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A collaborative endeavor to design cholinesterase-based catalytic scavengers against toxic organophosphorus esters☆

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ABSTRACT

Wild-type human butyrylcholinesterase (BuChE) has proven to be an efficient bioscavenger for protection against nerve agent toxicity. Human acetylcholinesterase (AChE) has a similar potential. A limitation to their usefulness is that both cholinesterases (ChEs) react stoichiometrically with organophosphosphorus (OP) esters. Because OPs can be regarded as pseudo-substrates for which the dephosphylation rate constant is almost zero, several strategies have been attempted to promote the dephosphylation reaction. Oxime-mediated reactivation of phosphylated ChEs generates a turnover, but it is too slow to make pseudo-catalytic scavengers of pharmacological interest. Alternatively, it was hypothesized that ChEs could be converted into OP hydrolases by using rational site-directed mutagenesis based upon the crystal structure of ChEs. The idea was to introduce a nucleophile into the oxyanion hole, at an appropriate position to promote hydrolysis of the phospho-serine bond via a base catalysis mechanism. Such mutants, if they showed the desired catalytic and pharmacokinetic properties, could be used as catalytic scavengers.

The first mutant of human BuChE that was capable of hydrolyzing OPs was G117H. It had a slow rate. Crystallographic study of the G117H mutant showed that hydrolysis likely occurs by activation of a water molecule rather than direct nucleophilic attack by H117. Numerous BuChE mutants were made later, but none of them was better than the G117H mutant at hydrolyzing OPs, with the exception of soman. Soman aged too rapidly to be hydrolyzed by G117H. Hydrolysis was however accomplished with the double mutant G117H/E197Q, which did not age after phosphonylation with soman.

Multiple mutations in the active center of human and *Bungarus* AChE led to enzymes displaying low catalytic activity towards OPs and unwanted kinetic complexities. A new generation of human AChE mutants has been designed with the assistance of molecular modelling and computational methods. According to the putative water-activation mechanism of G117H BChE, a new histidine/aspartate dyad was introduced into the active center of human AChE at the optimum location for hydrolysis of the OP adduct. Additional mutations were made for optimizing activity of the new dyad. It is anticipated that these new mutants will have OP hydrolase activity.

Abbreviations: AChE, acetylcholinesterase; BuChE, butyrylcholinesterase; CaE, carboxylesterase; ChE, cholinesterase; DFP, diisopropylfluorophosphate; DFPase, diisopropylfluorophosphate hydrolase; OP, organophosphorus compound; OPAH, organophosphorus acid anhydride hydrolase; PON1, paraoxonase.

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1. Introduction

Organophosphorus (OP) compounds are among the most toxic compounds synthesized. They have been used as pesticides and drugs for several decades. Certain of them are dangerous nerve agents [1,2]. For example, based on animal data the calculated lethal dose of VX, the most toxic nerve agent, is 0.3 mg (inhalation route) and 5 mg (percutaneous absorption) in humans. After rapid absorption mostly through skin and/or lungs, OPs distribute from the blood compartment into biological targets, tissue depot sites and sites of elimination. OPs are irreversible inhibitors of acetylcholinesterase (EC. 3.1.1.7; AChE) and butyrylcholinesterase (EC. 3.1.1.8; BuChE). Inhibition of AChE is responsible for acute toxicity [3,4]. However, OPs interact with numerous biological systems, and reactions with secondary targets are responsible for non-cholinergic and/or delayed toxicity [5].

After some 60 years of research, medical countermeasures against OP poisoning are reaching their optimum [6–8]. Yet they are still imperfect. They prevent lethality, but not incapacitation and irreversible brain damage. It is unlikely that more potent and universal drugs and antidotes devoid of side effects will be discovered in the near future. Therefore, bioscavengers represent an alternative approach to pharmacological pre- and post-exposure treatments. Scavengers can be antibodies, chemicals, e.g. functionalized cyclodextrins, and enzymes that sequester and inactivate highly toxic compounds before they reach their biological targets.

