

Human Serum Butyrylcholinesterase: A Bioscavenger for the Protection of Humans from Organophosphorus Exposure

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ABSTRACT

Human serum butyrylcholinesterase (Hu BChE) is currently under advanced development as a pretreatment drug for organophosphate (OP) poisoning in humans. Toward this effort, a procedure for the large-scale purification of Hu BChE from Cohn fraction IV-4 was developed, its pharmacokinetic properties were established, its shelf life was evaluated at various temperatures, and its safety and efficacy were examined. It was shown to protect mice, rats, guinea pigs, and monkeys against multiple LD₅₀ challenges of OP nerve agents by i.v. or s.c. bolus injections. Since inhalation is the most likely route of exposure on the battlefield or in public places, the aim of this study was to evaluate the efficacy of Hu BChE against whole-body inhalation exposure to sarin (GB) vapor. The study was conducted in minipigs because the pig offers many similarities in anatomy and physiology to humans. Male Göttingen minipigs were subjected to one of the following treatments: (1) Air exposure; (2) GB vapor exposure; and (3) pretreatment with 7.5 mg/kg of Hu BChE followed by GB vapor exposure. Hu BChE was administered by i.m. injection, 24 h prior to whole-body exposure to GB vapor at a concentration of 4.1 mg/m³ for 60 min or 11.4 mg/m³ for 10 min. EEG, ECG, and pupil size were monitored throughout exposure, and blood drawn from a surgically implanted jugular catheter before and throughout the exposure period was analyzed for acetylcholinesterase (AChE) and BChE activities, and the amount of GB present in plasma. Baseline blood AChE and BChE activities were 2.5 ± 0.1 U/ml and 0.14 ± 0.01 U/ml, respectively. Animals pretreated with 7.5 mg/kg of Hu BChE showed peak blood BChE activity of 41.2 ± 0.9 U/ml with a mean residence time of 287 ± 53 h. Similar retention times of 225 ± 19 h for monkey BChE in macaques and 8-11 days for Hu BChE in humans reported previously suggest that there is a high homology between human and pig enzymes. All animals exposed to GB vapor for 60 min showed signs of cardiac and neurological toxicity and died following exposure. Animals exposed to GB vapor for 10 min also died, but showed signs of cardiac toxicity only. All animals pretreated with 7.5 mg/kg of Hu BChE survived the GB exposure. Additionally, the amount of GB bound in plasma was 200-fold higher compared to that from plasma of pigs that did not receive Hu BChE, suggesting that Hu BChE was effective in scavenging GB in blood, and preventing it from inhibiting CNS AChE. Pretreatment with Hu BChE prevented cardiac abnormalities and seizure activity observed in untreated animals. These results provide convincing data that the use of Hu BChE would provide a capability for extended protection against a wide spectrum of nerve agents and would eliminate the need for extensive post-exposure therapy. In addition, the objective force will be able to take the advantage of these technologies, which will provide sustained maximum

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Human serum butyrylcholinesterase (Hu BChE) is currently under advanced development as a pretreatment drug for organophosphate (OP) poisoning in humans. Toward this effort, a procedure for the large-scale purification of Hu BChE from Cohn fraction IV-4 was developed, its pharmacokinetic properties were established, its shelf life was evaluated at various temperatures, and its safety and efficacy were examined. It was shown to protect mice, rats, guinea pigs, and monkeys against multiple LD50 challenges of OP nerve agents by i.v. or s.c. bolus injections. Since inhalation is the most likely route of exposure on the battlefield or in public places, the aim of this study was to evaluate the efficacy of Hu BChE against whole-body inhalation exposure to sarin (GB) vapor. The study was conducted in minipigs because the pig offers many similarities in anatomy and physiology to humans. Male Göttingen minipigs were subjected to one of the following treatments: (1) Air exposure; (2) GB vapor exposure; and (3) pretreatment with 7.5 mg/kg of Hu BChE followed by GB vapor exposure. Hu BChE was administered by i.m. injection, 24 h prior to whole-body exposure to GB vapor at a concentration of 4.1 mg/m³ for 60 min or 11.4 mg/m³ for 10 min. EEG, ECG, and pupil size were monitored throughout exposure, and blood drawn from a surgically implanted jugular catheter before and throughout the exposure period was analyzed for acetylcholinesterase (AChE) and BChE activities, and the amount of GB present in plasma. Baseline blood AChE and BChE activities were 2.5 ± 0.1 U/ml and 0.14 ± 0.01 U/ml, respectively. Animals pretreated with 7.5 mg/kg of Hu BChE showed peak blood BChE activity of 41.2 ± 0.9 U/ml with a mean residence time of 287 ± 53 h. Similar retention times of 225 ± 19 h for monkey BChE in macaques and 8-11 days for Hu BChE in humans reported previously suggest that there is a high homology between human and pig enzymes. All animals exposed to GB vapor for 60 min showed signs of cardiac and neurological toxicity and died following exposure. Animals exposed to GB vapor for 10 min also died, but showed signs of cardiac toxicity only. All animals pretreated with 7.5 mg/kg of Hu BChE survived the GB exposure. Additionally, the amount of GB bound in plasma was 200-fold higher compared to that from plasma of pigs that did not receive Hu BChE, suggesting that Hu BChE was effective in scavenging GB in blood, and preventing it from inhibiting CNS AChE. Pretreatment with Hu BChE prevented cardiac abnormalities and seizure activity observed in untreated animals. These results provide convincing data that the use of Hu BChE would provide a capability for extended protection against a wide spectrum of nerve agents and would eliminate the need for extensive post-exposure therapy.

