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Comparison of inhibition kinetics of several organophosphates, including some nerve agent surrogates, using human erythrocyte and rat and mouse brain acetylcholinesterase

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HIGHLIGHTS

- Inhibition kinetic assays were conducted with several highly relevant surrogates for sarin, VX and cyclosarin and insecticidal organophosphates in rat brain, mouse brain and purified human erythrocyte acetylcholinesterase.
- With the exception of chlorpyrifos-oxon, the nerve agent surrogates were more potent inhibitors than the other organophosphates.
- The potencies of the nerve agent surrogates were proportional to the toxicities of the nerve agents, cyclosarin > VX > sarin.

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ABSTRACT

Because testing of nerve agents is limited to only authorized facilities, our laboratory developed several surrogates that resemble nerve agents because they phosphylate the acetylcholinesterase (AChE) with the same moiety as the actual nerve agents. The inhibition kinetic parameters were determined for AChE by surrogates of cyclosarin (NCMP), sarin (NIMP, PIMP and TIMP) and VX (NEMP and TEMP) and other organophosphorus compounds derived from insecticides. All compounds were tested with rat brain and a subset was tested with mouse brain and purified human erythrocyte AChE. Within the compounds tested on all AChE sources, chlorpyrifos-oxon had the highest molecular rate constant followed by NCMP and NEMP. This was followed by NIMP then paraoxon and DFP with rat and mouse brain AChE but DFP was a more potent inhibitor than NIMP and paraoxon with human AChE. With the additional compounds tested only in rat brain, TEMP was slightly less potent than NEMP but more potent than PIMP which was more potent than NIMP. Methyl paraoxon was slightly less potent than paraoxon but more potent than TIMP which was more potent than DFP. Overall, this study validates that the pattern of inhibitory potencies of our surrogates is comparable to the pattern of inhibitory potencies of actual nerve agents (*i.e.*, cyclosarin > VX > sarin), and that these are more potent than insecticidal organophosphates.

1. Introduction

Following the introduction of organophosphates (OPs) as insecticides in the early 1940s, several OP compounds were developed into chemical weaponry and ultimately designated as nerve agents due to their neurotoxic mechanism of action (Johnson et al., 2009; Tucker, 2006). The use of nerve agents against civilian populations has been reported in several incidences including against the Kurdish minorities in Halabja, Iraq in 1988 and in the Tokyo subway attack by Aum Shinrikyo in 1995 (Gupta, 2009).

Abbreviations: AChE, acetylcholinesterase; ATCh, acetylthiocholine iodide; DFP, diisopropyl fluorophosphate; DTNB, 5,5′-dithio-bisnitrobenzoic acid; MOPS, 3-[N-morpholino] propane sulfonic acid; NCMP, nitrophenyl cyclohexyl methylphosphonate; NEMP, nitrophenyl ethyl methylphosphonate; NIMP, nitrophenyl isopropyl methylphosphonate; PIMP, phthalimidyl isopropyl methylphosphonate; TEMP, 3,5,6-trichloro-2-pyridinyl ethyl methylphosphonate; TIMP, 3,5,6-trichloro-2-pyridinyl isopropyl methylphosphonate; TRIS, tris[hydroxymethyl]-amino-methane.

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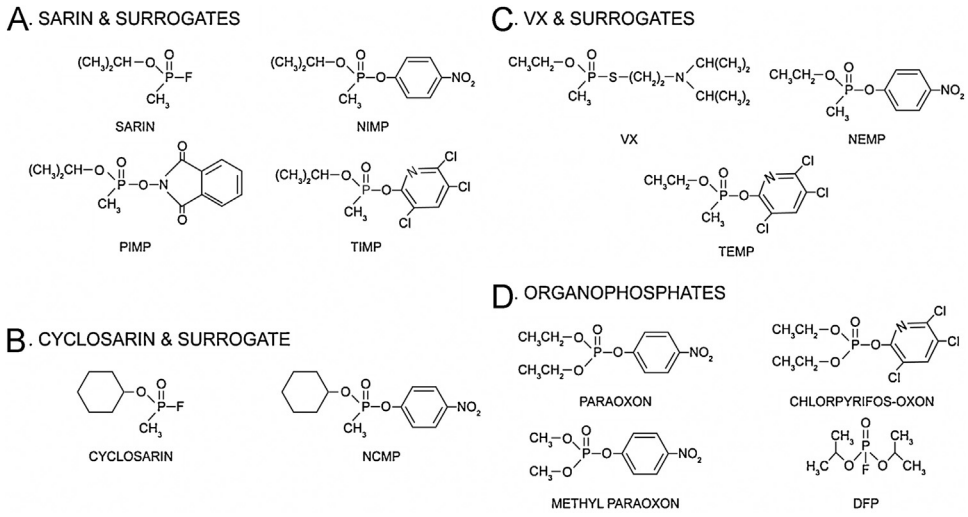

