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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 more a 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 $2xLD_{50}$ or $5xLD_{50}$ 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.

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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 LD_{50} 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 ^{31}P 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

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Table 1
Prophylactic efficacy of plasma HuBuChE in two species.

Species	OP Compound	Dose (LD_{50})	24 h survival
Guinea pig	VX	5.5	10/10
	GD	5.5	10/10
Cynomolgus monkey (N = 6)	GD	5.5	5/6

Animals were injected i.m. with HuBuChE (140 mg/kg for guinea pigs with GD, 30 mg/kg for guinea pigs with VX, and 28.3 mg/kg for cynomolgus macaques), then exposed to OP nerve agents either 10 (for monkeys) or 20 (for guinea pigs) hours later. Data are adapted from Lenz et al. [7].

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 °C. 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 (A_{412} 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 (T_{max}) and half time for elimination ($T_{1/2}$)

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):

$$C(t) = \frac{D}{\mathrm{Vd}} \frac{k_{01}}{k_{01} - k_{10}} (e^{-k}10^{t} - e^{-k}01^{t}) \quad (1)$$

where C = plasma concentration (mg/mL), t = time (min), D = dose (mg/kg), Vd = volume of distribution (mL/kg), k_{01} = absorption rate constant (min^{-1}), and k_{10} = 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 $2 \times LD_{50}$ (0.28 mg/kg, 1.0 μmol/kg; n = 20) or $5 \times LD_{50}$ (0.7 mg/kg, 2.6 μmol/kg; n = 10) of VX in 90% isopropyl alcohol. One hour later, nine animals exposed to the $5 \times LD_{50}$ dose of VX and half

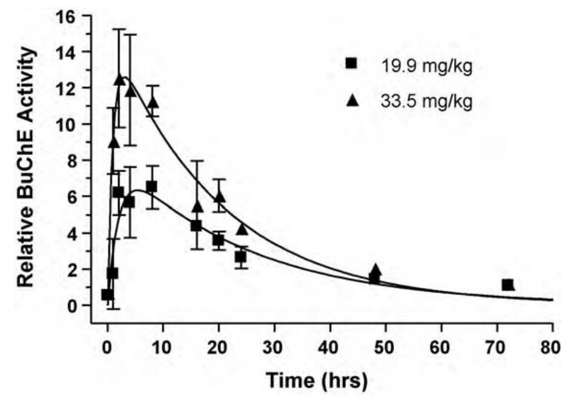

Fig. 1. Pharmacokinetics of rHuBuChE administered intramuscular (i.m.) in guinea pigs (n = 3).

of the animals exposed to the $2 \times LD_{50}$ 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 $2 \times LD_{50}$ 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 to 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 T_{max} was ∼4.2 h with >60% of the circulatory concentration of the enzyme available within 2 h. The estimated $T_{1/2}$ was 15.2 h. These results are in marked contrast to plasma-derived BuChE, which in guinea pigs has a circulatory T_{max} of 20 h after i.m. administration and a $T_{1/2}$ of ∼72 h [7].

3.2. Therapy experiments

Guinea pigs exposed to a $2 \times LD_{50}$ 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 $2 \times LD_{50}$ 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 $2 \times LD_{50}$ of VX followed by rHuBuChE died roughly 22 h after VX exposure. All guinea pigs exposed to $5 \times LD_{50}$ 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

Table 2
rHuBuChE as a post-exposure therapy in guinea pigs after percutaneous VX.