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protection to soldiers under the most adverse battlefield condition, without performance degradation or interruption of OPTEMPO.

1.0 INTRODUCTION

OP nerve agents such as soman (GD), GB, VX, and tabun, exert their toxicity by inhibiting acetylcholinesterase (AChE) in the central nervous system (CNS). The resultant increase in acetylcholine levels at cholinergic synapses, particularly in the brain and diaphragm, produces an acute cholinergic crisis characterized by miosis, increased tracheobronchial and salivary secretions, bronchoconstriction, bradycardia, fasciculations, behavioral incapacitation, muscular weakness, and convulsions, culminating in cardiorespiratory failure and death. Current medical countermeasures against OP nerve agent poisoning include a combination of pretreatment with a carbamate, pyridostigmine bromide, to protect a fraction of AChE from irreversible inhibition by OPs followed by post-exposure treatment with anticholinergic drugs such as atropine sulfate, to counteract the effects of excess acetylcholine and oximes such as 2-PAM chloride, to reactivate OP-inhibited AChE. Due to their inability to cross the blood-brain barrier, these antidotal regimens have met with limited success. While successful in preventing fatality of animals from OP poisoning, they do not preclude post-exposure seizure activity and convulsions, which lead to long-term CNS damage [1-3]. In addition, the timely delivery of this treatment is critical to achieving maximal therapeutic effect, which makes its feasibility questionable under battlefield conditions. These problems stimulated the development of enzyme bioscavengers, which prevent in vivo toxicity of OPs by sequestering them in circulation before they reach the CNS.

Among the enzymes that hold promise as scavengers of highly toxic OP nerve agents, cholinesterases (ChEs) have been extensively examined. The exogenous administration of AChE from fetal bovine serum and BChE from equine and human (Hu) serum, has been successfully used as a safe and efficacious prophylactic treatment to prevent poisoning by OP compounds, in both rodent and non-human primate models [4]. Of the three ChEs evaluated so far, 'self' Hu BChE has the most advantages as a potential candidate for human use. It provides a broad range of protection for all OP nerve agents, is readily absorbed from sites of injection, displays long-lasting stability in human circulation, and being from a human source the enzyme is not expected to produce any adverse immunological responses upon repeated administration into humans [5]. A dose of 200 mg of Hu BChE is envisioned as a prophylactic treatment in humans that can protect from exposure of up to 2 X LD₅₀ of GD [5]. In addition to its use as a pretreatment for OP nerve agent toxicity, it also has potential use for treating pesticide overexposure, cocaine-overdose, or succinylcholine-induced apnea [5]. We recently developed a procedure for the large-scale purification of Hu BChE, which yielded 6 g of purified enzyme from 120 kg of Cohn fraction IV-4 paste [6]. Purified Hu BChE exhibited a remarkable shelf life, displayed long-lasting stability in the circulation of rodents and non-human primates, and was devoid of any toxic side effects [7]. The efficacy of the enzyme as a pretreatment against 5 X LD₅₀ of GD and VX was demonstrated in guinea pigs [8]. Similarly, the enzyme was shown to protect cynomolgus monkeys against 3.5-5.5 X LD₅₀ of GD.

In this study, we investigated the utility of Hu BChE as a prophylactic measure against inhalation toxicity of GB vapor, which is a more realistic simulation of battlefield exposure. This study was conducted in the Göttingen minipig, which is widely accepted as a surrogate for cardiorespiratory physiology in man, and is an attractive animal model for investigating neurotoxicology. Although, the ability of exogenously administered Hu BChE to alleviate toxicity due to intranasal exposure to GD vapor, was reported previously in guinea pigs [9], this is the first report examining the efficacy of this enzyme against whole-body inhalation exposure in a higher mammalian species. The results of this study demonstrated that pretreatment with 7.5 mg/kg of Hu

BChE alone increased survivability and prevented cardiac abnormalities and seizure activity in minipigs exposed to GB vapor (4.1 mg/m³ for 60 min).

2.0 MATERIALS AND METHODS

Research was conducted under protocols approved by the United States Army Edgewood Chemical Biological Center Institutional 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*.