Fig. 1. Structures of (A) sarin and its surrogates NIMP (nitrophenyl isopropyl methylphosphonate), TIMP (3,5,6-trichloro-2-pyridinyl isopropyl methylphosphonate) and PIMP (phthalimidyl isopropyl methylphosphonate); (B) cyclosarin and its surrogate NCMP (nitrophenyl cyclohexyl methylphosphonate); C) VX and its surrogates NEMP (nitrophenyl ethyl methylphosphonate) and TEMP (3,5,6-trichloro-2-pyridinyl ethyl methylphosphonate); and; (D) Organophosphates: paraoxon, chlorpyrifos-oxon, methyl paraoxon, and DFP (diisopropylfluorophosphate).

More recently, suspected use of nerve agents against Syrian civilians in 2013 has been reported (Pita and Domingo, 2014; Eisenkraft et al., 2014). There is currently a concern with the threat of terrorist organizations utilizing nerve agents, and possibly other OPs, as weapons in their attacks.

OPs exert toxicity by inhibiting the enzyme acetylcholinesterase (AChE; EC 3.1.1.7), a serine hydrolase that converts the neurotransmitter acetylcholine (ACh) into choline and acetate. Inhibition of AChE causes ACh to accumulate in synaptic and neuromuscular junctions resulting in hyperactivation within the cholinergic nervous system (Ecobichon, 2001). Spasm of respiratory muscle, bronchoconstriction, increased secretion of mucus and shutdown of the brain's respiratory control center lead to death in mammals from respiratory failure in lethal dose exposures to OP anticholinesterases.

OP nerve agents are highly toxic and research on the actual nerve agents has been limited to only authorized facilities by the Chemical Weapons Convention. It would be beneficial to have chemicals that behave similarly to the nerve agents but are less toxic in order to allow laboratories that are not authorized to use the actual nerve agents to conduct research relevant to nerve agents. Therefore our laboratories developed surrogates for sarin and VX (Meek et al., 2012) and more recently to cyclosarin which substitute the nerve agent leaving group with another moiety (*i.e.,* 4-nitrophenyl, 3,5,6-trichloro-2-pyridinyl, or phthalimidyl) (Fig. 1). These surrogates are highly relevant for AChE inhibition studies because they phosphylate the serine residue of the AChE active site with the same moiety as the actual nerve agents.

Other OPs are not directly toxic as nerve agents. The phosphorothioate insecticides (e.g., parathion) must undergo bioactivation via cytochrome P450-mediated desulfuration to create their corresponding oxon metabolites (e.g., paraoxon) to be toxic. These oxons are substantially more potent anticholinesterases than the parent insecticide (Hodgson et al., 1991). These chemicals are also considered a potential terrorist tool and could be utilized to poison civilian populations (Ballantyn, 2009). For this reason, the oxons of three OP insecticides (parathion, methyl parathion and chlorpyrifos) were included in this study.

The present study was designed to assess the anticholinesterase potency through steady state kinetics investigating several OP molecules, including seven nerve agent surrogates and three insecticidal oxons. In addition to the surrogates synthesized in our laboratories, diisopropylfluorophosphate (DFP), which is commonly used as a surrogate of nerve agents by a number of investigators, was also studied. Three different enzyme sources were investigated: preparations of rat and mouse whole brain, and commercially available purified AChE isolated from human erythrocytes. The need to perform basic research on nerve agent effects as well as research to identify potential therapies for nerve agents must necessarily involve the use of laboratory animals. Thus information on the similarities of inhibition of AChE of two common laboratory rodents to that of humans will be valuable in extrapolating the animal data to humans.

1.1. Materials and methods

1.1.1. Chemicals

AChE from human erythrocytes, DFP and all reagent grade chemicals were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO). The insecticidal oxons paraoxon and methyl paraoxon were synthesized as described previously (Chambers and Chambers, 1989) from commercially available intermediates from Sigma-Aldrich Chemical Co. and chlorpyrifos-oxon was synthesized by similar methods from 3,5,6-trichloro-2-pyridinol generously donated by Dow Chemical Co. (Midland, MI). The nerve agent surrogates were synthesized as described below.

1.1.2. Synthesis of nerve agent surrogates

1.1.2.1. Nitrophenyl ester homologs. The synthesis reaction was as described by Meek et al. (2012). Briefly, triethylamine in benzene was slowly added to a mixture of methylphosphonic dichloride and 4-nitrophenol in benzene (80 ml) at room temperature. Then triethylamine in benzene and 2-propanol, ethanol, or cyclohexanol for NIMP (sarin surrogate), NEMP (VX surrogate), and NCMP (cyclosarin surrogate), respectively, were added slowly (Fig. 2A).