Protection	Dose (LD_{50})	No. of survivors at 48 h	Impairment to survivors	Time to recovery
rHuBuChE	2	9/10	Mild to none	4–8 h
None	2	2/10	Severe	>48 h
rHuBuChE	5	3/9	Severe	>48 h
None	5	0/1	N/A	None

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 minor to moderate pathology only in the brains of the two animals exposed to $2 \times LD_{50}$ of VX followed by saline and the three animals that survived exposure to $5 \times LD_{50}$ of VX followed by rHuBuChE. The nine animals exposed to $2 \times LD_{50}$ 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 medial 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 T_{max} for this material was ~6 h with >60% of the circulatory concentraton 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 T_{max} 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 $2 \times LD_{50}$ 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 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 $5 \times LD_{50}$ 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 stoichiometery 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

[1] Y. Ashani, S. Shapira, D. Levy, A.D. Wolfe, B.P. Doctor, L. Raveh, Butyrylcholinesterase and acetylcholinesterase prophylaxis against soman poisoning in mice, Biochem. Pharmacol. 41 (1991) 37–41.
[2] C.A. Broomfield, D.M. Maxwell, R.P. Solana, C.A. Castro, A.V. Finger, D.E. Lenz, Protection by butyrylcholinesterase against organophosphorus poisoning in nonhuman primates, J. Pharmacol. Exp. Ther. 259 (1991) 633–638.
[3] D.M. Cerasoli, E.M. Griffiths, B.P. Doctor, A. Saxena, J.M. Fedorko, N.H. Greig, Q.S. Yu, Y. Huang, H. Wilgus, C.N. Karatzas, I. Koplovitz, D.E. Lenz, *In vitro* and *in vivo* characterization of recombinant human butyrylcholinesterase (Protexia) as a potential nerve agent bioscavenger, Chem. Biol. Interact. 157 (2005) 363–365.
[4] D.M. Cerasoli, C.L. Robison, I.Y. Nwaneri, B. Somsamayvong, C.N. Karatzas, H. Wilgus, Y. Huang, J.H. McDonough, D.E. Lenz, L.A. Lumley, Pretreatment with pegylated Protexia protects against exposure to the nerve agents VX and soman. Abstracts, vol. 31, Society for Neuroscience, 2005, 337.19.
[5] B.P. Doctor, D.W. Blick, G. Caranto, C.A. Castro, M.K. Gentry, R. Larison, D.M. Maxwell, M.R. Murphy, M. Schutz, K. Waibel, A.D. Wolfe A.D., Cholinesterases as scavengers for organophosphorous compounds: protection of primate performance against soman toxicity, Chem. Biol. Interact. 87 (1993) 285–293.