2.1 Preparation of Minipigs for Whole Body Exposure to GB Vapor

Sexually mature male Göttingen minipigs (*Sus scrofa*) were obtained from Marshall Farms USA (North Rose, NY) and maintained in a temperature and humidity controlled facility. Surgeries to implant indwelling catheters into the external jugular veins of the minipigs were performed as described previously [10]. The minipigs were allowed to recover for at least three days before they were used for any exposures. During that time, the patency of the catheter was maintained by flushing with heparinized saline as needed.

2.2 Setup for Whole Body Exposure to GB Vapor

Whole body exposures were conducted in a 1000-l dynamic airflow inhalation chamber. The vapor generation system was located at the chamber inlet and was contained within a stainless steel glove box maintained under pressure. GB vapor was generated by delivering liquid agent into a spray atomizer using a gas-tight syringe. The concentration of GB in the exposure chamber was determined by trapping vapor using solid sorbent tubes (Tenax/Haysep), followed by thermal desorption and gas chromatographic analysis.

Pigs were placed in a sling constructed of canvas (Lomir Biomedical, Inc., Malone, NY, or Canvas and Awning supplies, White Marsh, MD) and fitted to accept the animal through 4 leg holes. Pigs were gently restrained in the sling by two straps that secured over the shoulders and hips. A muzzle harness was placed over each animal's snout, and secured both laterally and ventrally to the stainless-steel framing, to prevent the animals from freely moving their heads. The jugular line was exteriorized from the exposure chamber, which allowed collection of blood samples throughout the exposure period. Bipolar dermal electroencephalographic (EEG) and standard limb electrocardiographic (ECG) leads (I, II, and III) were attached and exteriorized from the chamber and utilized to continuously record tracings throughout the agent exposure period using a Bio-Logic headbox. The output was fed into a Dell personal computer at a sampling rate of 512 Hz. Infrared images of pupil were taken through the plexiglass walls of the exposure chamber.

2.3 Pharmacokinetics and Bioavailability of Hu BChE in Göttingen Minipigs

Minipigs (n=6) were administered Hu BChE at a dose of 3.0 or 7.5 mg/kg by i.m. injection. Blood samples were drawn at different time periods and assayed for BChE activity [11]. Data was processed by noncompartmental analysis of the residual BChE activity as a function of time following Hu BChE injection using PK Solutions 2000 software (Summit Research Services, Montrose, CO). The following pharmacokinetic parameters were determined: mean residence time (MRT), peak BChE activity in blood (C_{max}), time to reach peak plasma level following i.m. load (T_{max}), and area under the time course curve (AUC).

2.4 Efficacy of Hu BChE in Göttingen Minipigs

Minipigs were subjected to one of the following treatments: (1) saline injection followed by air exposure (n=9); (2) saline injection followed by GB vapor exposure (n=4); (3) pretreatment with 3.0 mg/kg of Hu BChE followed by GB vapor exposure (n=3); (4) pretreatment with 6.5 mg/kg of Hu BChE followed by GB vapor exposure (n=3); and (5) pretreatment with 7.5 mg/kg of Hu BChE followed by GB vapor exposure (n=5). Hu BChE or saline was administered by i.m. injection, 24 h prior to whole-body exposure to GB vapor. The concentration of GB vapor used was 4.1 mg/m³ for 60 min or 11.4 mg/m³ for 10 min, doses that were estimated to be lethal to 99% of untreated exposed pigs. EEG, ECG, and pupil size were monitored throughout exposure, and blood drawn from a surgically implanted jugular catheter before and throughout the exposure period was analyzed for AChE and BChE activities, and the amount of GB present in red blood cells (RBCs) and plasma.

2.5 Data Analysis

ECG: ECG data were analyzed using ECG-AUTO software (Emka Technologies, Falls Church, VA) after conversion of data output into IOX format. Twenty to 70 waveforms were defined to build custom libraries for each subject, and analyzed in 3 min contiguous time blocks, with a minimum of 20 valid beats required to qualify the data block for statistical analysis. For each block, RR, PR, and QT intervals were quantified.

EEG: EEG data were analyzed using the Sleepscan II and Insight software modules of the Bio-logic system. The fronto-central montage was initially screened by visual inspection to disqualify regions of the tracing exhibiting electromuscular contamination or other artifact. Low- (<0.5 Hz) and high-band (>35 Hz) filters, as well as a 60 Hz notch filter were then applied to the tracings. Following acclimation of pigs to the inhalation chamber, a pre-exposure baseline and a 60 minute exposure period following introduction of agent were recorded. These were fragmented into 5 min bins, and a 60 second data block from each bin was then subject to spectral analysis after applying Fast Fourier Transformation. EEG power in each of the classical frequency bands was determined by integrating the resulting frequency spectrum using the following limits: delta = 0.5 – 4.5 Hz, theta = 4.5 – 8.5 Hz, alpha = 8.5 – 13.5 Hz, beta 1 = 13.5 – 21.5 Hz, and beta 2 = 21.5 – 35.5 Hz.

