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Kinetic analysis of interactions of different sarin and tabun analogues with human acetylcholinesterase and oximes: Is there a structure–activity relationship?

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ABSTRACT

The repeated misuse of highly toxic organophosphorus compound (OP) based chemical warfare agents in military conflicts and terrorist attacks poses a continuous threat to the military and civilian sector. The toxic symptomatology of OP poisoning is mainly caused by inhibition of acetylcholinesterase (AChE, E.C. 3.1.1.7) resulting in generalized cholinergic crisis due to accumulation of the neurotransmitter acetylcholine (ACh) in synaptic clefts. Beside atropine as competitive antagonist of ACh at muscarinic ACh receptors oximes as reactivators of OP-inhibited AChE are a mainstay of standard antidotal treatment. However, human AChE inhibited by certain OP is rather resistant to oxime-induced reactivation. The development of more effective oxime-based reactivators may fill the gaps. To get more insight into a potential structure–activity relationship between human AChE, OPs and oximes in vitro studies were conducted to investigate interactions of different tabun and sarin analogues with human AChE and the oximes obidoxime and HI 6 by determination of various kinetic constants. Rate constants for the inhibition of human AChE by OPs, spontaneous dealkylation and reactivation as well as reactivation by obidoxime and HI 6 of OP-inhibited human AChE were determined. The recorded kinetic data did not allow a general statement concerning a structure–activity relationship between human AChE, OP and oximes.

1. Introduction

The high number of fatalities due to poisoning by organophosphorus compound-based (OP) pesticides and the continuing threat of misuse of highly toxic OP-type chemical warfare agents (nerve agents) emphasize the necessity for an effective medical treatment. The toxic symptomatology of OP poisoning is mainly caused by inhibition of AChE by phosphylation of its active site serine [1]. The failure of inhibited AChE to hydrolyze the neurotransmitter acetylcholine results in an endogenous acetylcholine intoxication followed by an over-stimulation of muscarinic and nicotinic ACh receptors in the autonomic, peripheral and central nervous system. Consequences are massive disturbances of numerous body functions finally resulting in death due to central and peripheral respiratory arrest.

Presently, standard treatment of OP poisoning includes the administration of a muscarinic antagonist, e.g. atropine, and of a reactivator of inhibited AChE (oxime) [2]. Hereby, anti-muscarinic drugs act only symptomatically while oximes may restore the enzyme function. At present, the oximes obidoxime, pralidoxime and HI 6 are introduced in different countries as antidotes against nerve agent poisoning. However, human AChE inhibited by certain OP, e.g. soman and tabun, is rather resistant towards reactivation by oximes while AChE inhibited by structurally different OP, e.g. sarin and paraoxon, can be easily reactivated [3,4]. The development of more effective oximes may fill the gaps of the actually used therapeutic countermeasures in OP poisoning. A growing number of studies have been performed to give a structural basis for the development of improved oximes [5–9]. In order to get more insight into potential structural requirements for the reactivatability of OP-inhibited AChE by oximes in vitro studies were conducted to investigate interactions of different sarin (Fig. 1) and tabun (Fig. 2) homologues with human AChE and obidoxime as well as HI 6 by determination of various kinetic constants.

2. Materials and methods

2.1. Materials

Acetylthiocholine iodide (ATCh) and 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) were obtained from Sigma. Obidoxime dichloride (obidoxime) was purchased from Merck (Darmstadt, Germany), HLö 7 dimethanesulfonate was a custom synthesis by Dr. Braxmeier (Chemisches Labor, Döpshofen, Germany) and HI 6

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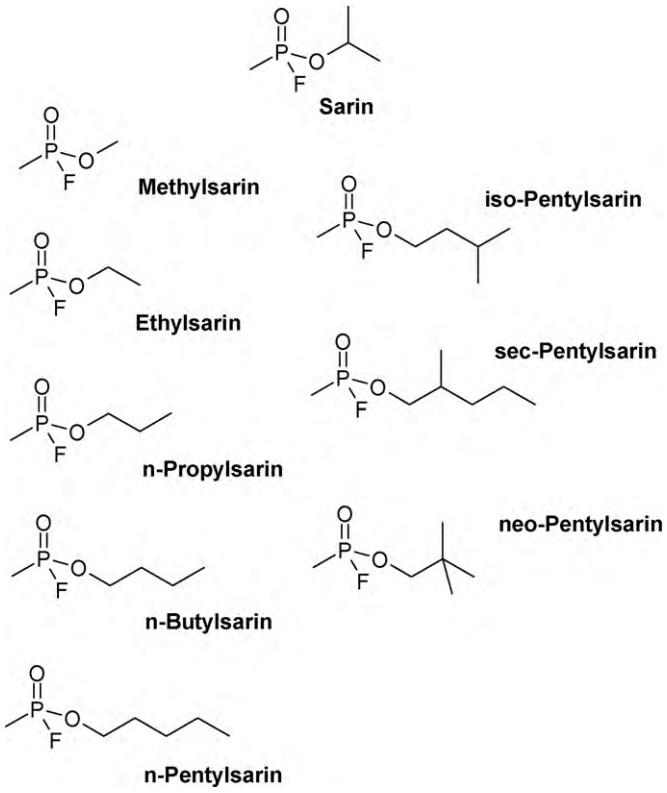

Fig. 1. Structure of sarin homologues.

