Butyrylcholinesterase as a therapeutic drug for protection against percutaneous VX

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Butyrylcholinesterase as a therapeutic drug for protection against percutaneous VX

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Abstract

The administration of purified human plasma-derived butyrylcholinesterase (HuBuChE) as a pretreatment has been demonstrated to enhance survival and protect against decreased cognitive function after exposure to organophosphorus poisons (OPs). Based on efficacy data obtained with guinea pigs and non-human primates and the lack of behavioral side effects, plasma-derived HuBuChE has been granted investigational new drug status by the US Food and Drug Administration. The recent availability of a recombinant form of HuBuChE (rHuBuChE) from the milk of transgenic goats has now allowed us to determine the pharmacokinetics of that material in guinea pigs and use it as a therapy following exposure to the VX. The rHuBuChE was expressed as a dimer and following intramuscular (i.m.) administration had a more rapid adsorption and clearance profile in guinea pigs than the plasma-derived material. Based on those data, we administered rHuBuChE i.m. 1 h after a percutaneous exposure of guinea pigs to either 2xLD₅₀ or 5xLD₅₀ of VX. Post-exposure therapy with rHuBuChE provided improved survival at both challenge levels, 90% and 33% respectively versus 20% or 0% respectively for animals that did not receive therapy. These studies showed that BuChE can be efficacious as a therapy against percutaneous exposure to VX.

1. Introduction

The efficacy of acetyl- and butyrylcholinesterase as protein drugs capable of providing protection against in vivo exposure to highly toxic organophosphorus compounds such as the chemical warfare agents soman, sarin or VX has been amply demonstrated over the past 15 years in a variety of animal model systems [1–13]. While mouse and macaque acetylcholinesterase and human butyrylcholinesterase have been used in pharmacokinetic studies and immunologic studies, plasma-derived human butyrylcholinesterase (HuBuChE, 7) and recombinant human butyrylcholinesterase (rHuBuChE) from the milk of transgenic goats [14,15] have longer in vivo residence as determined by pharmacokinetic studies in either guinea pigs or monkeys. The capacity of this pretreatment approach to provide protection (assessed as survival after exposure to an otherwise lethal dose of agent) is readily apparent (Table 1).

While bioscavengers are efficacious in providing protection against nerve agent poisoning when used as pretreatments, their value as post-exposure therapeutics has received little attention. To address that deficit, we carried out a limited study in which rHuBuChE was administered to guinea pigs one hour after a multiple LD₅₀ percutaneous exposure to VX. The ability of BuChE to provide protection, as measured by enhanced survival, offers a clear in vivo demonstration of the therapeutic use of a bioscavenger to protect against an OP nerve agent.

2. Methods

2.1. Materials

The nerve agent VX (O-ethyl-S-(2-isopropylaminoethyl) methylphosphonothiolate) was obtained from the Research Development and Engineering Center, Aberdeen Proving Ground, MD. The compound was >97% pure by 31P NMR analysis. The goat milk derived rHuBuChE was supplied as a non-PEGylated purified solution in saline at 75 mg/mL as a gift from Nexia Biotechnologies (now PharmAthene Inc., Annapolis, MD). The rHuBuChE was diluted in physiologic saline to the desired concentration immediately before use. Butyrylthiocholine, acetylthiocholine and 5,5′-dithiobis-(2-nitrobenzoic acid) were purchased from Sigma Chemicals (St. Louis, MO).

2.2. rHuBuChE pharmacokinetics

Two doses of rHuBuChE (19.9 and 33.5 mg/kg) were injected intramuscularly (i.m.) in guinea pigs to determine pharmacokinetics in blood. The rHuBuChE was diluted and administered in a...
total volume of 1.0 mL i.m. in guinea pigs, with the dose being split equally between the quadriceps. Blood samples were collected by toenail clip into heparinized containers prior to the injection of rHuBuChE (time 0) and at approximately 1, 2, 4, 8, 16, 20, 24, 48 and 72 h after injection. Plasma was isolated from each sample and stored frozen at −80 ºC. Plasma samples from three guinea pigs in each dose group were analyzed for BuChE activity as a function of time after administration using a microtiter plate modification of the method of Ellman et al. [16]. Butyrylthiocholine was used as the assay substrate, and colorimetric (A412 nm) responses were detected using a SpectraMax Plus 384 (Molecular Devices, Sunnyvale, CA).