3.0 RESULTS AND DISCUSSION

3.1 Pharmacokinetics and Bioavailability of Hu BChE

Minipigs that were administered Hu BChE by i.m. injection showed a rapid increase in BChE activity, which reached peak levels at ~24 h. Time courses for 3.0 and 7.5 mg/kg of Hu BChE, are shown in Figure 1(left panel). Purified Hu BChE exhibited circulatory stability profiles similar to those observed in mice, guinea pigs, and rhesus monkeys [7]. Baseline AChE and BChE activities in blood were 2.5 ± 0.1 and 0.14 ± 0.01 U/ml, respectively. Doses of 3.0 and 7.5 mg/kg of Hu BChE resulted in peak BChE activities of 14.8 ± 2.1 and 41.2 ± 0.9 U/ml, respectively. Regardless of the dose of administration, the enzyme displayed a MRT of 287 ± 53 h. Such long retention times were previously observed for homologous injections of monkey BChE into macaques (MRT=225 $\pm$ 19 h; [12]) and for Hu BChE into humans (8-11 days; [13, 14]). As expected, the administration of Hu BChE did not affect the levels of circulating AChE (Figure 1, right panel).

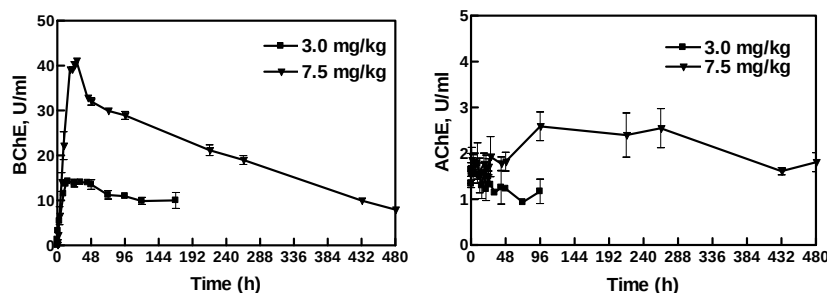


Figure 1: Average BChE (left panel) and AChE (right panel) levels in the blood of minipigs following i.m. injections of two doses of Hu BChE.

3.2 Dose of Hu BChE Needed for Protection

In the first study, minipigs were pretreated with saline, 3.0, 6.5, or 7.5 mg/kg of Hu BChE by i.m. injection and challenged with air or GB vapor (4.1 mg/m^3 for 60 min) 18-20 h later. All untreated control animals or those treated with 3.0 or 6.5 mg/kg of Hu BChE died following exposure to GB vapor, while all animals pretreated with 7.5 mg/kg of Hu BChE survived exposure to GB vapor. Circulating RBC AChE activity in untreated control animals was completely inhibited in <30 min into exposure to GB vapor. On the other hand, in animals that were pretreated with Hu BChE, the complete inhibition of AChE occurred 40-60 min into exposure, depending on the dose of Hu BChE used. Results demonstrate that pretreatment with 3.0 or 6.5 mg/kg of Hu BChE did not provide complete protection to minipigs against an inhalation exposure to GB vapor. However, pretreatment with 7.5 mg/kg of Hu BChE was effective in preventing toxicity due to GB vapor by sequestering it in circulation. This was also supported by the results of a fluoride ion-based regeneration assay that measured the amount of GB bound to RBCs and plasma at 60 min into exposure. The amount of GB detected in RBCs was similar in control and all Hu BChE pretreated pigs. However, the amount of GB detected in plasma increased with the dose of Hu BChE, and was 200-fold greater in pigs pretreated with 7.5 mg/kg of Hu BChE.

In the second study, minipigs were pretreated with saline or 7.5 mg/kg of Hu BChE by i.m. injection and challenged with air or GB vapor (11.4 mg/m^3 for 10 min) 18-20 h later. All untreated control animals died, while all animals pretreated with 7.5 mg/kg of Hu BChE survived exposure to GB vapor. In animals that were pretreated with Hu BChE, 75 % of circulating AChE activity was inhibited at 10 min into exposure and a maximal inhibition of 85 % was observed at 2 h. Results demonstrate that pretreatment with 7.5 mg/kg of Hu BChE was also effective in preventing toxicity due to an acute exposure to GB vapor.

