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Reactivation kinetics of a series of related bispyridinium oximes with organophosphate-inhibited human acetylcholinesterase—Structure–activity relationships

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This article is devoted to Prof. Dr. Peter Eyer on the occasion of his 70th birthday.

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ABSTRACT

Despite extensive research in the last six decades, oximes are the only available drugs which enable a causal treatment of poisoning by organophosphorus compounds (OP). However, numerous in vitro and in vivo studies demonstrated a limited ability of these oximes to reactivate acetylcholinesterase (AChE) inhibited by different OP pesticides and nerve agents. New oximes were mostly tested for their therapeutic efficacy by using different animal models and for their reactivating potency with AChE from different species. Due to the use of different experimental protocols a comparison of data from the various studies is hardly possible. Now, we found it tempting to determine the reactivation kinetics of a series of bispyridinium oximes bearing one or two oxime groups at different positions and having an oxybismethylene or a trimethylene linker under identical conditions with human AChE inhibited by structurally different OP. The data indicate that the position of the oxime group(s) is decisive for the reactivating potency and that different positions of the oxime groups are important for different OP inhibitors while the nature of the linker, oxybismethylene or trimethylene, is obviously of minor importance. Hence, these and previous data emphasize the necessity for thorough kinetic investigations of OP-oxime-AChE interactions and underline the difficulty to develop a broad spectrum oxime reactivator which is efficient against structurally different OP inhibitors.

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1. Introduction

Despite extensive research in the last six decades, oximes are the only available drugs which enable a causal treatment of poisoning by organophosphorus compounds (OP) [1]. The primary mechanism of action of oximes is the reactivation of OP-inhibited acetylcholinesterase (AChE), the main target of OP inhibitors [2–4]. At present, only few oximes, i.e. obidoxime, pralidoxime and TMB-4, are used clinically. However, numerous in vitro and in vivo studies demonstrated a limited ability of these oximes to reactivate AChE inhibited by different OP pesticides and nerve agents [1,5,6].

This situation led to the search for more effective oximes in the past decades and up to now a countless number of oximes has been synthetized [7]. A major contribution to this effort was made by Prof. I. Hagedorn from the University of Freiburg, Germany, who synthetized more than 1000 oximes including some of the most effective reactivators, e.g. obidoxime, HI-6 and HLö 7 [8].

New oximes were mostly tested for their therapeutic efficacy by using different animal models and for their reactivating potency with AChE from different species [7,9,10]. In view of the use of different experimental protocols it is hardly possible to compare data of the various studies in order to quantify the ability of oximes to reactivate inhibited AChE.

Evaluation of the reactivating potency of oximes requires the determination of the reactivation kinetics including the dissociation and reactivity constants [11]. Hereby, the determination of meaningful kinetic constants is a major challenge and requires the use of appropriate experimental procedures. Recently, we established a protocol which allows the investigation of oximes with largely different affinities and reactivities [12]. Now, we found it tempting to determine the reactivation kinetics of a series of bispyridinium oximes with human AChE inhibited by structurally different OP, i.e. the organophosphate paraoxon, the organophosphonate cyclosarin and the phosphoramidate tabun (Fig. 1) at identical experimental conditions. For this attempt oximes bearing one or two oxime functions at different positions and having an oxybismethylene or a trimethylene linker were selected (Table 1), namely the Hagedorn oximes obidoxime, HI-6, HS 3 and HS 4 [13–15], TMB-4 [16], ICD585 [17] and K005 [18] which was first described by [19]. With these data at hand it should be possible to

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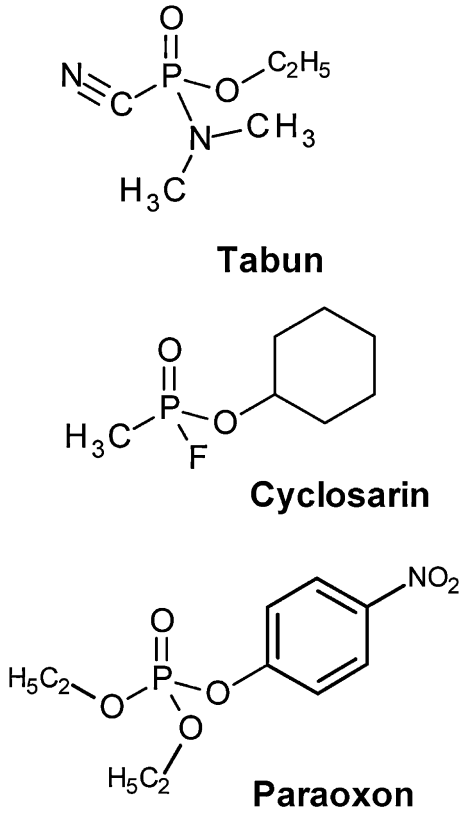


Fig. 1. Chemical structure of OP inhibitors used in this study.

get more insight into structural requirements for reactivation and to provide a database for investigating structure–activity relationships.

