivo*. Presence of high OP concentrations, e.g. in intentional self-poisoning by pesticides, may lead to re-inhibition of reactivated AChE thus impairing net reactivation[45]. In addition, premature and ongoing aging of OP-inhibited AChE affects oxime effectiveness[46]. Finally, the *in vivo* efficacy of oximes is significantly determined by a proper dosing and an adequate duration of oxime administration[26].

Declaration of interest

The authors declare that they do not have any conflicts of interest

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