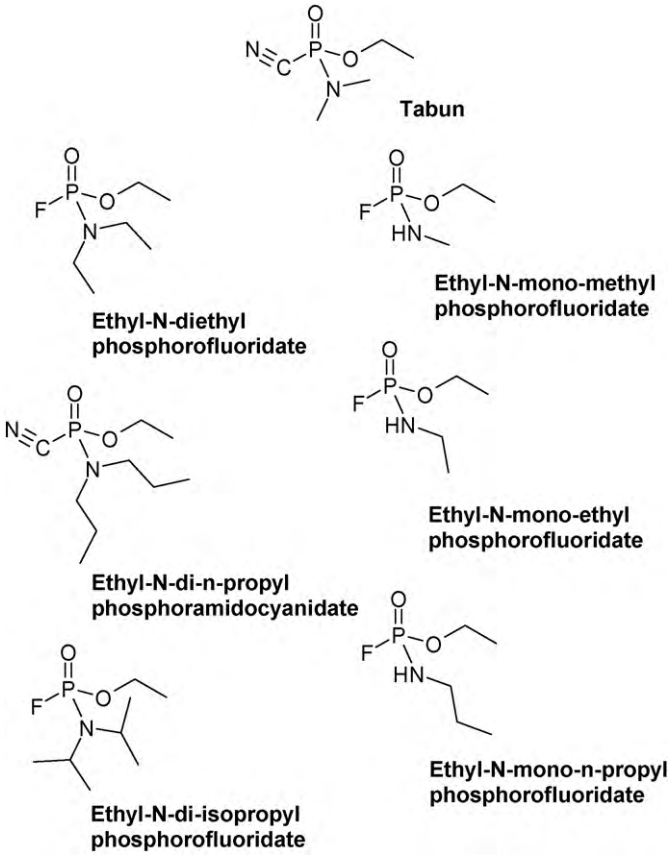

Fig. 2. Structure of tabun homologues.

dichloride was kindly provided by Dr. Clement (Defence Research Establishment Suffield, Ralston, Alberta, Canada).

Methylsarin, ethylsarin, n-propylsarin, n-butylsarin, n-pentylsarin, iso-pentylsarin, sec-pentylsarin and neo-pentylsarin as well as tabun and its analogues (>98% by GC–MS, ^{1}H NMR and ^{31}P NMR, Figs. 1 and 2) were made available by the Ministry of Defence (Bonn, Germany). All other chemicals were purchased from Merck Eurolab (Darmstadt, Germany). OP stock solutions (0.1%, v/v) were prepared weekly in 2-propanol, stored at 4 °C, and were appropriately diluted in distilled water just before use. Oximes (200 mM) were prepared in distilled water, stored at −80 °C and diluted daily as required in distilled water at the day of experiment.

Hemoglobin-free erythrocyte ghosts, serving as AChE source, were prepared as described before [10]. Aliquots of the erythrocyte ghosts were stored at −80 °C until use. Just before the experiment thawed ghosts were homogenised on ice with a Sonoplus HD 2070 ultrasonic homogenator (Bandelin Electronic, Berlin, Germany), three-times for 5 s with 30 s intervals, to achieve a homogeneous matrix for the kinetic studies.

In order to prevent AChE denaturation during long-term experiments at 37 °C AChE was stabilized by addition of human plasma that was incubated with soman (100 nM) for 30 min at 37 °C to ensure complete inhibition and aging of BChE [11]. The inhibited plasma was dialyzed (phosphate buffer, 0.1 M, pH 7.4) overnight at 4 °C to adjust pH and to remove residual inhibitor.

2.2. Enzyme assays

AChE activities were measured spectrophotometrically (Cary 3Bio, Varian, Darmstadt) with a modified Ellman assay [12,13]. The assay mixture (3.15 ml) contained 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. Inhibition of human AChE

Human erythrocyte ghosts were incubated with a small volume (1%, v/v) of appropriate OP concentrations for 15 min (sarin and analogues) and 30 min (tabun and analogues) at 37 °C to achieve an AChE inhibition by >80%. The samples treated with tabun and analogues were dialyzed (phosphate buffer, 0.1 M, pH 7.4) overnight at 4 °C to remove residual inhibitor. Then, the absence of inhibitory activity was tested by incubation of treated and control ghosts (30 min, 37 °C).

2.4. Determination of inhibition rate constants (k_i) of sarin and analogues

The second-order inhibition rate constants (k_i) of sarin and its analogues were determined with human AChE in the presence of substrate [14]. In brief, 10 μl erythrocyte ghosts and 5 μl diluted OP were added to a cuvette containing phosphate buffer, DTNB and ATCh (final volume 3.165 ml), the resultant OP concentrations were 2–75 nM. ATCh hydrolysis was continuously monitored for up to 30 min. The recorded curves were analyzed by non-linear regression analysis and used for the further determination of $k_i = k_2/K_D$ [14,15].

2.5. Determination of rate constants for aging (k_a) and spontaneous reactivation (k_s)

OP-treated human ghosts were mixed with equal volumes of soman-treated, dialyzed human plasma to prevent denaturation of AChE during long-term experiments at 37 °C [3]. Samples of