2. Materials and methods

2.1. Materials

Paraoxon-ethyl (paraoxon, Fig. 1) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), tabun and cyclosarin (>98% by GC–MS, ^{1}H NMR and ^{31}P NMR, Fig. 1) were made available by the German Ministry of Defence. The tested oximes (>95% by ^{1}H NMR; Table 1) were from different sources: obidoxime dichloride was purchased from Merck (Darmstadt, Germany) and TMB-4 from Sigma–Aldrich (Taufkirchen, Germany), HI-6 dichloride monohydrate was provided by Dr. Clement (Defence Research Establishment Suffield, Ralston, Alberta, Canada), K005 dibromide by Dr. Kuca (Faculty of Military Health Sciences, Hradec Kralove, Czech Republic), ICD585 by Prof. Taylor (University of California, San Diego, USA) and HS 3 and HS 4 were made available by Prof. Eyer (University of Munich, Munich, Germany). 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide (ATCh) were supplied by Sigma–Aldrich. All other chemicals were from Merck (Darmstadt, Germany).

Tabun (6.2 mM) and cyclosarin stock solutions (5.5 mM) were prepared in acetonitrile and paraoxon stock solutions (10 mM) in 2-propanol and were stored at 20 °C and −80 °C, respectively. Oxime stock solutions (200 mM) were prepared in distilled water and were stored at −80 °C. Working solutions were appropriately diluted in distilled water just before the experiment and were kept on ice until use.

Hemoglobin-free human erythrocyte ghosts were prepared as described from heparinized human blood and served as AChE source [20]. Aliquots of the erythrocyte ghosts with an AChE activity adjusted to that found in whole blood were stored at −80 °C and aliquots were homogenized prior to use to achieve a homogeneous matrix for the kinetic studies.

2.2. AChE assay

AChE activities were measured with a modified Ellman assay [21] at 412 nm (Cary 50, Varian, Darmstadt) using polystyrol cuvettes, 0.45 mM ATCh as substrate and 0.3 mM DTNB as a chromogen in 0.1 M phosphate buffer (pH 7.4).

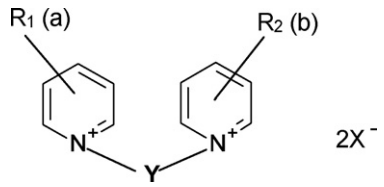
All experiments were performed at 37 °C and pH 7.4. All concentrations refer to final concentrations.

2.3. Reactivation of OP-inhibited human AChE by oximes

Human erythrocyte ghosts were incubated for 15 min at 37 °C with a small volume (≤1%, v/v) of paraoxon (100 nM), tabun (100 nM) or cyclosarin (20 nM final concentration) to achieve an AChE inhibition of >95%. Then, the treated ghosts were dialyzed (phosphate buffer, 0.1 M, pH 7.4) overnight at 4 °C to remove residual inhibitor followed by incubation of treated and control ghost aliquots (30 min, 37 °C) to verify the absence of inhibitory activity. Aliquots were stored at −80 °C until use.

150 μl inhibited AChE were mixed with 150 μl phosphate buffer containing 0.2% gelatin in order to stabilize AChE activity during prolonged incubation at 37 °C. At $t = 0$ 5 μl oxime was added to initiate reactivation. After specified time intervals (2–60 min) 20 μl aliquots were transferred to tempered cuvettes

Table 1
Chemical structure and acidity of tested oximes.



Code	a	R_1	Y	b	R_2	X	pK_{a1}	pK_{a2}	Refs.
TMB-4	4	CHNOH	$(CH_2)_3$	4	CHNOH	Br	7.78	8.61	[a]
Obidoxime	4	CHNOH	CH_2OCH_2	4	CHNOH	Cl	7.54	8.12	[b]
HS 3	2	CHNOH	CH_2OCH_2	4	CHNOH	Cl	7.23	8.24	[c]
HS 4	2	CHNOH	CH_2OCH_2	2	CHNOH	Cl	7.04	7.95	[d]
K005	2	CHNOH	$(CH_2)_3$	2	CHNOH	Br	7.13	8.55	[e]
HI-6	2	CHNOH	CH_2OCH_2	4	$CONH_2$	Cl	7.28	–	[f]
ICD585	2	CHNOH	$(CH_2)_3$	4	$CONH_2$	Cl	7.7	–	[g]

pK_a values were taken from: [a] [36]; [b,f] [31]; [c] [37]; [d] [27]; [e] [18]; [g] unpublished data.

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