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Human serum butyrylcholinesterase: In vitro and in vivo stability, pharmacokinetics, and safety in mice☆

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Abstract

The use of exogenously administered cholinesterases (ChEs) as bioscavengers of highly toxic organophosphate (OP) nerve agents is now sufficiently well documented to make them a highly viable prophylactic treatment against this potential threat. Of the ChEs evaluated so far, human serum butyrylcholinesterase (HuBChE) is most suitable for human use. A dose of 200 mg (3 mg/kg) of HuBChE is envisioned as a prophylactic treatment in humans that can protect from an exposure of up to $2 \times LD_{50}$ of soman. In addition to its use as a prophylactic for a variety of wartime scenarios, including covert actions, it also has potential use for first responders (civilians) reacting to terrorist nerve gas release. We recently, developed a procedure for the large-scale purification of HuBChE, which yielded approximately 6 g of highly purified enzyme from 120 kg of Cohn fraction IV-4. The enzyme had a specific activity of 700–750 U/mg and migrated as a single band on SDS–PAGE. To provide data for initiating an investigational new drug (IND) application for the use of this enzyme as a bioscavenger in humans, we established its pharmacokinetic properties, examined its safety in mice, and evaluated its shelf life at various temperatures. In mice administered various doses up to 90 mg/kg, enzyme activity reached peak levels in circulation at 10 and 24 h following i.p. and i.m. injections, respectively. The enzyme displayed a mean residence time (MRT) of 40–50 h, regardless of the route of administration or dose of injected enzyme. Mice were euthanized 2 weeks following enzyme administration and tissues were examined grossly or microscopically for possible toxic effects. Results suggest that HuBChE does not exhibit any toxicity in mice as measured by general observation, serum chemistry, hematology, gross or histologic tissue changes. The shelf life of this enzyme stored at 4, 25, 37, and 45 °C was determined in lyophilized form. The enzyme was found to be stable when stored in lyophilized form at −20, 4, 25, or 37 °C to date (2 years), as measured by specific activity and SDS polyacrylamide gel electrophoresis. The effect of storage on circulatory stability was determined by measuring MRT in mice; there was no change in the MRT of lyophilized enzyme stored at −20 °C to date (2 years). These results provide convincing data that HuBChE is a safe bioscavenger that can provide protection against all OP nerve agents. Efforts are now underway to prepare the required documentation for submission of an IND application to the United States Food and Drug Administration (USFDA).

Keywords: Organophosphorus compounds; Human serum butyrylcholinesterase; Bioscavenger; Pharmacokinetics; Safety

1. Introduction

Although current antidotal regimens for organophosphate (OP) poisoning are effective in preventing lethality of animals from OP poisoning, they do not prevent post-exposure incapacitation, convulsions, performance deficits or in many cases, permanent brain damage [1–3]. These problems stimulated the development of enzyme

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bioscavengers as a pretreatment to sequester highly toxic OPs before they reach their physiological targets and prevent the in vivo toxicity of OPs and post-exposure incapacitation [3]. Among the enzymes examined as potential scavengers of highly toxic OP nerve agents, significant advances have been made using cholinesterases (ChEs). Of the ChEs evaluated so far, human serum butyrylcholinesterase (HuBChE) has several advantages as an exogenously administered prophylactic for human use [4]. First, it reacts rapidly with all highly toxic OPs, offering a broad range of protection for nerve agents including, soman, sarin, tabun, and VX. Second, it possesses a very long retention time in human circulation and is readily absorbed from sites of injection. Third, since the enzyme is from a human source, it should not produce any adverse immunological responses upon repeated administration into humans. A dose of 200 mg of HuBChE is envisioned as a prophylactic treatment in humans that can protect from exposure of up to $2 \times LD_{50}$ of soman [4].

The foremost requirement to advance HuBChE as a bioscavenger for human use was to obtain sufficient amounts of purified enzyme for conducting animal and clinical studies. A procedure for the large-scale purification of HuBChE was developed, which yielded gram quantities of purified enzyme from Cohn fraction IV-4 paste [5]. The objective of the current effort was to provide pre-clinical pharmacological information for conducting phase I clinical trials of HuBChE in humans. Therefore, we investigated the pharmacokinetics as well as safety and toxicity of purified HuBChE in mice. Blood was sampled at various time intervals to characterize the pharmacokinetics of HuBChE in mice following i.p., or i.m. administration. The safety and toxicity of HuBChE was measured by general observation, serum chemistry and hematology. Animals were euthanized at the end of 2 weeks and tissues were examined grossly or microscopically for possible toxic effects. The stability of the enzyme stored at 4, 25, 37, and 45 °C was determined in lyophilized form. The effect of storage at −20 °C on circulatory stability was also determined by measuring the mean residence time (MRT) of enzyme in mice. These initial results provide convincing data that HuBChE is a safe bioscavenger that can protect humans against all OP nerve agents.

2. Materials and methods

2.1. Purification of HuBChE

HuBChE was purified from Cohn Fraction IV-4 using a two-step procedure, which employed affinity batch extraction of enzyme followed by anion-exchange chromatography. Approximately, 6 g of purified enzyme was obtained from 120 kg of Cohn fraction IV-4 using this procedure [5] and stored in lyophilized form at −20 °C. The specific activity of the enzyme was 700–750 U/mg as measured in 50 mM sodium phosphate buffer at pH 8.0, at 25 °C, using 1 mM butyrylthiocholine as the substrate [6].

2.2. Pharmacokinetics, bioavailability, and safety, of HuBChE in mice

Research was conducted under protocols approved by the Walter Reed Army Institute of Research Animal Care and Use Committee, in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals, experiments involving animals, and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*.

Thirty-six CD-1 mice (8 weeks old, equal number of male and female, weight 25–30 g) were divided into six groups ($n = 6$). Animals in each group were injected with HuBChE (0.1, 1, or 3 mg) by i.m. or i.p. injections. Two extra groups of animals injected with saline only by either i.m. or i.p. injection, served as controls. Following enzyme administration, 10 μl of blood was drawn at various time intervals and diluted 20 times with water for the determination of blood BChE activity [6]. The following pharmacokinetic parameters were determined from the time course curve of blood BChE concentration: MRT, maximal concentration (C_{max}), time to reach the maximal concentration (T_{max}), elimination half-life ($T_{1/2}$), and area under the plasma concentration time curve extrapolated to infinity (AUC), using a Windows-based program for non-compartmental analysis of pharmacokinetic data [7]. The animals were observed for any abnormal physiological or behavioral signs for 2 weeks; they were euthanized and blood was collected for determining hematology and serum chemistry parameters. A complete necropsy was also performed and a full set of tissues, including brain, heart, lung, liver, intestine, kidney, eye, spleen, and muscle injection sites, were examined for any gross or histological changes.

2.3. In vitro and in vivo stability of HuBChE

Aliquots of enzyme (1 mg) were stored in lyophilized form at 4, 25, 37, or 45 °C. Samples were resuspended in 1 ml of 50 mM sodium phosphate buffer, pH 8.0 at various time intervals and assayed for BChE activity as described [6]. In vivo circulatory stability of the enzyme