Mean plasma BuChE time–concentration data were fit to standard pharmacokinetic models using GraphPad Prism nonlinear regression software (version 4.03, 2005, GraphPad Software, Inc., La Jolla, CA). Pharmacokinetic parameter estimates and predicted rBuChE concentrations as a function of time were calculated from the raw data using an appropriate mathematical model. The following criteria were utilized as guidelines for determining the appropriate model: minimal sum of squared residuals, high correlation coefficient, small standard deviations of parameter estimates and unbiased distribution patterns of residuals for estimates of observed versus predicted values. Parameter estimates generated were time to maximum plasma concentration (Tmax) and half time for elimination (T1/2).

The pharmacokinetics of rHuBuChE after i.m. administration to guinea pigs were best described by a one-compartment model with first-order absorption and elimination described by Eq. (1) below:

\[ C(t) = \frac{D \cdot k_{01}}{V_d \cdot k_{01} - k_{10}} (e^{-k_{10} t} - e^{-k_{01} t}) \] (1)

where C = plasma concentration (mg/mL), t = time (min), D = dose (mg/kg), Vd = volume of distribution (mL/kg), k01 = absorption rate constant (min⁻¹), and k10 = elimination rate constant (min⁻¹).

### 2.3. Therapy experiments

#### 2.3.1. Animals

Male Hartley albino guinea pigs (Cavia porcellus; Charles River Laboratories, Kingston, NY) weighing 300–500 gm were used. Animals were quarantined and observed for a minimum of five days for evidence of disease in accordance with the stipulations mandated for an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility prior to being put on study. Guinea pig ration and water were provided ad libitum.

#### 2.3.2. Procedure

Male Hartley guinea pigs were shaved on the left flank in a roughly 2 in. × 2 in. patch. Animals were anesthetized by injection (s.c.) with 32 mg/kg ketamine and 4 mg/kg xylazine. Five minutes later, animals were exposed at the shaved patch to 2 × LD50 (0.28 mg/kg; 1.0 µmol/kg; n = 20) or 5 × LD50 (0.7 mg/kg, 2.6 µmol/kg; n = 10) of VX in 90% isopropyl alcohol. One hour later, nine animals exposed to the 5 × LD50 dose of VX and half of the animals exposed to the 2 × LD50 dose of VX were injected i.m. with 220 mg/kg (2.6 µmol/kg) of goat milk derived rHuBuChE. Guinea pigs received a maximum injected volume of 1 mL, and any injected volume that was >0.3 mL was split equally (between the two quadriceps). The remaining animals exposed to 2 × LD50 of VX were injected with an equivalent volume of saline. Animals were observed for signs of OP intoxication for at least 3 h, and were checked intermittently for toxic signs during the next 48 h. Body weight was measured at 24 h intervals. The experiment was terminated at 48 h; after euthanasia, organs were collected from animals that had survived this time for pathology assessment.

### 3. Results

#### 3.1. rHuBuChE pharmacokinetics

The pharmacokinetics of rHuBuChE in guinea pigs followed a one-compartment model with a single elimination phase (Fig. 1). The estimated Tmax was ~4.2 h with >60% of the circulatory concentration of the enzyme available within 2 h. The estimated T1/2 was 15.2 h. These results are in marked contrast to plasma-derived BuChE, which in guinea pigs has a circulatory Tmax of 20 h after i.m. administration and a T1/2 of ~72 h [7].

#### 3.2. Therapy experiments

Guinea pigs exposed to a 2 × LD50 percutaneous dose of VX (n = 20) all displayed mild signs of intoxication after 1 h. Those animals that received saline at 1 h post VX (n = 10) displayed progressively more severe toxic signs. Eight of the 10 saline-treated animals died, with an average time until death of just over 5 h (range 3.5–10 h) after VX exposure. The two surviving saline-treated animals displayed continued impairment, and had lost ~15% of their initial body weight at 48 h post VX exposure, at which time the experiment was terminated. Among guinea pigs that were exposed to 2 × LD50 of VX followed an hour later by an i.m. injection with rHuBuChE (n = 10), 9/10 survived for 48 h (Table 2). The severity of toxic signs among the VX challenged survivors increased for 1–2 h after injection of enzyme, and then lessened during the following 1–2 h. All signs of toxicity among surviving animals in this set had resolved by 5 h post VX exposure, and at 48 h animals had lost on average 5% of initial body weight. A single animal exposed to 2 × LD50 of VX followed by rHuBuChE died roughly 22 h after VX exposure. All guinea pigs exposed to 5 × LD50 of VX (n = 10) displayed mild to severe signs of intoxication after 40 min. One animal in this set died at 50 min after exposure to VX, and the remaining 9 were administered rHuBuChE. Three of these nine animals survived to 48 h, displaying continued impairment and loss of ~15% of initial body weight at 48 h.
body weight. The other six rHuBuChE-treated animals died with a mean time to death of ~9 h (range 3.5–22 h) after VX exposure. Histological examination of organs from animals that had survived 48 h revealed minimal to moderate pathology only in the brains of the two animals exposed to 2 × LD₅₀ of VX followed by saline and the three animals that survived exposure to 5 × LD₅₀ of VX followed by rHuBuChE. The nine animals exposed to 2 × LD₅₀ of VX followed by rHuBuChE displayed no detectable pathology in either brain or peripheral organs.

