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Reversible inhibition of acetylcholinesterase by carbamates or huperzine A increases residual activity of the enzyme upon soman challenge

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Abstract

The treatment options in soman poisoning are very limited due to rapid aging of the inhibited acetylcholinesterase, which makes the enzyme essentially intractable. Hence, oxime treatment probably comes too late in realistic scenarios. As an alternative, protecting part of the enzyme by reversible inhibition prior to soman exposure has been proposed. This strategy was successfully tested in animal experiments, but its efficacy still awaits complete understanding. In particular, it is unclear whether survival is improved by a higher residual activity of acetylcholinesterase during the acute phase, when the reversible and irreversible inhibitors are present together. In previous experiments with carbamate pre-treatment and paraoxon challenge we noticed an increased residual activity of erythrocyte acetylcholinesterase compared to non-pre-treatment. This result was encouraging to also test for comparable effects when using soman. Immobilized human erythrocytes were continuously perfused for real-time measurement of acetylcholinesterase activity by a modified Ellman method using 0.45 mM acetylthiocholine. After having established the inhibition rate constant of soman, we tested the prophylactic potential of physostigmine, pyridostigmine and huperzine A. Pre-treatment with the reversible inhibitors inhibited the enzyme by 20–95%. Additional perfusion with 10 nM soman for 30 min resulted in a residual activity of 1–5%, at low and high pre-inhibition, respectively. The residual activity was markedly higher than in the absence of reversibly blocking agents (0.1%). After discontinuation of soman and the reversible inhibitors, enzyme activity recovered up to 30% following pre-inhibition by 50%. The experimental data agreed with computer simulations when feeding the kinetic-based model with the established rate constants. The results with soman essentially agreed with those obtained previously with paraoxon.

Keywords: Carbamate prophylaxis; Nerve agents; Soman; Acetylcholinesterase; Pyridostigmine; Physostigmine; Huperzine A; Kinetic analysis; Computer simulation

1. Introduction

Standard treatment of poisoning by organophosphorus compounds includes the administration of an antimuscarinic agent, e.g. atropine, and of an acetylcholinesterase (AChE) reactivator (oxime) (Worek et al., 2005). The latter component is effective in sarin and VX poisoning, less effective in tabun and quite ineffective

Abbreviations: AChE, acetylcholinesterase (EC 3.1.1.7); AU, absorbance units; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); RBC, red blood cell

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in soman poisoning due to rapid 'aging' of the inhibited AChE. After spontaneous dealkylation of the pinacolyl-AChE-conjugate this enzyme species is considered as irreversibly inhibited and resistant towards reactivation by oximes (Shafferman et al., 1996). Unfortunately, the pinacolyl-AChE-conjugate of primates exhibits particularly short aging half-lives in the range of 1–2 min (Talbot et al., 1988; Shafferman et al., 1996). Hence, the useful time for effective oxime administration is very short in soman poisoning of humans and oxime treatment probably comes too late in realistic scenarios.

In the early 1960s, when the soman threat and the inability of proper treatment were recognized during the Cold War, other strategies were searched for. One means of protecting against soman poisoning is the prophylactic use of certain reversible inhibitors (carbamates) of AChE. Koster was the first to employ this approach by using physostigmine to protect animals from the lethal effect of diisopropylfluorophosphate (Koster, 1946). Since then several carbamates, including those that are thought not to pass through the blood–brain barrier, have been used in conjunction with atropine to protect animals from the lethal effects of soman (Berry and Davies, 1970; Gordon et al., 1978). Recently, non-acylating inhibitors, such as huperzine A, have been successfully tested (Lallement et al., 2002; Gordon et al., 2005). The antidotal action of reversible cholinesterase inhibitors were referred to their common property of being able to inhibit AChE reversibly and protect the enzyme from irreversible phosphylation. Provided that the absorbed organophosphorus compound was rapidly eliminated, free enzyme would be gradually regenerated to allow survival. This assumption plausibly explains why animals having survived acute soman poisoning may recover more rapidly following carbamate pre-treatment compared to those without pre-treatment. It does not explain, however, how they survive the acute phase when both inhibitors are present. During this phase, both inhibitors are expected to work additively. This view has already been presented by Berry and Davies (1970) who wrote: "If this is the mechanism of the protective action, there is obviously a period during which a great proportion of the enzyme would be inactivated, both reversibly and irreversibly. During this critical period a lethal accumulation of acetylcholine could occur at the nerve endings."

To our knowledge, AChE activity was rarely measured at the onset of intoxication (usually within 2 min) and in cases when AChE activity was reported methods for preventing spontaneous reactivation during work-up were not presented. Guinea-pigs challenged with VX and treated with atropine 1 min later had about 3% and 5% of red blood cell (RBC) AChE activity after saline or pyridostigmine pre-treatment, respectively (Koplovitz et al., 1992). Since AChE was determined radiometrically, which usually lasts several minutes, spontaneous reactivation during measurement may have obscured the result. Given a decarbamylation half-life of 15–30 min (Eckert et al., 2006b), 5% of active enzyme will appear in 1.1–2.2 min, respectively, a period that will almost always elapse when performing the usual enzyme assays.

Recently, we introduced a dynamically working in vitro model using immobilized human red blood cells (RBC) as an enzyme source (Eckert et al., 2006a). This model allowed continuous determination of AChE activity in real-time. Pre-treatment with short acting inhibitors had a protective effect on RBC-AChE when further challenged with paraoxon (Eckert et al., 2006b). Thus, it was tempting to adopt the perfusion model for analysis of the time course of AChE activity during soman exposure and of the influence of pre-treatment with reversible AChE inhibitors. The question was whether residual AChE activity remained higher during a simultaneous challenge with a reversible inhibitor and soman after pre-treatment in comparison to non-pre-treatment.

For the sake of simplicity, we use the term "reversible inhibitor" for the carbamates and huperzine A, because the inhibition of AChE is completely reversible. In fact, huperzine A is a competitively acting, true reversible inhibitor while the acylating carbamates are pseudo-reversible inhibitors since the carbamate is not recovered unchanged upon spontaneous reactivation of the enzyme.

2. Materials and methods

Customary chemicals were obtained from commercial sources at the purest grade available.

(−)-Physostigmine (eserine hemisulfate) and pyridostigmine bromide were purchased from Sigma (Deisenhofen, Germany), (−)-huperzine A from Calbiochem-Merck (Darmstadt, Germany).

Racemic soman was made available by the German Ministry of Defence.

Particle filters employed were Millex®-GS, 0.22 μm (Millipore; Eschborn, Germany).

2.1. General experimental procedure

Experimental procedure was performed using the recently described dynamic model (Eckert et al., 2006a,b). In short, human erythrocytes were layered onto a particle filter (Millex®-GS, 0.22 μm, Ø 33 mm) resulting in a stable enzyme reactor. Maximum AChE activity was determined by continuously perfusing the enzyme reactor with the medium, consisting of acetylthiocholine (0.45 mM), DTNB (Ellman's