[6] B.P. Doctor, A. Saxena, Bioscavengers for the protection of humans against organophosphate toxicity, Chem. Biol. Interact. 157–158 (2005) 167–171.
[7] D.E. Lenz, D.M. Maxwell, I. Koplovitz, C.R. Clark, B.R. Capacio, D.M. Cerasoli, J.M. Federko, C. Luo, A. Saxena, B.P. Doctor, C. Olson, Protection against soman or VX poisoning by human butyrylcholinesterase in guinea pigs and cynomolgus monkeys, Chem. Biol. Interact. 157–158 (2005) 205–210.
[8] D.E. Lenz, C.A. Broomfield, D.T. Yeung, P. Masson, D.M. Maxwell, D.M. Cerasoli, Nerve agent bioscavengers; progress in development of a new mode of protection against organophosphorus exposure, in: J.A. Romano, B. Lukey, H. Salem (Eds.), Chemical Warfare Agents: Toxicity at Low Levels, 2nd edition, CRC Press, Boca Raton, 2007, pp. 175–202.
[9] D.M. Maxwell, A.D. Wolfe, Y. Ashani, B.P. Doctor, Cholinesterase and carboxylesterase as scavengers for organophosphorous agents, in: J. Massoulie, F. Bacou, E. Barnard, A. Chatonnet, B.P. Doctor, D.M. Quinn (Eds.), Cholinesterases: Structure, Function, Mechanism, Genetics and Cell Biology, American Chemical Society, Washington, 1991, pp. 206–209.
[10] L. Raveh, J. Grunwald, D. Marcus, Y. Papier, E. Cohen, Y. Ashani, Human butyrylcholinesterase as a general prophylactic antidote for nerve agent toxicity, Biochem. Pharmacol. 45 (1993) 2465–2474.
[11] L. Raveh, E. Grauer, J. Grunwald, E. Cohen, Y. Ashani, The stoichiometry of protection against soman and VX toxicity in monkeys pretreated with human butyrylcholinesterase, Toxicol. Appl. Pharmacol. 145 (1997) 43–53.
[12] A.D. Wolfe, R.S. Rush, B.P. Doctor, I. Koplovitz, D. Jones D., Acetylcholinesterase prophylaxis against organophosphate toxicity, Fund. Appl. Toxicol. 9 (1987) 266–270.
[13] A.D. Wolfe, D.W. Blick, M.R. Murphy, S.A. Miller, M.K. Gentry, S.L. Hartgraves, B.P. Doctor, Use of cholinesterases as pretreatment drugs for the protection of rhesus monkeys against soman toxicity, Toxicol. Appl. Pharmacol. 117 (1992) 189–193.
[14] Y.-J. Huang, Y. Huang, H. Baldassarre, B. Wang, A. Lazaris, M. Leduc, A.S. Bilodeau, A. Bellemare, M. Côté, P. Herskovits, M. Touati, C. Turcotte, L. Valeanu, N. Lemee, H. Wilgus, I. Bégin, B. Bhatia, K. Rao, N. Neveu, E. Brochu, J. Pierson, D.K. Hockley, D.M. Cerasoli, D.E. Lenz, C.N. Karatzas, S. Langermann, Recombinant Human butyrylcholinesterase from milk of transgenic animals to protect against organophosphate poisoning, Proc. Natl. Acad. Sci. 104 (2007) 13603–13608.
[15] Y.-J. Huang, P.M. Lundy, A. Lazaris, Y. Huang, H. Baldassarre, B. Wang, C. Turcotte, M. Côté, A. Bellemare, A.S. Bilodeau, S. Brouillard, M. Touati, P. Herskovits, I. Begin, N. Neveu, E. Brochu, J. Pierson, D.K. Hockley, D.M. Cerasoli, D.E. Lenz, H. Wilgus, C.N. Karatzas, S. Langermann, Substantially improved pharmacokinetics of recombinant human butyrylcholinesterase by fusion to human serum albumin, BMC Biotechnol. 8 (2008) 50.
[16] G.L. Ellman, K.D. Courtney, V. Andres Jr., R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (1961) 88–95.
[17] N.H. Barakat, X. Zheng, C.B. Gilley, M. MacDonald, K. Okolotowicz, J.R. Cashman, S. Vyas, J.M. Beck, C.M. Hadad, J. Zhang, Chemical synthesis of two series of nerve agent model compounds and their stereoselective interaction with human acetylcholinesterase and human butyrylcholinesterase, Chem. Res. Toxicol. 22 (2009) 1669–1679.
[18] C.A. Castro, V.C. Gresham, A.V. Finger, D.M. Maxwell, R.P. Solana, D.E. Lenz, C.A. Broomfield, Behavioral decrements persist in rhesus monkeys trained on a serial probe recognition task despite protection against soman lethality by butyrylcholinesterase, Neurotoxicol. Teratol. 16 (1994) 145–148.
[19] E.D. Clarkson, J.P. Hackley Jr., M. Skvorak, S. Shutz, S. Schulz, R. Railer, M. Schons, R. Macalalag, Characterization of cutaneous exposure to chemical warfare agents GD, VX and VR, in: Society for Neuroscience Meeting Abstracts, 2001, p. 11 (Abstract #478).
[20] E.D. Clarkson, S.M. Schulz, R.F. Railer, M.A. Sigler, T.P. Logan, S.I. Baskin, Cutaneous exposure to GD and VX: Timing of antidotes, in: The Toxicologist: 45th Annual Meeting and ToxExpo, 2006, p. 17 (Abstract #81).
[21] H. Mumford, M.E. Price, J.R. Wetherell, A novel approach to assessing percutaneous VX poisoning in the conscious guinea-pig, J Appl Toxicol. 28 (2008) 694–702.