3.3 Effect of Hu BChE Pretreatment on Sarin-Induced Cardiotoxicity

The present study also demonstrates that in addition to increasing survivability, pretreatment with Hu BChE was effective in preventing acute cardiac toxicity due to GB vapor. The accumulation of acetylcholine at central as well as peripheral cholinergic synapses due to the inhibition of AChE by OP nerve agents, causes alterations in autonomic drive which produces cardiac pathophysiology. Three phases of cardiac OP intoxication have been described [15]: (a) An increase in sympathetic tone, presumably mediated by stimulation of sympathetic ganglionic receptors, is reflected in progressive sinus tachycardia. Tachycardia is also among the most common cardiac abnormalities observed in clinical cases of sub-lethal OP poisoning

[16]; (b) Arrhythmia, most likely produced by alterations in AV conduction. Allon et al. [17] reported that AV conduction abnormalities were hallmarks of OP intoxication, and AV block was noted to occur in guinea pigs after upper airway inhalation of GD [18]. Arrhythmias were reported to be a consistent feature of GD intoxication in baboons, and could be the first indication of cardiac toxicity [19]; and (c) QT prolongation, ventricular tachycardia and fibrillation are reportedly observed immediately prior to cardiac death. QT prolongation is commonly reported in survivors of OP poisoning [16, 20].

Normal baseline data for cardiac heart rate, rhythmicity, RR, PR, and QT intervals were established using air-exposed control animals. A comparison of these values with similar data from previously published data showed that the mean RR and QT intervals observed in this study were within the 95% confidence limits of referenced values. PR intervals for air-exposed pigs were also similar to reference values. Upon exposure to GB vapor (4.0 mg/m^3 for 60 min), untreated control animals exhibited a variety of cardiac abnormalities, starting with tachycardia and a corresponding shortening of QT intervals, followed by bradycardia, and ultimately death. Pretreatment with Hu BChE delayed the onset of cardiac abnormalities in a dose-dependent manner, with 7.5 mg/kg preventing cardiac abnormalities observed in untreated animals and those treated with lower doses of Hu BChE (Figure 2).

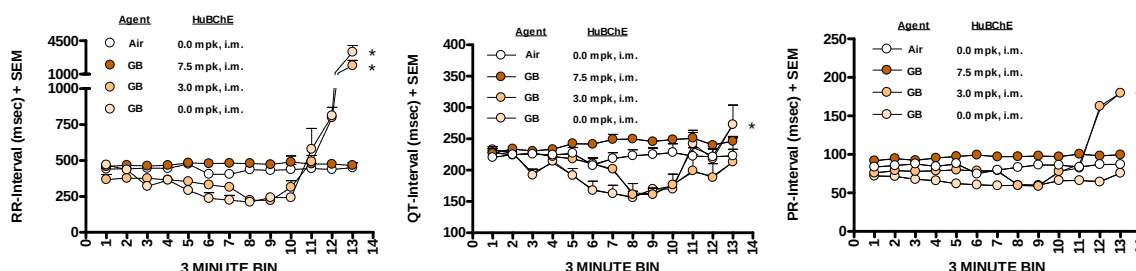


Figure 2: Effect of Hu BChE pretreatment on RR, QT, and PR intervals for minipigs exposed to GB vapor (4.0 mg/m^3 for 60 min). Gottingen minipigs were treated with saline or Hu BChE 24 h prior to exposure to air or GB vapor. RR (A), QT (B), and PR (C) intervals were analyzed as a function of time relative to death. * indicates that treatment as a function of time is significantly different from saline-injected, air-exposed control animals. Gaps in the data prevented the calculation of variance estimates in the group that received 6.5 mg/kg Hu BChE. This group was therefore not included in the analysis.

In the second study, whole body inhalation exposure to GB vapor (11.4 mg/m^3 for 10 min) also resulted in the rapid development of a variety of cardiac abnormalities in the minipig (Figure 3). Tachycardia began within several min of GB exposure. Heart rate accelerated throughout the 10 min exposure period, reaching nearly 200 beats/min. PR and uncorrected QT intervals were largely unchanged during this time. This was followed by the development of a profound bradycardia between 10-20 min following exposure onset. Interestingly, this period was associated with a slight shortening, rather than lengthening, of the PR interval suggesting the development of cardiac arrhythmia. In the final phase of toxicity, heart rate dramatically slowed concomitant with a marked lengthening of the PR interval. QT intervals remained stable until the animals expired. A regular, but progressively slowing junctional or ventricular rhythm was observed immediately prior to complete cardiac asystole. Of note, neither QT prolongation nor end-stage tachycardia were features of acute GB intoxication in the minipig. It remains unclear whether these features, as reported in human survivors of OP poisoning, reflect a species difference, differential effect of GB versus other OP nerve agents and pesticides, or the post-exposure time course. All animals ($n=4$) pre-treated with Hu BChE survived nerve agent exposure. Heart rate, PR intervals and corrected QT intervals remained unchanged from baseline levels

throughout the nerve agent exposure and subsequent 50 min post-exposure monitoring period (Figure 3). Thus, pretreatment of minipigs with 7.5 mg/kg Hu BChE appeared to completely prevent acute cardiac intoxication following exposure to GB vapor. No changes in heart rate, PR and corrected QT intervals were observed for up to 1 week following GB vapour exposure, confirming protection against chronic cardiotoxic effects resulting from GB vapor exposure (data not shown).

