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Emergence of catalytic bioscavengers against organophosphorus agents

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ABSTRACT

Bioscavengers are an effective alternative approach for pre- and post-exposure treatments of nerve agent (NA) poisoning. Bioscavengers are natural or recombinant enzymes, reactive proteins, and antibodies that neutralize NAs before they reach their physiological targets. They are administered by injection (protein or gene delivery vector) and react with NAs in the bloodstream. Other ways of delivery can be used: inhalation for pulmonary delivery, topical creams for skin protection, etc. Operational bioscavengers must be producible at low cost, not susceptible to induce immune response and adverse effects, and stable in the bloodstream, upon storage, and under field conditions.

First generation bioscavengers, cholinesterases and carboxylesterases, are stoichiometric bioscavengers. However, stoichiometric neutralization of NAs needs administration of huge doses of costly biopharmaceuticals. Second generation bioscavengers are catalytic bioscavengers. These are capable of detoxifying organophosphates regeneratively. By virtue of high turnover, much lower doses are needed for rapid neutralization of toxicants. The most promising catalytic bioscavengers are evolved mutants of phosphotriesterases (bacterial enzymes, mammalian paraoxonases), displaying enantiomeric preference for toxic NA isomers. However, engineering of cholinesterases, carboxylesterases, prolidases and other enzymes, e.g. phosphotriesterases-lactonases from extremophiles is of interest. In particular, association of cholinesterase mutants (not susceptible to age after phosphylation) with fast-reactivating oximes leads to pseudocatalytic bioscavengers. Thus, catalytic and pseudocatalytic bioscavengers are an improvement of bioscavenger-based medical countermeasures in terms of efficacy and cost.

1. Introduction

Bioscavengers are enzymes, antibodies, and reactive proteins, that sequester and inactivate highly toxic compounds before these molecules reach their biological targets. The idea that exogenous bioscavengers could be used for protection against NAs and treatment of poisoning was introduced some 30 years ago [1]. In fact, it was known for decades that endogenous enzymes react with OPs in the body and exert some protection against OP poisoning [2]. It was also known that numerous enzymes degrade and detoxify OPs, including nerve agents (NAs) [3,4].

We must consider three types of bioscavengers: 1) stoichiometric bioscavengers that neutralize OPs in a mole-to-mole reaction; 2) pseudocatalytic bioscavengers, which are stoichiometric bioscavengers that are continuously regenerated after phosphylation in a coupled nucleophile-mediated reaction, and 3) catalytic bioscavengers for which OPs are substrates.

First studies on exogenous bioscavengers demonstrated the protective effect of stoichiometric bioscavengers [1] and catalytic bioscavengers [5]). In the past 20 years, research was dominated by the development of first generation bioscavengers, mostly human butyrylcholinesterase (BChE) as an alternative to pharmacological drugs for pre-treatment of NA poisoning [6] and post-exposure treatment [7]. However, the trend is being reversed. Several recent reviews pointed out the interest of catalytic bioscavengers [8–11]. Moreover, during the joint international conference on cholinesterases and paraoxonases there were twice as many communications about catalytic bioscavengers than about stoichiometric bioscavengers (http://tox.umh.es/12thche/index.html). The present article illustrates the advantages of catalytic bioscavengers

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CaE, carboxylesterase; CSP, cresyl saligenyl phosphate; DFP, diisopropylfluorophosphate; GMP, Good Manufacturing Practice; NA, nerve agent; NTE, neuropathy target esterase; OP, organophosphorus compound; PLL, phosphotriesterase-like lactonase; PAF-AH, platelet-activating factor acetylhydrolase; PON, paraoxonase; PROL, prolidase; PTE, phosphotriesterase; QM/MM, combined quantum mechanics and molecular mechanics method; MD, molecular dynamics; TOCP, tri-*ortho*-cresyl phosphate.

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and the attraction they represent for treatments of OP poisoning.

2. Protective action of bioscavengers

After OP exposure, OP molecules penetrate into the body through the skin, lungs and eyes. OPs diffuse from the blood towards their physiological targets: central cholinergic synapses, ganglia and neuromuscular junctions, non-cholinergic targets, and depot sites (Fig. 1). Endogenous bioscavengers present in the skin, lungs, liver, blood and capillaries inactivate a part of the toxic dose. Certain OPs are converted by various organs in the body into potent cholinesterase inhibitors, e.g. thiono-OP activated into oxon, or tri-*ortho*-cresyl phosphate (TOCP) activated into cresyl saligenyl phosphate (CSP) [cf. ref.9 and references herein]. Pre-exposure administration of exogenous bioscavengers, noted «E» for «enzyme», neutralizes OP molecules either before their penetration into the body or transfer to targets (Fig. 1). Comprehensive cartoon representations of the detoxifying action of stoichiometric, pseudo-catalytic and catalytic bioscavengers are in ref 8. Intravenous or intramuscular injection of bioscavengers allows neutralization of OPs in the bloodstream [12]. Incorporation of bioscavengers into active topical skin protectants can protect the skin against liquid droplets of OPs [13]. Aerosolization of bioscavengers prevents penetration of volatile OPs and dusty NAs via the respiratory tract [14,15].

