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Catalytic bioscavengers in nerve agent poisoning: A promising approach?

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HIGHLIGHTS

- Catalytic bioscavengers are considered promising for treatment of nerve agent poisoning.
- Recent in vitro and in vivo data support the bioscavenger concept.
- Catalytic bioscavengers are substrate specific and do not cover the spectrum of threat agents.
- The biological stability of catalytic bioscavengers is inadequate and needs substantial improvement.
- Further research is needed to transfer catalytic bioscavengers into advanced development and human use.

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ABSTRACT

The repeated use of the nerve agent sarin against civilians in Syria in 2013 emphasizes the continuing threat by chemical warfare agents. Multiple studies demonstrated a limited efficacy of standard atropine–oxime treatment in nerve agent poisoning and called for the development of alternative and more effective treatment strategies. A novel approach is the use of stoichiometric or catalytic bioscavengers for detoxification of nerve agents in the systemic circulation prior to distribution into target tissues. Recent progress in the design of enzyme mutants with reversed stereo selectivity resulting in improved catalytic activity and their use in in vivo studies supports the concept of catalytic bioscavengers. Yet, further research is necessary to improve the catalytic activity, substrate spectrum and in vivo biological stability of enzyme mutants. The pros and cons of catalytic bioscavengers will be discussed in detail and future requirements for the development of catalytic bioscavengers will be proposed.

1. Introduction

The recent, large-scale homicidal use of the chemical warfare nerve agent sarin (Fig. 1) in Syria drew the attention to the continuous threat by these highly toxic organophosphorus compounds (OP; Pita and Domingo, 2014; Eisenkraft et al., 2014). The high toxicity of OP nerve agents (Fig. 1) is caused by covalent binding to a serine hydroxyl group at the active site of the pivotal enzyme acetylcholinesterase (AChE; Aldridge and Reiner, 1972). Subsequent accumulation of the neurotransmitter acetylcholine and overstimulation of muscarinic and nicotinic cholinergic receptors results in the development of multiple, characteristic clinical signs and finally in death due to respiratory arrest (Sidell, 1997).

Standard therapy of nerve agent poisoning comprises the administration of atropine and an AChE reactivator (oxime) and is virtually unchanged since almost five decades (Namba and Hiraki, 1958; Eyer and Worek, 2007; Cannard, 2006). Huge research efforts were undertaken to replace the oximes pralidoxime (2-PAM) and obidoxime by more effective reactivators (Worek and Thiermann, 2013). However, despite the synthesis of several thousand compounds since the early 1950's no oxime with superior efficacy and extended spectrum to cover the broad range of nerve agents was identified so far.

Multiple in vitro and in vivo studies demonstrated that therapy of nerve agent poisoning by atropine and an oxime can at best improve survival in animals but has a very limited effect in preventing incapacitation of the victim (Sidell, 1992). This unsatisfying situation induced intensified research efforts for alternative therapeutic concepts. These include adjuncts to basic

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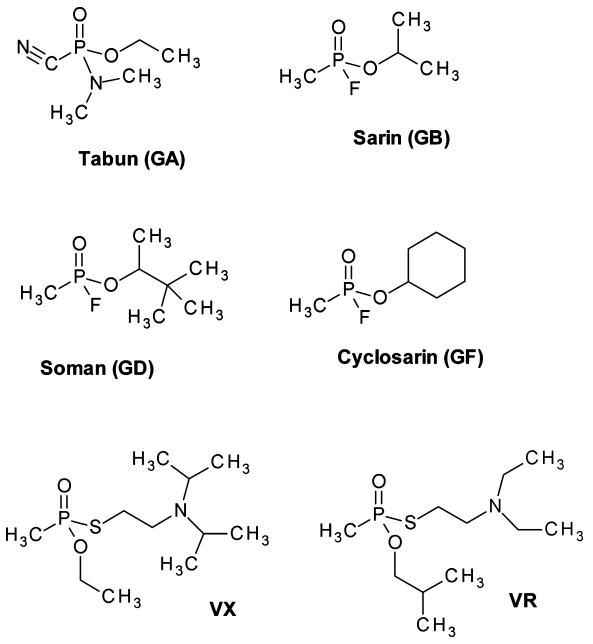

Fig. 1. Chemical structures of classical nerve agents.

atropine and oxime therapy, receptor active compounds to improve neuromuscular transmission at respiratory muscles, small molecule scavengers and enzymes which can bind or hydrolyse nerve agents (Wetherell et al., 2007; Seeger et al., 2012; Timperley et al., 2012; Turner et al., 2011; Bierwisch et al., 2014; Worek et al., 2014c; Zengerle et al., 2011; Nachon et al., 2013).

Recent in vivo studies provided clear evidence that stoichiometric, i.e., human AChE and butyrylcholinesterase (BChE), and catalytic bioscavengers can be highly effective in preventing nerve agent toxicity (Nachon et al., 2013; Masson and Rochu, 2009; Lenz et al., 2007). In view of quite recent data on the therapeutic effectiveness of phosphotriesterase (PTE) mutants this paper will discuss advantages and limitations of catalytic bioscavengers.