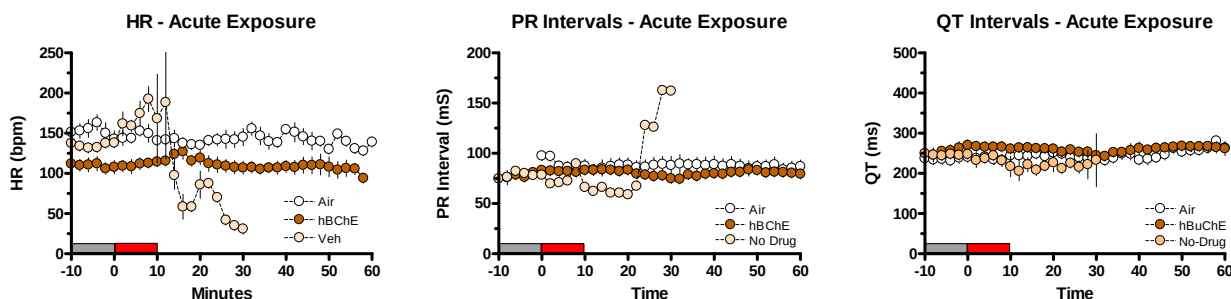


Figure 3: Effect of Hu BChE pretreatment on RR, QT, and PR intervals for minipigs exposed to GB vapor (11.4 mg/m³ for 10 min). HR, PR and non-corrected QT intervals for minipigs pretreated with 7.5 mg/kg Hu BChE or vehicle and exposed to GB vapor. Vehicle-treated, air-exposed pigs are included for reference. Grey bar indicates baseline. Time of agent exposure is indicated by red bar.

3.4 Effect of Hu BChE Pretreatment on Sarin-Induced Neurological Toxicity

Following OP exposure, the accumulation of acetylcholine in the CNS likely precipitates the development of seizure activity [21]. Focal spike and wave discharges are likely to initiate in particularly sensitive brain regions, for example the limbic telencephalon, generalize through recruitment of excitatory amino acid neurotransmission, and then rapidly progress to sustained convulsive seizures or status epilepticus [22]. A s.c. exposure of rats to GD was shown to produce alterations in EEG coincident with the development of convulsions, which were characterized by an increase in EEG amplitude from 300 to >900 μ V, with an increase evident in all spectral bands [23].

Consistent with these observations, a typical EEG power spectrum obtained from an air-exposed minipig demonstrated low amplitude (peak < 300 μ V) and long wavelength energy predominantly within the delta and theta frequency bands. A published EEG spectrum for pigs was not available for comparison, but this spectrum was comparable to that published for rhesus monkeys [24]. EEG power spectra of control pigs were stable throughout the period of air exposure. As expected, whole body exposure to GB vapor (4.1 mg/m³ for 60 min) resulted in the development of generalized tonic-clonic seizures in minipigs (Figure 4). Minipigs that received saline administration in lieu of Hu BChE exhibited a sharp increase in total power (peak amplitude of 500 μ V) with the onset of generalized seizures 30-40 min into GB exposure. Seizure onset was associated with redistribution of the power spectra away from delta and into higher frequency bands. Notably, administration of 7.5 mg/kg Hu BChE completely prevented seizure activity (Figure 4).