4. Discussion

Butyrylcholinesterases from a variety of sources have been shown to have similar kinetic properties with respect to substrates and inhibitors [3,4,7]. This enzyme has also been shown to provide in vivo protection against poisoning by several different chemical warfare nerve agents when given as a pretreatment [2,6–8]. The extent of protection afforded is hypothesized to be directly related to simple stoichiometry, where 1 mole of enzyme will bind, and hence neutralize, 1 mole of nerve agent. Protection also has been demonstrated against exposure to multiple medical lethal doses of GD or VX. Animals thus protected are asymptomatic and show either no or very transient signs of poisoning, with no behavioral side effects [4,7,17]. Due to the rapid onset of toxic signs after poisoning with G-type nerve agents in animals, BuChE has not been used in a therapeutic modality with these agents. In order to address the potential utility of HuBuChE when administered therapeutically, we chose to administer a rapidly absorbed dimeric form of rHuBuChE obtained from the milk of transgenic goats. Our pharmacokinetic studies of the rHuBuChE administered i.m. in guinea pigs revealed that the Tₘ₉₅ for this material was ~6 h with >60% of the circulatory concentrator of the enzyme available within 2 h and considerable material available as early as 1 h after administration. This is in marked contrast to the plasma-derived BuChE which has a circulatory Tₘ₉₅ of 20 h after i.m. administration [7] and much smaller percentage of material in circulation at 2 h.

VX has been shown to have a slow percutaneous absorption resulting in delayed onset of signs after exposure of at least 1 h [18–20]. When we exposed anesthetized guinea pigs to a 2 × LD₅₀ dose of VX and then administered rHuBuChE intramuscularly 60 min later, nine out of ten animals survived with minimal and transient signs of intoxication without the administration of standard therapy of atropine, oxime or diazepam. While all of these animals displayed mild signs of poisoning following VX, the signs had resolved in 5 h and by 24 h post-exposure all animals appeared normal; over the next 24 h period they proceeded to gain weight and exhibit normal behavior. Given that these animals received a 2.6-fold mole excess of rHuBuChE versus VX, the extent of survival is in good agreement with that observed when HuBuChE was used as a pretreatment [7]. A matched set of control animals exhibited severe signs of poisoning, with only two out of 10 animals surviving for 48 h, where both survivors lost >15% of their initial weight and displayed continued signs of poisoning. Histopathological examination of the brain, heart, and other critical organs of the 48 h survivors indicated moderate injury in the two control animals, but no detectable abnormalities in the rHuBuChE treated animals. For the group of animals that received 5 × LD₅₀ of VX percutaneously, there was a less than equal mole ratio of administered rHuBuChE to VX due to the fact that the challenge took place before the maximal circulatory concentration of rHuBuChE had been achieved (Fig. 1). The resultant decrease in the number of survivors is reflective of this slightly <1:1 stoichiometry of rHuBuChE to VX. These results are in good agreement with those of Mumford et al. [21] who have recently demonstrated similar protection against VX with plasma-derived HuBuChE in a free-ranging non-anesthetized instrumented guinea pig model. Combined, these two studies demonstrate that bioscavengers administered under appropriate conditions (e.g. following exposure to a slow-onset nerve agent) can provide protection therapeutically as well as when given as a pretreatment.

The capability of bioscavengers to provide not only pretreatment protection but also (as reported here) therapeutic protection against nerve agent exposure offers an expanded range of use for this protein drug as a nerve agent antidote.

Conflict of interest
None.

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Investigators adhered to the Guide for the Care and Use of Laboratory Animals (1996) by the Institute of Laboratory Animal Resources, National Research Council, in accordance with stipulations for AAALAC accredited facilities. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed.

References