3. Requirements for a bioscavenger

An operational bioscavenger must fulfil a numbers of constraints:

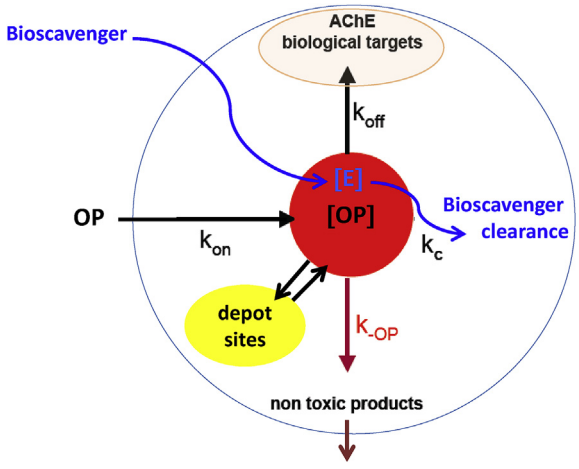

Fig. 1. Biological fate of organophosphorus compounds (OPs) and bioscavengers (E) in the body. Routes of penetration of OPs are absorption through the skin, eyes, and/or respiratory tract (nerve agents, pesticides, flame retardants), or ingestion (accidental or intentional self-poisoning). OP molecules distribute from the blood compartment into tissues, including depot sites, biological targets (peripheral and central cholinergic synapses and secondary targets), and sites of elimination (liver and kidney). Acetylcholinesterase (AChE) is the physiological target in acute poisoning. Reaction with secondary targets (carboxylesterases, serine-amidases, neuropathy target esterase, serine-peptidases, lipases, and various proteins) is responsible for non-cholinergic sublethal effects of OPs, delayed toxicity (neuropathies), and chronic toxicity at low dose exposure. Certain targets are endogenous bioscavengers. Among them, butyrylcholinesterase (BChE), present in numerous tissues, acts as an endogenous stoichiometric bioscavenger; its concentration in human plasma is about 50 nM. Therapeutic enzymes (exogenous stoichiometric/catalytic bioscavengers), mostly administered by i.v. or i.m. injection, act in the bloodstream after absorption and distribution. The blood clearance of bioscavengers has to be slow.

1) Biochemical constraints: a) because the maximum OP concentration in blood is always very low, even in the most severe cases of poisoning, much lower than the dissociation constant of bioscavenger-OP complex ($[OP] \ll K_d$), the neutralization reaction ($-d[OP]/dt$) takes place under pseudo-first-order conditions. The rate of reaction is:

$$-d[OP]/dt = (k_{-OP}/K_d).[E].[OP] \quad (1)$$

In Eq (1), $(k_{-OP}/K_d).[E]$ is the first-order rate constant. For stoichiometric bioscavengers, k_{-OP}/K_d is the bimolecular rate constant of phosphylation. For catalytic bioscavengers, k_{-OP}/K_d is the specificity constant k_{cat}/K_d. The bimolecular rate constant must be as high as possible; b) the spectrum of activity must be as large as possible, and ideally should cover G and V agents and OP pesticides; c) the enantiomeric preference must be towards toxic stereoisomers.

2) Biological and safety constraints: a) long biological life ($t_{1/2}$ or mean residence time in the bloodstream for injected bioscavengers), i.e. slow clearance; b) immuno-tolerance; c) no iatrogenic effects; d) no contaminants (e.g. virus, coagulation factors).
3) Industrial constraints: a) available from natural sources or expression systems and production under good manufacturing practice (GMP) conditions; b) thermal stability of liquid or lyophilized formulations upon storage and under field conditions of use; c) delivery system for injection, aerosolization, and other uses. 4) Economic constraint: affordable cost.

4. Stoichiometric bioscavengers

Cholinesterases (i.e. fetal bovine serum AChE, human BChE, and human AChE) are first generation bioscavengers. They are irreversibly inhibited by OPs through phosphylation of their catalytic serine. Therefore, the mole-to-mole reaction implies administration of huge doses of enzyme for pre-exposure and post-exposure treatments. In 2006, plasma-derived human BChE was approved by the FDA as an investigational new drug (IND) for pre-treatment of NA poisoning.

A dose of 200 mg of human BChE protects a 70 kg human against 2 × LD_{50} racemic soman [16]. It was estimated that the cost of such a dose is of the order of $20 000.

New purification methods of human BChE tetramer from side products of plasma fractionation, using the highly selective affinity ligand, huprine [17] will reduce considerably the cost of production. New expression systems for high level production of recombinant tetrameric human BChE in CHO cells [18] or in plants [19] will also reduce the cost down to $ 400–1200 per dose [20]. However, this cost is still too high for protection of personnel (e.g. first responders) or long term post-exposure treatment of severe poisoning (e.g. suicide or accidental poisoning by OP pesticides). The main limitation of stoichiometric bioscavengers is the cost/dose of single-use enzyme.

5. Catalytic bioscavengers

Catalytic bioscavengers by virtue of their turnover are multiple-use enzymes. Comparing a stoichiometric bioscavenger with a catalytic bioscavenger displaying the same efficacy (identical rate -d[OP]/dt) against a given OP concentration (Eq (2))

$$(k_{-OP}/K_d).[E_{stscav}].[OP] = (k_{cat}/K_m).[E_{catscav}].[OP] \quad (2)$$