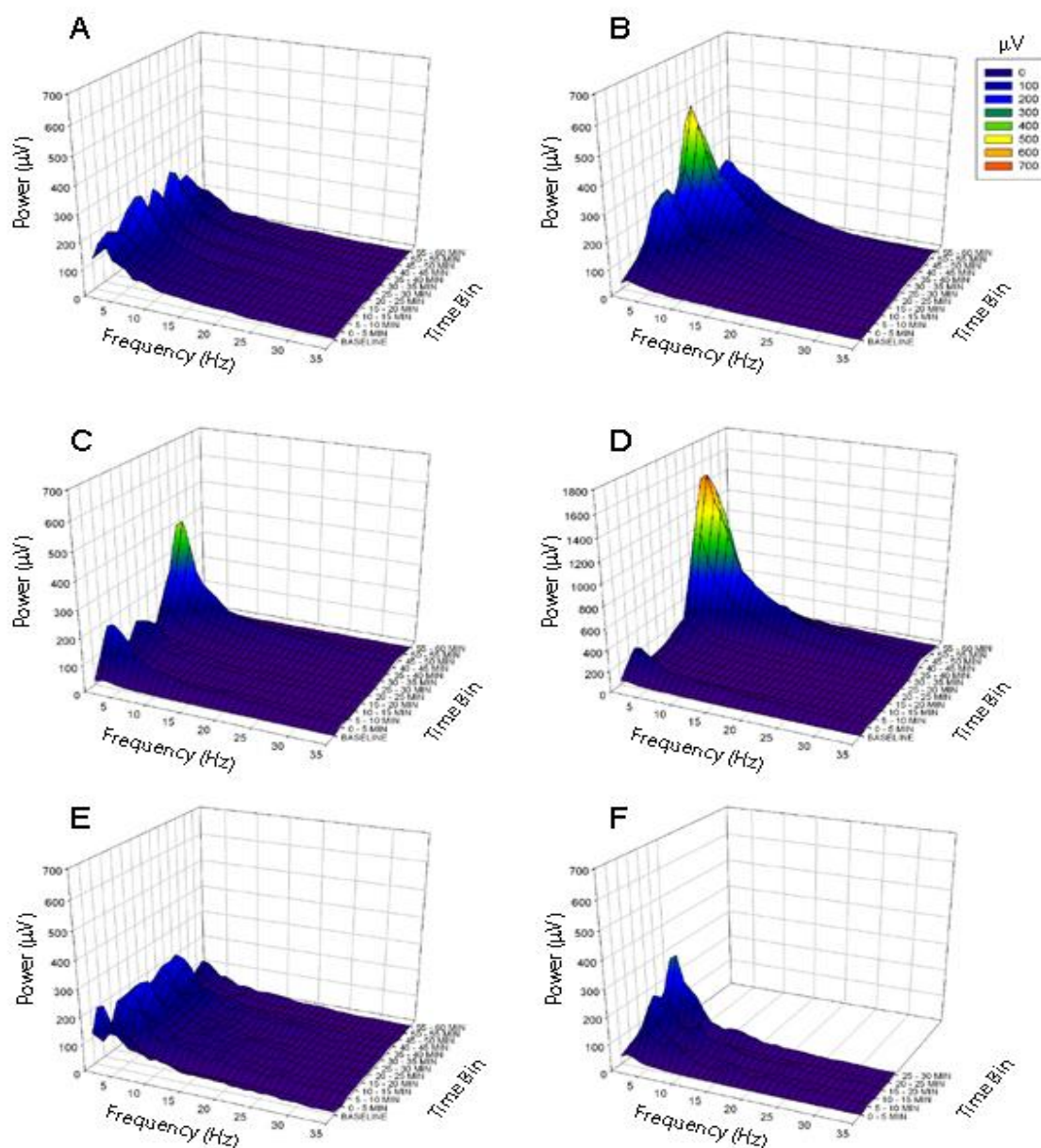


Figure 4: 3D plots of EEG power spectra as a function of time from onset of exposure (4.1 mg/m^3 for 60 min). (A) Saline-injected, air-exposed control pigs feature stable low amplitude, long wavelength energies. (B) Untreated pigs exposed to GB vapor exhibit marked increases in EEG power concurrent with onset of seizure activity. Minipigs pretreated with 3.0 (C) and 6.5 (D) mg/kg Hu BChE also exhibited seizure activity, although high amplitude EEG energy was dose-dependently delayed in onset. Pretreatment with 7.5 mg/kg Hu BChE (E) prevented the manifestation of high amplitude EEG energy, and baseline spectra were similar to saline injected animals. Spectra were also normal 1 week after pretreatment and GB exposure (F).

In the second study, minipigs were exposed to substantially higher GB vapor concentrations (11.4 vs. 4.1 mg/m³) albeit over a shorter time frame (10 vs. 60 min). As indicated above, all vehicle-treated, GB-exposed minipigs rapidly succumbed to the nerve agent. During this time however, EEG spectra remained relatively normal (Figure 5). There was a slight increase in overall power over baseline with the onset of vapor exposure in both Hu BChE and vehicle-treated animals. Peak power, however, remained predominantly in the delta frequency band, below 400 μ V, and on par with the spectral distribution of air-exposed pigs. Thus, it does not seem likely that the observed increase in spectral power reflects a nerve agent-specific event, but perhaps is related to stimulus presentation, e.g. airflow changes in the inhalation chamber. In light of the rapid development of cardiac toxic signs and the rapid demise of the untreated animals, it is clear that the lack of EEG findings is not related to an issue with agent exposure. Moreover, real-time monitoring of the chamber atmosphere confirmed the concentration of GB vapor as 11.4 mg/m³, approximately three times that which produced seizures. Thus, it seems most likely that the untreated animals in this study simply succumbed to intoxication prior to the onset of seizure activity. Because of the absence of seizures in the untreated group, the present study does not directly address protection of the CNS under these exposure conditions. It is, however, encouraging to note that despite an otherwise lethal GB exposure, Hu BChE-treated minipigs failed to develop abnormal EEG signs at any point during the post exposure period or in a 1 week follow-up examination.