2. Catalytic bioscavengers

As early as 1946 Mazur realized that mammalian blood and tissue contains enzymes which hydrolyse the OP compound diisopropylfluorophosphate (DFP; Mazur, 1946). Subsequent studies verified the initial observation and characterized OP hydrolyzing enzymes (Adie, 1956; Erdös and Boggs, 1961; Zech and Zürcher, 1974; Reiner et al., 1989). Catalytic bioscavengers are usually named according to the substrate they were discovered with resulting in an inconsistent and confusing nomenclature (Prokop et al., 2006). All of the OP hydrolyzing enzymes belong to the phosphotriesterase class (PTE, EC 3.1.8) subdivided into two groups. Enzymes with a preference for P–O bond cleavage are referred to as OP hydrolases (e.g., paraoxonase and bacterial phosphotriesterase, EC 3.1.8.1) and those with a preference for P-F or P-CN bonds are termed diisopropylfluorophosphatases (e.g., DFPase; EC 3.1.8.2) In the meantime, research focused on human paraoxonase (PON1) and bacterial PTE as potential catalytic bioscavengers (Masson and Rochu, 2009), although other enzymes, e.g., prolidase, laccase and other bacterial enzymes, are under investigation as well (Amitai et al., 1998; Rezk et al., 2015; Otto et al., 2013). In fact, the prophylactic administration of OP hydrolases in different animal species provided protection against various OP compounds in different species (Ashani et al., 1991; Bird et al., 2008; Gresham et al., 2010; Valiyaveettil et al., 2011; Worek et al., 2014a; Raveh et al., 1992).

The mechanism of nerve agent detoxification is the major difference between stoichiometric and catalytic bioscavengers. Human AChE and BChE bind nerve agents irreversibly and are thus consumed while catalytic enzymes hydrolyze nerve agents by cleaving various phosphorus–ester bonds (P–F, e.g. sarin, soman, cyclosarin, P–CN, e.g., tabun, P–S, e.g., VX and P–O, e.g., paraoxon) without being inhibited by the toxic compounds. The secession of the leaving groups results in formation of virtually non-toxic nerve agent metabolites.

Wild-type PON1 and bacterial PTE exhibit a low catalytic efficacy toward nerve agents with k_{cat}/K_M values of usually less than $10^6\,M^{-1}\,min^{-1}$ with G-agents and substantially lower values with VX and other V-agents (Masson et al., 1998; Masson and Rochu, 2009; Lenz et al., 2007; Wales and Reeves, 2012; Goldsmith et al., 2012; Cherny et al., 2013). In addition, wild-type enzymes hydrolyze preferentially the less toxic P(+) nerve agent enantiomers as coordination of the P=O oxygen activates the phosphorus for a nucleophilic attack simultaneously stabilizing the tetrahedral intermediate which – in a stereogenic environment – results in discrimination of chiral substrates. This unsatisfactory situation led to research for the investigation of the mechanism of nerve agent hydrolysis, the elucidation of the three dimensional structure and the development of mutants with reversed stereoselectivity and higher catalytic efficacy (Masson and Rochu, 2009; Nachon et al., 2013).

Quite recently, research groups using rational design and directed evolution succeeded to generate enzyme mutants with substantially higher catalytic efficacy toward G-type nerve agents, with k_{cat}/K_M values up to $5 \times 10^7\,M^{-1}\,min^{-1}$, and in part reversed stereoselectivity (Kirby et al., 2013; Goldsmith et al., 2012). Moreover, Cherny et al. (2013) were successful in the design of *Brevundimonas diminuta* PTE mutants with a maximum k_{cat}/K_M value of $5 \times 10^6\,M^{-1}\,min^{-1}$ for the toxic P(−) VX enantiomer (Cherny et al., 2013). In the end, the intensified research of the past few years resulted in the design of chimeric PON1 and bacterial PTE mutants with k_{cat}/K_M values which are close to or even exceed the desired $1 \times 10^7\,M^{-1}\,min^{-1}$ (Ashani et al., 2011; Josse et al., 2001).

2.1. In vivo efficacy of catalytic bioscavengers

There is only limited information on the in vivo efficacy of catalytic bioscavengers in the open literature. The prophylactic injection of wild-type *Pseudomonas* sp. PTE protected mice against multiple LD_{50}s of tabun, paraoxon and diethylfluorophosphate (Ashani et al., 1991; Raveh et al., 1992). *Agrobacterium radiobacter* OpdA, a PTE with a rather low catalytic activity toward nerve agents but capable to hydrolyze a broad range of OP pesticides (Horne et al., 2002; Wille et al., 2012), was injected i.v. to rats prior to poisoning by $3LD_{50}$ parathion, methyl-parathion or dichlorvos (Bird et al., 2008; Gresham et al., 2010). A single OpdA dose resulted in 24 h survival of all dichlorvos and of approx. 60% of parathion poisoned rats while all methyl-parathion exposed rats survived 16 h but none 24 h. Prophylactic administration of encapsulated wild-type PTE protected mice from multiple LD_{50}s of paraoxon and diisopropylfluorophosphate (Wales and Reeves, 2012). Recombinant wild-type PON1 given 30 min prior to $1.2LCT_{50}$ sarin or soman resulted in a significantly higher survival rate compared to agent control guinea pigs (Valiyaveettil et al., 2011) while *Trichoplusia ni* larvae expressed rePON1 failed to protect guinea pigs from $2LD_{50}$ tabun, sarin soman or cyclosarin (Hodgins et al., 2013).

Recently, the chimeric PON1 mutant IIG1 (Goldsmith et al., 2012) was tested in an anesthetized guinea pig model against $\sim 2LD_{50}$ cyclosarin (Worek et al., 2014a). Pre-exposure prophylaxis with 1 mg/kg IIG1 60 min prior to cyclosarin resulted in a plasma

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