The bioscavenger concept for challenging OPs originated with the observation by Main [9] that "A esterase" (an old name for PON 1), injected i.v. into rats, "significantly reduced the i.v. toxicity of paraoxon". Because of concern that soman intoxication was resistant to conventional treatment, a search for catalytic soman scavengers was begun that extended over the course of several years. "DFPases" (a general designation for any enzyme that would catalyse the hydrolysis of organophosphorus cholinesterase inhibitors) from a variety of sources were discovered [10–12] and some of them tested [13–16], but practical considerations dictated that human enzymes would be most suitable for use as scavengers in humans. Subsequent studies including active immunization and passive immunization with monoclonal antibodies, revealed that antibodies, by their nature, bind their substrates without changing them and thus might bind an OP and take it out of circulation temporarily but eventually will release it to exercise its toxicity [17,18]. At that point it was realized that catalytic activity was not needed; a stoichiometric scavenger could protect an animal if present in a high enough concentration. The problem was to obtain a relatively large amount of an enzyme that would react with an OP rapidly and irreversibly and would be innocuous in the circulation at artificially high concentrations. This challenge was met initially by Alan Wolfe, who isolated enough AChE from foetal bovine serum to attempt a test in mice, using VX for the challenge. Indeed, administration of foetal bovine serum AChE to mice provided protection against nerve agent poisoning. It was concluded that highly toxic OPs could be sequestered *in vivo*, thus providing a new approach to treatment of OP intoxication [19]. Numerous enzymes have been found to react with OPs [5]. OPs are either irreversible inhibitors or substrates of these enzymes. Skin and plasma BuChE, erythrocyte AChE and liver carboxylesterases are endogenous stoichiometric bioscavengers. Plasma PON1 hydrolyzes OPs, liver glutathione *S*-transferase and cytochrome P450 (CYP2P) oxidize OP side-chains. Though albumin, the most abundant plasma protein (0.6 mM), reacts with OPs, its reactivity is too slow to contribute significantly to detoxification of these compounds in acute poisoning [20]. Recent reviews on the bioscavenger approach for protection against organophosphorus exposure are available [21–24].

2. Stoichiometric scavengers versus catalytic scavengers

An effective bioscavenger detoxifies OP molecules in the blood compartment before they reach their biological targets. Four overall constants control the concentration of OP in blood (Fig. 1): the overall infusion or feeding constant of OP molecules across the skin (or other routes, e.g. lungs, stomach) toward the circulation system (k_{on}), the diffusion constant from blood toward biological targets (k_{off}), the inactivation constant of reactive enzymes present in the blood (k_{-OP}), and the clearance constant (k_c) of these enzymes. Efficient detoxification by bioscavengers requires that k_{-OP} to be as high as possible. In addition, k_{-OP} has to be much higher than k_{off}, and k_c must be very slow.

There are two types of scavenger to consider. There are stoichiometric scavengers, which react on a one-to-one basis with the OP, thereby inactivating one OP per scavenger. And, there are catalytic scavengers that hydrolyze the OP, thereby inactivating multiple OP per scavenger.

Butyrylcholinesterase (EC. 3.1.1.8; BuChE) is the most important stoichiometric scavenger in human plasma. The concentration of BuChE in blood (about 50 nM) is 20 times higher than the concentration of erythrocyte AChE

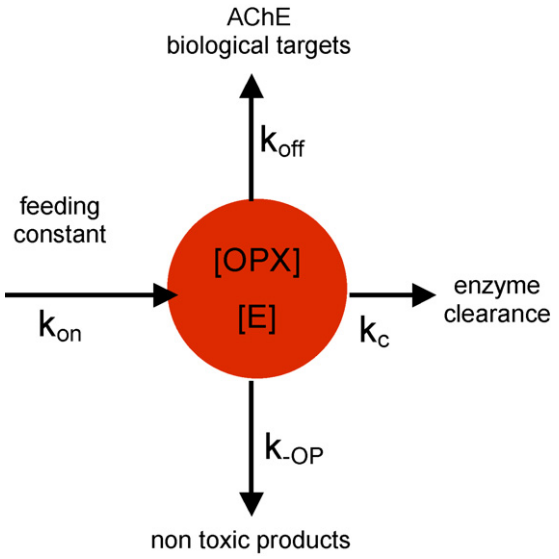

Fig. 1. Kinetic control of OP concentration ([OPX]) in human blood.