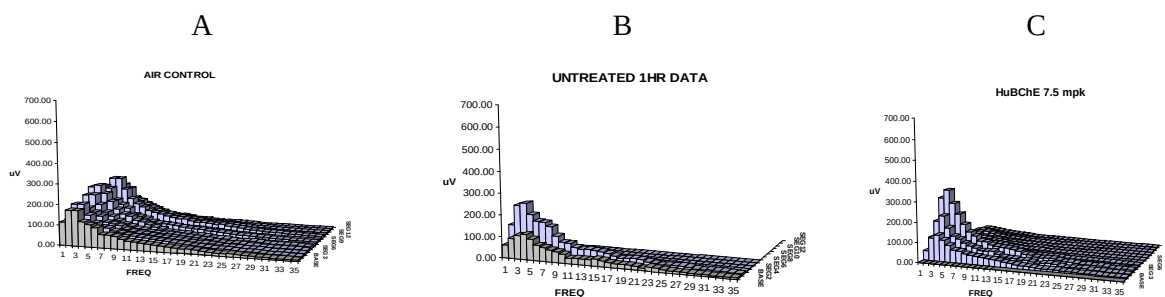


Figure 5: EEG spectral analysis of minipigs treated with vehicle (A, B) or 7.5 mg/kg Hu BChE (C) and exposed to air (A) or GB vapor (B, C). Although there is an increase in delta band power in GB-exposed minipigs, peak voltage is indistinguishable from air-exposed controls.

3.5 Effect of Hu BChE Pretreatment on Sarin-Induced Miosis

It was reported that a direct effect of OP nerve agent exposure is miosis [25]. Consistent with this report, all untreated control minipigs exposed to GB vapor had pinpoint pupils 5-6 min into exposure (Figure 6 A). The onset or magnitude of the miosis observed upon exposure to GB vapor was not altered by pretreatment with Hu BChE (Figure 6 B). A similar time course of miosis in both treated and untreated pigs suggests that miosis could be used as a biomarker of exposure in Hu BChE pretreated individuals.

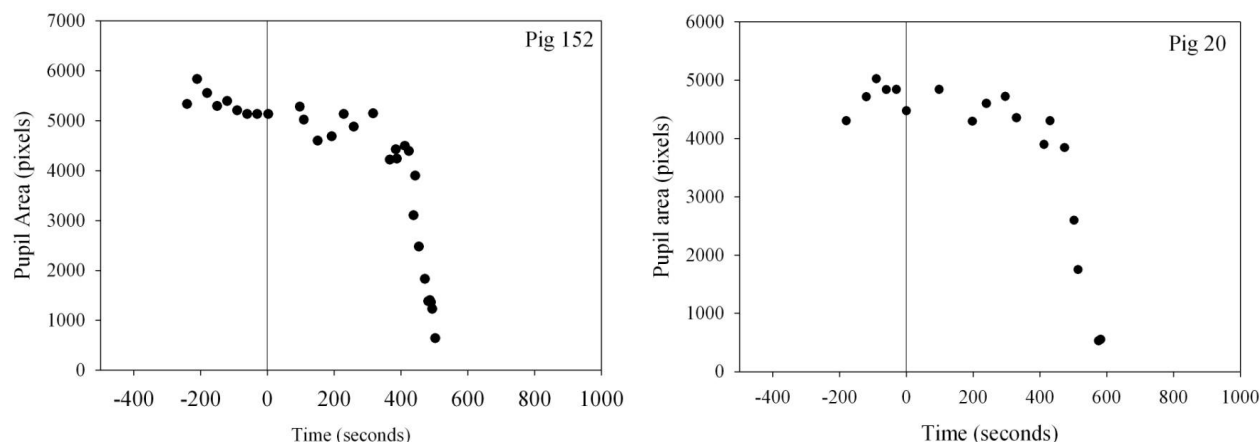


Figure 6: Representative tracings showing the change in pupil size in (A) a minipig exposed to GB vapor alone, and (B) a minipig pretreated with 7.5 mg/kg of Hu BChE by i. m. injection prior to GB vapor exposure.

4.0 CONCLUSIONS

The results of this first report examining the utility of Hu BChE as a prophylactic measure against whole-body inhalation exposure demonstrate that pretreatment with Hu BChE alone was sufficient to protect Göttingen minipigs not only from lethality due to GB vapor, but also against cardiac and neurological toxicity. Although the pharmacokinetic behavior of Hu BChE in minipigs was similar to that reported in mice, rats, guinea pigs and monkeys, it displayed a much longer MRT in minipigs. As with s.c. and i.v. exposures in rodents and non-human primates, the protection of minipigs against inhalation exposure could be easily followed by monitoring blood ChE levels. Pretreatment with Hu BChE delayed the inhibition of RBC AChE by GB in a dose-dependent manner. A dose of 7.5 mg/kg of Hu BChE was sufficient to completely sequester GB, and protect minipigs from toxicity due whole body exposure to GB vapor. This study not only highlights the universal applicability of protection by Hu BChE against OP toxicity regardless of the route of exposure, but will aid the reliable prediction of protective dose in humans.

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